# EFFECT OF NUTRIENT INTAKE DURING GESTATION ON ESTRUS OF BEEF COWS AND POSTNATAL GROWTH AND DEVELOPEMNT OF CALVES

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Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
May, 2008

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# CHAPTER I

# INTRODUCTION

The beef industry is an important economic entity in the United States and in Oklahoma. There are 104.8 million cattle in the United States (USDA 2007a) and Oklahoma has the third largest beef herd in the United States with just over 2 million cows (USDA 2007b). Because the beef industry is so important to this state and nation, producers must become as efficient as possible as all input costs increase due to increased transportation and feed costs. Reproduction is one of the most important factors affecting profitability of cow calf producers. If a cow is to produce a calf every year they must rebreed by 85 d after calving. A major factor that prevents a calf every year is postpartum anestrus in beef cows.

Reproductive inefficiency due to a long anovulatory interval after calving is a major economic loss for beef cattle producers. Reproductive disease and suboptimum reproductive performance costs the beef industry 441 to 502 million annually (Bellows et al., 2002). The greatest portion of this cost is cows that do not rebreed during a set breeding season. One fourth of all cows culled are sold because of reproductive failure (NAHMS, 1997).

Feed costs are one of the largest inputs for any phase of beef cattle production. Nutrition has a profound impact on reproduction in beef cows (reviewed by Wettemann

et al., 2003) but can affect other economically important traits. Level of nutrition during gestation may influence the growth and development of the calf. The changes in development of the fetus may persist in the offspring into later life. Therefore an understanding of nutritional effects on both postpartum reproduction of cows and on the offspring is needed to fully understand the role of nutrition in a beef cow operation.

The objectives of the work presented herein were to determine the effects of days after calving and BCS at calving on estrous and luteal responses of suckled anestrous beef cows after treatment with estradiol, to evaluate the effect of nutrient restriction during early gestation on postnatal growth, glucose regulation, and DNA, protein and gene expression in tissues of steers.

# CHAPTER II

# REVIEW OF LITERATURE

## Introduction

Cost of purchased supplemental feed is the second largest expense for a cow calf operation and accounts for 14% of all expenses (Bevers, 2007). The largest expense is depreciation of cows and equipment and is not usually included in economic analysis of cow calf operations. With feed making up such a large portion of the expenses of a cow calf operation, it is only logical to try to minimize the feed used. This can be done by allowing cows to decrease BCS during certain periods of production and then gain it back when forage is at its greatest quantity and quality. This is a common practice in fall calving herds in Oklahoma. Cows are rebred starting in December and once pregnant are allowed to decrease BCS and BW until grasses begin growing in the spring. With more producers wanting to decrease supplement costs and allow cows to decrease BW and BCS during certain periods of the production cycle it is important to fully understand all effects of nutrition on all aspects of a cow calf operation. Nutrition of the cow has a substantial impact on reproduction and may influence the performance of calves.

## The effects of nutrition on reproduction in beef cows

Numerous studies have examined the affect of nutrition on postpartum reproduction in cattle. Wiltbank et al. (1962) and Dunn et al. (1969) determined the effects of nutrient intake during gestation on pregnancy rate after calving. Several reviews have elucidated the effects of nutrition on reproduction of beef cows (Dunn and Kaltenbach, 1980; Short and Adams, 1988; Randel 1990; Wettemann et al., 2003; Hess et al., 2005). The effects of nutrition on reproduction are dependent on timing relative to calving. The effects of nutrition can be mediated by body energy reserves indicated by BCS.

### Postpartum reproduction

The average length of gestation for beef cows is 283 days with variation from 281 to 294 d due to breed of the cow (Cundiff et al., 1993). In addition there is genetic variation for length of gestation within a breed (Cundiff et al., 1993). For a cow to have a calf every 365 d with a average gestation length leaves 82 d after calving for a cow to successfully rebreed. There are several physiological mechanisms that control duration of anestrus and infertility in postpartum beef cows (reviewed by Short et al., 1990). Postpartum anestrus can include silent heat in which ovulation preceded the first observed estrus (Humphrey et al., 1983, Perry et al., 1991). However, suckling and nutritional factors are the two primary factors causing postpartum anestrus. Short estrous cycles are the second factor that contributes to anestrus and usually occurs during the first 30 to 40 days after calving. Short estrous cycles result from either a corpus luteum (CL) that is not capable of functioning normality or premature regression signal. The CL

formed during a short estrous cycle is smaller, secretes less progesterone and is less responsive to stimulation with LH (Short et al., 1974; Rutter and Rander 1984; Carruthers et al., 1986). Another reason for a short cycle postpartum is due to a premature CL regression signal (PGF2α, Garverick et al., 1992). Then there is uterine involution, which often plays a relatively small part; however, it is still a barrier to fertility during the first 30 d after calving (Graves et al., 1968). Finally there is general infertility that is associated with any estrus regardless of its time during the lifetime of the cow and regardless of physiologic state (Short et al., 1990).

**Body condition score**

Body condition score (BCS) is a numerical 1 to 9 scoring system with 1 being severely emaciated and 9 being very obese that estimates the amount of fat and therefore energy reserves in a cow (Wagner et al., 1988). BCS has a strong relationship with carcass fat ($R^2 = 0.82$) and carcass energy ($R^2 = 0.85$, Wagner et al., 1988). This system allows producers to visually access the BCS and thus the energy reserves of cows at any point in the production system.

Cows with a greater BCS at calving have increased pregnancy rate when compared with cows that calve in a thinner body condition (Rakestraw et al., 1986; Richards et al. 1986; Selk et al., 1988). Pregnancy rates were improved in primiparous cows that calved in greater BCS (56, 80, 96% for BCS 4, 5, and 6 respectively; Spitzer et al. 1995). First service conception rate was not affected by BCS at calving; however, overall pregnancy rate was greater for cows that calved with greater BCS (Lake et al., 2005). Cows that calved in a BCS of 5 or greater had a greater pregnancy rate regardless of whether BCS

increased or decreased from 6 mo of gestation until parturition (Morrison et al., 1999). There is a negative correlation between BCS at calving and duration of postpartum anestrus (Richards et al., 1986; Ciccioli et al., 2003).

Nonlactating cows with a BCS ≤ 4 had reduced weights of ovaries, corpus lutea and follicular fluid compared to cows in moderate to good body condition (Rasby et al., 1991). Cows with a lower BCS at calving had reduced numbers of large follicles after calving compared with cows in good body condition (Perry et al., 1991)

**Prepartum nutrition**

Prepartum nutrition has a major effect on BCS at calving. Therefore it is difficult to separate the effects of prepartum nutrition from the effects of BCS at calving. If cows are fed greater amounts of protein and energy before calving they have a shorter interval from calving to estrus and ovulation (Perry et al., 1991) and a shorter interval from calving to pregnancy (Dunn et al., 1969). Increased nutrient intake during the prepartum period increased the percentage of cows exhibiting estrus during the breeding interval (Corah et al., 1979; Spitzer et al., 1995) and increased pregnancy rates (Selk et al., 1988; Marston et al., 1995). If nutrient intake is reduced before calving, cows are thinner at parturition, have a prolonged period from calving until estrus, and fewer cows express estrus during the breeding season (Wiltbank, 1962; Bellows and Short, 1978; Dunn and Kaltenbach, 1980).

## Postpartum nutrition

The effects of postpartum nutrition on reproduction are dependent on the BCS of cows at calving. If cows calve with adequate BCS, then postpartum dietary energy intake has less effect on the length of the interval from calving to first estrus (Richards et al., 1986; Marston et al., 1995, Spitzer et al., 1995). When cows calve with a lower BCS, then the interval from calving to first estrus can be shortened by feeding greater amounts of energy after calving (Wiltbank et al., 1964; Spitzer et al., 1995) and increased intake decreased the interval from calving to first normal luteal phase (Lalman et al., 2000). When cows calved with a BCS of 4 to 5 and were fed to gain 0.9 kg/d for 71 d postpartum, they had a shorter interval from calving to estrus and ovulation, a larger dominate follicle at first ovulation, and increase pregnancy rates after AI at first estrus than cows that were fed to gain 0.45 kg/d (Ciccioli et al., 2003). Even cows calving in good condition are not exempt to the effects of inadequate nutrient intake during the postpartum period. Rakestraw et al. (1986) observed that cows fall calving with good BCS, and were subsequently exposed to inadequate nutrient intake after calving due to season decrease in forage availability and quality, had fewer cows exhibiting estrus during the first 70 d postpartum.

## Anestrus

Anestrus is a physiological condition where ovulation and estrous behavior are absent. This is normal during the postpartum period in beef cows and is a significant factor in decreased postpartum reproduction (Wettemann et al., 1980; Yavas and Walton,

2000b; Wiltbank et al., 2002). Its length can be affected by many factors including pre- and post-partum nutrition and also body condition at calving.

**Ovarian Characteristics**

Follicular waves are not detected during the final three weeks of pregnancy (Ginter et al., 1996). A large dominant follicle is present on the ovary 10 d after calving in beef cows; however, only 11% of the dominant follicles ovulated at approximately 10 d postpartum (Murphy et al., 1990). Beef cows have 3 to 8 follicular waves after calving before ovulation occurs (Murphy et al., 1990; Stagg et al., 1995). Although dominant follicles are present during postpartum anestrus, ovulation does not occur. Dominant follicles on the ovary during the postpartum period may not respond to gonadotropins. Not all of the dominant follicles of postpartum anovulatory cows treated with LH (Duffy et al., 2000) or GnRH (reviewed by Wettemann et al., 1980; Yavas and Walton, 2000a) will ovulate, indicating that not all dominant follicles have the capacity to respond to LH. Estrogen production by dominant follicles increases with time after parturition (Spicer et al., 1986b). The dominant follicles present during the postpartum anovulatory period produce less estrogen than preovulatory follicles during normal estrous cycles (Braden et al., 1986). Estrous behavior does not occur before the first postpartum ovulation in most beef cows (Murphy et al., 1990; Looper et al., 1997, Ciccioli et al., 2003).

Luteal function that is shorter than the usual duration is common after the first postpartum ovulation. The life span of a corpus luteum after the first postpartum ovulation is normally less than 10 d (Corah et al., 1975; Werth et al., 1996; Looper et al.,

2003). This short lived corpus luteum is due to a premature luteotropic signal (Garverick et al., 1992).

**Endocrine function**

Concentrations of LH and FSH in plasma are minimal in cows during late gestation (Crowe et al., 1998). On d 1 to3 after parturition, concentrations of FSH increase and surges of FSH are associated with recruitment of follicular waves (Crowe et al., 1998). Concentrations of GnRH in the hypothalamus remain constant during the postpartum anovulatory period (Nett et al 1988).

Pulses of LH are not detectable during the early postpartum period (reviewed by Wettemann et al., 1980; Yavas and Walton, 2000) and resume some time around d 25 postpartum and continue to increase during the postpartum period (Rawlins et al., 1980; Riley et al., 1981; Lueng et al. 1986). Pituitary stores of LH are replenished back to that of cycling cows by d 30 postpartum (Nett et al., 1988). Plasma concentrations of LH vary and are affected by BCS of cows (Rutter and Randel, 1984; Bishop et al., 1994). Exogenous GnRH can increase LH pulses and concentrations in postpartum anestrous beef cows (Echternkamp et al., 1978; Spicer et al., 1986a). A single injection of GnRH induced an LH surge and caused 100% of cows to ovulate their first postpartum dominant follicle (Crowe et al., 1993). Two injections of GnRH 10 d apart induced cyclic activity in beef cows (Cobb et al., 1977). A single injection of GnRH ovulated well developed follicles (Fonseca et al., 1980; Irvin et al., 1981; Carruthers et al., 1986). The corpus luteum (CL) produced by a single administration of GnRH usually has a shorter life span then spontaneously-formed CL (Copelin et al., 1988; Pratt et al., 1982; Wettemann et al.,

1982) and reduced responsiveness to LH in vitro (Kesler et al., 1981). Infusion of GnRH every 2 hr increased size and number of large follicles (Spicer et al., 1986a) and induced preovulatory type surges in LH (Jagger et al., 1987). Administering GnRH every 2 h for either 2 or 4 d resulted in 80 and 73%, respectively of cows ovulating at greater than 20 d postpartum (Riley et al. 1981; Walter et al., 1982). Continuous infusion of GnRH beginning on d 9 to 35 after calving induced an LH surge and ovulation in suckled beef cows (Lofstedf et al., 1981; Jagger et al., 1987; D'Occhio et al., 1989). Hourly pulses of LH resulted in 50% of treated cows to ovulate their first postpartum dominate follicle (Duffy et al., 2000). This indicated that the major cause for 3 to 8 postpartum follicular waves before the first postpartum ovulation is a dysfunction in the hypothalamus and an inability to release GnRH to cause ovulation.

**Use of estrogens to influence reproduction in cattle**

Endogenous estrogens have been used to study physiological mechanisms that regulate reproduction in domestic animals and also to manipulate the reproductive cycle to improve reproduction efficiency. At this time there is not an estrogen compound approved for use in cattle in the United States.

**Estrous behavior**

Estrus in the bovine is a limited period of time in which the female will stand to be mounted by the male. The estrous period is associated with dramatic alterations in hormones that control estrus (reviewed by Allrich, 1994). Estradiol-17β is produced and secreted by the dominant follicle (Moor, 1973; Staigmiller et al., 1982).

Treatment of ovariectomized heifers (Ray, 1965; Rajamahendran et al., 1979; Cook et al., 1986) or cows (Nessan and King, 1981; Cook et al., 1986) with estradiol benzoate induced standing estrus. Estrogen treatment induces other signs of estrus besides standing, including vulva sniffing, head-head contact with herd mates and mounting other animals (Katz et al., 1980). Treatment of dairy cows with estradiol benzoate induced estrus in 12 to 16 h (Cook et al., 1986). Administration of estradiol valerate ( a part of Synchro-Mate B) resulted in standing estrus in ovariectomized heifers and cows (McGuire et al., 1990) and in anestrus suckled beef cows > 62 d postpartum (Walters et al., 1982). Estradiol benzoate administration to suckled anestrous beef cows resulted in estrus at 25 to 50 d postpartum (Fike et al., 1997).

Amount of estrogen administered can affect the estrus response in animals of similar physiological states. When large doses of estrogen were administered to ovarectomized cows all cows responded with estrus (Short et al., 1973; Nessan and King, 1981; Cook et al., 1987). However, when low doses of estrogen were given to ovariectomized cows either estrus was not elicited (Nessan and King, 1981) or only a small percentage of cows responded with estrus (Cook et al., 1986). When a moderate dose of estrogen was given a greater number of cows responded with estrus (Cook et al., 1986). Postpartum cows do not respond to estrogen like ovarectomized cows. Nancarrow et al. (1977) observed that cows greater than 21 d postpartum responded with estrus after administration of 500 μg of estradiol benzoate. However, when the same dose of estradiol benzoate was administered to suckled cows, estrus was not initiated until 6 wk after parturition while their unsuckled counter parts were in estrus as soon as two wk postpartum (Radford et al., 1976; 1978). Administration of 1 mg of estradiol

cypionate (ECP) caused 58% of 40 d postpartum anestrous suckled cows to respond with estrus (Rubio et al., 2004). It appears that suckled cows do not respond to estrogen administration with estrus as early after calving as unsuckled cows.

Estrogens initiate estrous behavior by acting on receptors in the brain. If electric lesions are placed in the ventral hypothalamus above the median eminence estrus is abolished in ewes (Clegg at al., 1958). Implants containing estrogen did not induce estrus when placed in the preoptic area but did induce estrus when placed in the mediobasal hypothalamus (Blanhe et al., 1991).

**Ovarian Function**

Luteinizing Hormone is a glycoprotein that is responsible for stimulating ovulation and initiates formation of the corpus luteum on the ovary. Estrogens elicit the release of an LH surge in ovariectomized cows (Short et al., 1973; Forrest et al., 1981) and prepubertal Holstein heifers (Swanson and McCarthy, 1978). However, in postpartum suckled beef cows the effect of estrogen on release of LH from the pituitary is not established. Treatment of suckled and unsuckled beef and dairy cows with estrogen at 2 wk after calving can release LH (Short et al., 1979; Nancarrow et al., 1977; Zaied et al., 1981). However, administration of estrogen to suckled beef cows did not induce an LH surge for at least 6 wk after calving (Radford et al., 1976; 1978). Similarly, anestrous lactating beef cows at 40 d postpartum did not respond with luteal activity after a 1 mg dose of ECP (Rubio et al., 2004). Suckling appears to have an effect on the ability of estrogen to cause a release of LH in postpartum beef cows.

## Introduction to fetal programming

Beef cow in the US are mostly maintained on pastures that are unfit for crop production and therefore not irrigated. The lack of irrigation leaves beef cows susceptible to the effects of drought or seasonal low rain fall and also the decline in forage quality associated with season and stage of maturity. If these periods of nutritional deficits are during gestation or shortly before breeding then this can lead to possible insults to the fetus. The effect of insults during gestation to the fetus has been known to cause effects at all time points in the fetus's prenatal and postnatal life. In 1992, Hales and Barker released the thrifty phenotype hypothesis, which was an expansion of the earlier thrifty genotype hypothesis (Neel, 1962). The thrifty phenotype hypothesis stated that when the fetal environment is poor, there is an adaptive response in the fetus which optimizes the growth of key body organs and tissues at the expense of other less important tissues. This leads to altered postnatal metabolism, which may give offspring a greater chance of survival when nutrients are not available. However, when nutrients are more abundant this altered metabolism can be detrimental (Hales and Barker, 1992; Hales and Barker, 2001). An element of this idea is the concept of a critical or sensitive period during which perturbations (nutritional or other) may cause long term changes in development (Barraclough and Gorski, 1961; Ferguson and Joanen, 1982; Maxfield et al., 1998a). In placental mammals, the prenatal growth trajectory is sensitive to direct and indirect effects of maternal nutrition from oocyte maturation to birth (Rehfeldt et al., 2004; Ferguson, 2005).

Another term that had been coined for this biological process is metabolic imprinting (Waterland and Garza, 1999). Metabolic imprinting states that an insult that

occurs during a specific window of gestation may produce a specific outcome which may differ among individuals and is measurable and persists through adulthood and outcome displays a dose response or threshold relationship. There has been debate as to the appropriateness of these two terms, so another term that has been suggested is developmental plasticity, the ability of a single genotype to produce more than one alternate form of structure, physiological state, or behavior in response to different environmental conditions (Barker, 2004). In animal science and production, intrauterine growth retardation (IUGR) is a frequently used term. It is impaired growth and development of embryo/fetus or alterations of organ size and weight during pregnancy (Wu et al., 2006). It is most often measured by weight of the fetus and its organs or birth weight and can be absolute weight or weight corrected for gestational length and or fetal weight.

**Experimental methods used to alter fetal growth**

There are several ways to experimentally influence the fetus during gestation. Maternal undernutrition of ewes during the last 40 to 50 d of gestation decreased fetal growth rate by 30 to 70% and in some cases stopped fetal growth (Wallace, 1946; Mellor and Matheson, 1979). Undernutrion can be for either a specific nutrient like protein or a vitamin or can be a reduction of the global diet to a point that protein, energy, vitamins, and minerals are deficient. Maternal overnutrition is another nutritional approach utilized to produce insults to the fetus (Wallace et al., 1996). Heat stress has also been used to alter fetal growth in animal models (Alexander, 1974; Reynolds et al., 1985). Hypoxia or decreased oxygen in the air or blood has been used to cause reduced fetal growth.

Hypoxia is produced in animals by housing animals at high altitude (Xiao et al., 2001) or by manipulating the oxygen to $CO_2$ ratio in the air an animal inhales (Gheorghe et al., 2007). Surgical removal of caruncles before mating has been used to cause a decreased surface area for placental attachment in sheep (Robinson et al., 1979). The use of either artery ligation (Newnham et al., 1986) or embolism of placenta arteria (Clapp et al., 1982) has been used to fetal programming.

**The effects of maternal nutrition on the placenta**

The placenta is responsible for transporting nutrients and oxygen to the fetus and metabolic waste products away from the fetus during pregnancy. The growth of the placenta is crucial for fetal growth (Gootwine, 2004; Reynolds et al., 2005). The placenta also provides an immune interface between mother and fetus. It's also a source of protein and steroid hormones that influence fetal, placental and maternal metabolism and development.

**Development and Morphology**

The major growth of the placenta occurs during the first half of gestation, with the majority of fetal growth occurring during late gestation. In the sheep, polycotyledonary epitheliochorial placentation is fully established by 30 days after conception, and the number of placentomes attached is fixed at this time. Rapid hyperplastic growth occurs until d 55, and then growth proceeds at a declining rate at approximately 75 d of gestation (Ehrhardt and Bell, 1995). In the cow, placentation and placental growth is similar to that of the ewe except for modest placental growth during the third trimester that consists

mainly of the maternal (caruncular) component (Bell et al., 1999). An increase in placental growth in sheep is associated with enhanced fetal growth (Gootwine, 2004). Uterine and placental blood flow and volume increase during gestation to meet the needs of the fetus (Reynolds et al., 2005). During late gestation, umbilical blood flow increases in sows, ewes and cows to meet the metabolic need of the fetus/fetuses (Ford, 1995; Pere and Etienne, 2000). Thus, impaired placental growth is associated with IUGR (Mellor, 1983; Schoknecht et al., 1994; Wallace et al., 1996; Wallace et al., 2003).

Maternal nutrient intake in the ewe may have an effect on the placenta before it is even formed. The trophoblast, a layer of cells of the blastocyst that eventually differentiate into the fetal membranes and placenta, can be affected by maternal intake. Rhind et al. (1989) observed that ewes receiving 0.5 times maintenance requirements from mating had average trophoblast lengths of 500 μm on d 11 of gestation compared to 1,400 μm from ewes that received 1.5 times maintenance requirements. Since the trophoblast develops into the placenta it would be appropriate to interject that this difference in trophoblast development, if maintained under nutritional restriction, could account for at least a part of the difference in placenta development later in gestation. A 70% reduction in feed intake from 45 d prior to mating until 7 d postmating in the ewe results in an inverse relationship between ewe weight gain and uteroplacenta growth in twin pregnancy indicating a disruption of placental growth (MacLaughlin et al., 2005).

One of the easiest ways to measure placental development is to weigh the placenta. In ewes, placental weight was reduced at d 80 of gestation by maternal energy restriction from d 28 to 80 of gestation (Dandrea et al., 2001). If ewes were subjected to severe nutrient restriction from d 85 to 90 of gestation, placental weights were decreased on d

135 of gestation (McMullen et al., 2005). Ewes exposed to hyperthermia from d 64 of gestation until d 136 to 141 of gestation had a 46% reduction in placenta weight compared with ewes maintained in a thermoneutral environment (Early et al., 1991). This was associated with a reduction in total placenta content of protein, RNA, and DNA, indicating a reduced number of cells in the placenta. Protein restriction in the sow from mating until d 63 of gestation reduced placenta weight at d 63 of gestation (Schoknecht et al., 1994). If nutrient intake was restored to above maintenance requirements after nutrient restriction the placenta compensated and became larger than the placenta of control ewes (Heasman et al., 1998; Dandrea et al., 2001; Whorwood et al., 2001).

When singleton–bearing adolescent ewes were fed approximately twice maintenance requirements for energy, the growth of the placenta was impaired (Wallace et al., 1996; Wallace et al., 2004). In late gestation, total placentome mass in the overfed ewe compared with control ewes was reduced by 45 to 50% (Wallace et al., 2000; Wallace et al., 2002a). Placental and uterine blood flow was reduced approximately 35% and absolute placenta glucose transport capacity was lower due to a smaller placenta size in overnourished adolescent sheep (Wallace et al., 2002a; Wallace et al., 2002b). Gestation length was shortened by 3 days and birth weight of lambs was reduced by 30% (Wallace et al., 2004).

In cows decreased nutrient intake from d 145 to 259 of gestation resulted in increased chorioallantoic and cotyledonary weight of the placenta and reduced fructose in the amniotic fluid at 259 d of gestation (Rasby et al., 1990). A diet that is inadequate for protein during the first trimester of pregnancy in the cow increased dry cotyledon weight at term (Perry et al., 1999). A change from either low or adequate levels of dietary

protein in the cow during first trimester to either adequate or low protein level during the second trimester increased trophectoderm volume and decreased blood vessel volume and volume density in fetal villi compared to animals that were maintained with either an adequate or restricted level of dietary protein during both trimesters of gestation (Perry et al., 1999). Global nutrient restriction from d 30 to 125 increased caruncular capillary surface density and decreased cotyledonary capillary density, capillary number density, and capillary surface density at 250 d of gestation compared with cows with adequate nutrition (Vonnahme et al., 2007).

Ovine placentomes can be characterized into an A through D morphology (Vatnick et al., 1991). Inverted placentomes are categorized as Type A. Type D placentomes are everted and resemble the morphology of late gestation bovine placentome. Severe nutrition restriction (30% of maintance energy requirements) from d 85 to 90 of gestation shifted placentomes towards the everted phenotype (Type D, McMullen et al., 2005). A 15% reduction in nutrient intake for the first 70 d of gestation increased the growth of the fetal side of the placenta and shifted the type of placentomes from A in the controls to Type D in the nutrient restricted ewes at d 130 of gestation (Steyn et al., 2001). Either Low (adrenalectomized and cortisol administered to produce lower then normal plasma cortisol concentrations) or high cortisol (cortisol infusion) from d 112 to 130 of gestation altered placental morphology, with increased type B placentomes at d 130 of gestation (Jensen et al., 2005).

Animals may develop mechanisms to compensate for periods of maternal nutrient deficit if they have enough time to evolve or adapt to this type of environment. Vonnahme et al. (2006) used ewes that had been adapted to a low nutrient environment

for 30 yrs (Beggs sheep) and ewes that had a sedentary lifestyle and always consumed a diet that met or exceeded NRC recommendations for over 30 yrs (UW sheep). Ewes from both groups were bred to the same male and paired together and exposed to either nutrient restriction or adequate nutrition from d 28 to78 of gestation. At d 78 of gestation there was a decrease in the fetal weight and blood glucose concentration in the nutrient restricted verses control UW ewes. However, there was no difference in fetal measurements in the Beggs regardless of nutrient intake. The placenta of the Beggs ewes had a reduced number of type A placentones and an increased number of type B, C, and D placentomes compared with the UW ewes. Placenta efficiency was different for the nutrient restricted vs control UW ewes but was not different for the Beggs ewes. This indicates that the Beggs ewes were able to convert placentones to more advanced types (B, C, and D) at an accelerated rate and this may function to maintain normal nutrient delivery by increased placenta surface area to the developing fetus during periods of nutrient restriction. It also appears that Beggs ewes are able to maintain fetal concentrations of amino acids that enable normal fetal growth thought augmenting placental efficiency for amino acid transport (Jobgen et al., 2007).

**Enzymes**

Enzymes that are affected by maternal nutrition or other insults that affect fetal growth fall into three classes: vascular growth factors and enzymes effecting blood flow, growth factors, and enzymes that function in steroid metabolism.

Nitric oxide synthase (NOS) is an enzyme that converts L-arginine to nitric oxide ($NO^5$). Nitric oxide regulates angiogenesis (Ziche et al., 1994) or the formation and

development of blood vessels and capillaries. Protein restriction from d 0 to 40 or 60 of gestation in sows resulted in decreased concentrations and activity of both forms of inducible NOS ($Ca^{2+}$ dependent and independent) in the placenta (Wu et al., 1998). Endothelial NOS was decreased in the umbilical artery but increased in the fetal cotyledon at d 132 of gestation in ewes that had been exposed to hyperthermia from d 35 to 115 of gestation (Arroyo et al., 2006). Hyperthermia from d 40 to 90 of gestation in the ewe reduced cotyledon NOS protein content by 50% on d 90 of gestation (Galan et al., 2001).

The vascular endothelial growth factor (VEGF) family of proteins are protein signaling molecules that promote capillary growth, increase vascular permeability and regulate placenta blood flow (Cheung et al., 1995; Reynolds and Redmer, 2001; Regnault et al., 2002). Vascular endothelial growth factor acts through VEGFR-1 and VEGFR-2 receptors. Another family of signaling proteins are the angiopoietins (Ang-1 and Ang-2) which act through a common receptor Tunica interna endothelial cell kinase 2 (Tie-2) (Maisonpierre et al., 1997). Angiopoietin -1 acts synergistically with VEGF to stimulate angiogenesis (Koblizek et al., 1998) by maturation and stabilization of developing vasculature (Suri et al., 1996). Ang-2 causes destabilization that is required for sprout formation and branching angiogeneses (Maisonpierre et al., 1997). Placental VEGF mRNA expression was reduced at d 90 of gestation in ewes exposed to severe nutrient restriction from d 85 to 90 of gestation (McMullen et al., 2005). Hyperthermia from d 40 to 120 of gestation increased mRNA for Ang-1, Ang-2, and Tie-2 in fetal cotyledons at d 55 of gestation while Tie-2 mRNA and concentrations of protein were decreased at d 135 of gestation (Hagen et al., 2005). Restriction of both energy and protein from d 30 to 125

of gestation in cows increased placental growth factor, a member of the VEGF family, along with its receptor, fms-like tyrosine kinase, at d 125 of gestation (Vonnahme et al., 2007).

In rodents, peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. PPARg is important for placenta development and acts in vascular development of the placenta (Barak et al., 1999). Treatment of rats from d 13 to 22 of gestation with dexamethasone decreased placenta PPARg mRNA by 35% in the labyrinth zone at d 22 of gestation (Hewitt et al., 2006b).

Severe nutrient restriction of ewes from d 85 to 90 of gestation resulted in decreased IGFBP3 and IGFBP2 mRNA at d 90 and 135 of gestation (McMullen et al., 2005). IGFBP influences the availability of insulin like growth factor (IGF) (Jones and Clemmons, 1995) and IGF plays a functional role in placental development (Wathes et al., 1998). Decreased growth of the placenta, decreased permeability of nutrients and a secondary increase of placenta AA transporters are observed in early pregnancy in IGF II knock out mice (Constancia et al., 2002). In later pregnancy Placenta AA transporters fail to compensate for reduced placenta size and fetal growth rate is severely reduced. Another important enzyme in placenta function is ornithine decarboxylase which regulates synthesis of polyamines from L-ornithine and is essential for placental growth (Hoshiai et al., 1981). Concentrations of ornithine decarboxylase were reduced in the placenta of gilts fed a low protein diet from d 0 to either d 40 or 60 of gestation (Wu et al., 1998). Secreted frizzled related proteins (SFRPs) inhibit the WNT pathway (Name derived from the *Drosophila Wingless* (*Wg*) and the mouse *Int-1* genes) by binding to

WNT ligand or frizzled receptors (Guo et al., 1998; Bafico et al., 1999). WNT pathway plays an important role in placental development and is required for fusion of the chorian and allantois during placental development (Galceran et al., 1999; Parr et al., 2001). Maternal dexamethasone treatment from d 13-22 of gestation in the rat resulted in increased expression of SFRP4 mRNA in basal and labyrinth zones of the placenta at d 22 of gestation (Hewitt et al., 2006a). This demonstrated that increased expression of SFRP4 is associated with reduced growth of the placenta.

Molecules that are responsible for or regulate steroid production in the placenta may be affected by maternal nutrient intake. Reduction in nutrient intake to 50% of recommended intake from d 28 to 77 of gestation in the ewe results in decreased concentrations of 11β-hydroxysteroid dehydrogenase 2 mRNA and protein concentration in the placenta at d 77 of gestation (Whorwood et al., 2001). There are two isoforms of 11β-hydroxysteroid (1 and 2) and they are responsible for conversion of cortisone to biologically active cortisol. Another molecule that may effect steroid production in the placenta is tumor necrosis factor α (TNFα) which is a cytokine with suspected function in female reproduction and embryonic development (Hunt et al., 1996). Tumor necrosis factor α may regulate placenta steroid production (Carbo et al., 1995). Iron deficiency from before mating to d 21 of gestation in the rat increased concentrations of TNFα and its receptor, TNFα type 1 receptor (Gambling et al., 2002). Vitamin A deficiency throughout pregnancy in the rat increased infiltration of neutrophils positive for TNFα and TNFα type 1 receptor positive trophoblast cells (Antipatis et al., 2002). This could indicate that TNFα is produced by neutrophils and that an immune interaction is playing a role in placenta steroid production during pregnancy.

**The effects of maternal nutrition on the fetus**

Maternal nutritional status influences nutrient partitioning in the gestating animal and therefore influences fetal growth and the development of the fetus (Wallace, 1948; Godfrey, 2002). Growth of the fetus is sensitive to the direct and indirect effects of maternal dietary intake at even the earliest stages of embryonic life when conceptus nutrient requirements are low (Robinson et al., 1999).

An important regulator of fetal growth, The IGF system comprises two ligands (IGF-I and IGF-II), two receptors (IGF-IR and IGF-IIR) and six binding proteins (IGFBP-1 to IGFBP-6; Jones and Clemmons, 1995). The concentrations of IGF-I and IGF-II in the fetus are positively correlated with birth weight in pigs, sheep and humans (Lee et al., 1993; Kind et al., 1995; Ong et al., 2000). The IGF-I and II genes are expresses in fetal tissues from pre-implantation to just before birth (Watson et al., 1994; Hill et al., 1998; Fowden et al., 1998). Fasting of ewes resulted in a 50% decrease in fetal concentration of plasma IGF-I and a 15 to 20% decrease in concentrations of plasma IGF-II (Oliver et al., 1996; Lee et al., 1997). Deletion of either IGF-I or IGF-II in mice reduced fetal growth by 40% (DeChiara et al., 1990; Baker et al., 1993). Deletions of both IGF-I and IGF-II in mice reduced fetal growth by 70% (Efstratiadis, 1998). When IGF-IR was deleted fetal growth of mice was reduced by 55% (Baker et al., 1993), this suggests that both IGFs act through the IGF-IR to stimulate tissue growth (Efstratiadis, 1998). Fetal growth was enhanced by IGF-II over expression caused by either deletion of the IGF-IIR which controls extracellular concentrations of IGF-II or by biallelic IGF-II expression (Lua et al., 1994; Ludwig et al., 1996).

**Effects of nutrition on ovine fetal growth**

The effects of maternal nutrient restriction of ewes during early and mid gestation are summarized in Table 1 and the effects during mid to late gestation are in Table 2. Maternal nutrient restriction during early to mid gestation had no effect on fetal growth at both the time of cessation of nutrient restriction and also later in gestation in many of the studies. However other reports indicate an effect of nutrient intake during early and mid gestation on fetal growth at the end of the nutrient restriction period (Everitt, 1964; Parr and Williams, 1982; Vonnahme et al., 2003), with the exception of Vincent et al (1985) who observed differences in birthweight due to maternal nutrient restriction from d 0 to 60 of gestation in the ewe. Even when fetal weight is similar for restricted and unrestricted ewes during early to mid gestation, crown rump length was reduced for fetuses whose dams were exposed to nutrient restriction (Ford et al., 2007). The effects of nutrient restriction during mid to late gestation usually resulted in a reduction in fetal weight (Table 2). The effects of maternal nutrient restriction during late gestation can be observed as soon as 3 d after the start of restriction in ewes at 115 d of gestation and the nutrient restriction resulted in a 30 to 44% decrease in fetal growth rate as measured by crown rump length (Mellor and Matheson, 1979). Nutrient restriction in the pregnancy ewe produced inconsistent fetal growth and this could be due to amount or length of nutrient restriction and the timing of nutrient restriction relative to gestation. Maternal live weight and composition at conception and the start of nutrient restriction may play a role in the outcome of nutritional restriction in the ewe (Russel et al., 1981).

Table 1. Effects of maternal undernutrition of sheep during early to mid gestation on fetal growth

| Author | Restriction period (d of gestation) | Fetal growth (slaughter) |
|---|---|---|
| Wallace (1948) | 28-91 | No effect (d 91) |
| Wallace (1948) | 0-90 | No effect (d 90) |
| Everitt (1964) | 0-90 | Reduced (d 90) |
| Parr and Williams (1982) | 1-35 | Reduced (d 35) |
| McCrabb et al., (1986) | 0-96 | No effect (d 96) |
| McCrabb et al., (1992) | 30-96 | No effect (d 96) |
| Arnold et al., (2001) | 50-90 | No effect (d 90) |
| Vonnahme et al., (2003) | 28-78 | Reduced (d 78) |
| Vincent et al., (1985) | 0-60 | Reduced (birth) |
| Heasman et al., (1998) | 28-77 | No effect (d 145) |
| Hawkins et al., (2000) | 0-70 | No effect (d 132) |
| Rae et al., (2001) | 0-30 | No effect (d 50, 65 or 110) |
| | 31-50 | No effect (d 50, 65 or 110) |
| | 0-50 | No effect (d 50, 65 or 110) |
| | 0-65 | No effect (d 65 or 110) |
| | 0-110 | No effect (d 110) |
| Steyn et al., (2001) | 0-70 | No effect (d 130) |
| Whorwood et al., (2001) | 28-77 | No effect (d 145) |
| MacLaughlin et al., (2005) | 0-7 | No effect (d 54) |
| McMullen et al., (2005) | 85-90 | No effect (d 90 or 135) |
| Ford et al., (2007) | 28-78 | No effect (birth) |
| Gilbert et al., (2007) | 28-78 | No effect (d 78 or 135) |

Adapted from Redmer at al., (2004)

Table 2. Effects of maternal undernutrition of sheep during mid to late gestation on fetal growth

| Author | Restriction period (d of gestation) | Fetal growth (slaughter) |
|---|---|---|
| Wallace (1948) | 91-144 | Reduced (d 144) |
| | 28-144 | Reduced (d 144) |
| Robinson (1977) | 0-145 | Reduced (d 145) |
| Robinson et al., (1979) | 0-145 | Reduced (d 145) |
| Holst et al., (1986) | 1-145 | Reduced (d 145) |
| Meller (1983; 1987)) | 90-145 | Reduced (d 145) |
| Faichney et al., (1987) | 50-135 | Reduced (d 135) |
| McCrabb et al., (1986) | 30-142 | No effect (d 142) |
| Kelly (1992) | 90-145 | Reduced (d 145) |
| Arnold et al., (2001) | 50-130 | Reduced (d 130) |

Adapted from Redmer at al., (2004)

The effects of maternal overnutrition are not as well studied as maternal undernutrition. The majority of research in this area has utilized the overnourished adolescent pregnant ewe as a model and has resulted in decreased fetal weight at birth (Wallace et al., 1996; Wallace et al., 2004). Maternal overnutrition of cattle throughout gestation had no effect on calf birth weight but did increase dystocia and neonatal death rate (Arnett et al., 1971).

**Effects of nutrition on porcine fetal growth**

Reduced intake of a complete ration by 50% for 2 estrous cycles before mating or from d 14 to 21 of lactation in sows before rebreeding results in decreased fetal weight and female fetal survival at 30 d of gestation (Ashworth, 1991; Vinsky et al., 2006). Birth weight of piglets decreased as dietary intake of energy decreased throughout gestation (Baker et al., 1969). Reduced nutrient intake after d 80 of gestation reduced fetal growth in gilts (Noblet et al., 1985).

**Effects of nutrition on bovine fetal growth**

The effects of nutrient restriction in cows during gestation are summarized in Table 3. Nutrient restriction that occurs before and during the third trimester of gestation (d 190 of gestation) reduced birth weight. There are a few studies that are exceptions to this statement. In these studies that reported no effect of nutrient restriction, the differences in cow weight at the end of nutritional treatment were not that different indicating that the nutrient restriction was not severe enough to cause differences in fetal growth (Bellows and Short, 1978; Doornbos et al., 1984; Shell et al., 1990). Heifers tend to respond to nutritional restriction with decreased calf birth weight more consistently then cows (Bellow and Short, 1978). Not only can nutritional restriction reduce calf birth weight it can also shorten gestation length (Hafez et al., 1968, Warrington et al., 1988). Protein restriction in isocaloric diets fed during the last 4 mo of gestation decreased gestation length by 8 days (Waldhalm et al., 1979). However, inadequate protein fed in an isocaloric diet does not reduce calf birth weight. Cows that calved in a BCS of 6 have calves with heaver birth weight then calves from cows that calved in a BCS of 4 (Spitzer et al., 1995). If the BCS difference is a 4 vs 5 or 4.6 vs 5.2 BCS, calf birth weights are not effected (Ciccioli et al., 2003; Martin et al., 2007), indicating that a BCS difference must be in excess of 1.5 BCS with one group having a BCS equal to or less then 4.

Table 3. Effects of maternal undernutrition of cows during gestation on fetal growth

| Author | restriction period (d of gestation) | restricted | BW[a] or BCS of dams precalving | calf weight |
|---|---|---|---|---|
| Hafez et al., 1968 | 45 to term | intake | heifers 441 vs 625 | reduced birth weight |
| Tudor, 1972 | 180 to term | intake | Δ BW during trt + 63.8 or -36.8 | reduced birth weight and gestation length |
| Corah et al., 1975 | 180 to term | intake | heifers ΔBW of + 36.1 vs -5.8 | reduced birth weight |
| Bellow and Short 1978 | 190 to term | intake | heifers 329 vs 378 | reduced birth weight |
| | 190 to term | intake | heifers 346 vs 361 | reduced birth weight |
| | 190 to term | intake | cows 422 vs 473 | no effect |
| Kroker and Cummins, 1979 | 190 to term | intake | heifers ΔBW 42.5 vs -12 vs -46 | reduced birth weight |
| Prior et al 1979 | 42 to term | intake | heifers 355 vs 421 vs 469 | no effect at 90, 125, 150, 180, 210, 240, and 255 d of gestation |
| Waldhalm et al., 1979 | 120 to term | protein | cows 371 vs 410 | reduced gestation length |
| Doornbos et al., 1984 | 220 to term | intake | heifers and cows 450 vs 456 | no effect |
| Anthony et al., 1986 | 205 to term | protein | heifers473 vs 498 | no effect |
| Boyd et al., 1987 | 230 to term | intake | cows 503 vs 527 | reduced birth weight |
| Carstens et al., 1987 | 190 to term | protein | heifers ΔBW 97 vs 180 | no effect |
| Warrington et al., 1988 | 90 to term | intake | heifers ΔBW of -3.75 vs -92 | reduced birth weight and gestation length |
| Hough et al., 1990 | 190 to term | intake | cows 643 vs 575 | no effect |
| Miner et al 1990 | 220 to term | intake | cows ΔBw 1.9 to 46.4 | no effect |
| Rasby et al., 1990 | 145 to 259 | intake | cows 419 vs 511 | no effect |
| Shell et al., 1990 | 100 to term | intake | cows 514 vs 571 | no effect |
| Spitzer et al., 1995 | 190 to term | intake | heifers calve at BCS of 4 or 6 | reduced birth weight |
| Martin et al., 1997 | 140 to term | protein | heifers 475 vs 357 | no effects |
| Perry et al., 1999 | 42 to 198 | protein | heifers (Not reported) | no effect at term |
| Freetly et al., 2000 | 90 to term | intake | cows 549 vs 590 | reduced birth weight |
| Ciccioli et al., 2003 | 190 to term | supplement | heifers calve at BCS of 4 or 5 | no effect |
| Martin et al., 2007 | 190 to term | supplement | 5.2 vs 4.6 BCS | no effect |

[a]kg

**Fetal organs and tissues affected by maternal nutritional intake**

More than the weight or morphometric measurements of a fetus can be affected by maternal nutrient intake. When the uterine environment is inadequate for normal fetal growth, the fetus adapts and promotes the growth of some organs and tissues at the expensed of other tissue and organs (Hales and Barker, 1992).

Nutrient intake of the dam influences growth of lungs. Restriction of nutrients of ewes from d 28 to 78 of gestation reduced weight of lungs in fetuses at d 78 of gestation (Vonnahme et al., 2003). Similarly, reduced nutrient intake of ewes from d 85 to 90 of gestation resulted in lighter fetal lungs at d 90 and 135 of gestation (McMullen et al., 2005). When cows were exposed to a protein deficient diet from d 140 of gestation until parturition, lungs and trachea of calves were lighter compared with calves from cows that received adequate protein (Martin et al., 1997).

Nutrient restriction (50% of requirement for energy, protein, vitamins, and minerals) of ewes from d 28 to 78 of gestation resulted in fetuses with smaller kidneys at d 78 of gestation (Vonnahme et al., 2003). Similarly when sows were fed protein deficient diets from d 0 to 63 of gestation the weights of fetal kidneys were reduced (Schoknecht et al., 1994). Nutrient restriction of ewes from d 28 to 77 of gestation followed by adequate nutrient intake, increased weight and width of the kidney but decreased kidney length at145 d of gestation (Whorwood et al., 2001). Male fetuses from ewes restricted from d 28 to 78 of gestation may be more sensitive to nutrient restriction and therefore have heavier fetal kidneys with decreased angiotensin II type 1 receptors at 135 d of gestation then their unrestricted male fetuses while female fetuses were unaffected by nutritional treatment (Gilbert et al., 2007).

In the fetal heart of sheep, myocyte size, intercapillary distance, and myocyte myofibrillar and mitochondrial volume increase, while capillary density, myocyte-to capillary ratio, and the myocyte matrix volume density decrease with increasing gestational age (Smolich et al., 1989). The percent of binucleated myocytes increase during gestation (Burrell et al., 2003). Before d 110 of gestation cardiac growth is via myocyte hyperplasia, after d 110 of gestation cardiac growth is due to both hyperplasia and hypertrophy of myocytes (Burrell et al., 2003). The growth of the heart between parturition and 4 to 6 wk of age is due to hypertrophy of myocytes (Burrell et al., 2003). The ratio of mononucleated to binucleated cells in the heart can be altered by environmental factors during fetal life (Barbera et al., 2000.). The fetal circulatory system may also be affected by maternal nutrient intake. Nutrient restriction in the ewe from d 28 to 78 of gestation increased both left and right ventricles of the fetal heart when expressed as a percentage of fetal weight on d 78 of gestation (Vonnahme et al., 2003). It also alters the expression of a range of genes that have been implicated in either cardiac hypertrophy or inhibition of cardiac remodeling (Han et al., 2004). Nutrient restriction from d 0 to 7 of gestation in the ewe produced increased femoral arteriol blood pressure at two separate periods (115 to 125 and 135 to 147 d of gestation) in the fetus (Edwards and McMillen, 2002). Reduced nutrient intake from mating to 70 d of gestation produced fetal lambs with reduced basal fetal heart rate and increased basal femoral artery vascular restriction at 128 d of gestation (Hawkins et al., 2000).

In sheep nutrient restriction (50% of maintenance requirements) from d 18 before to d 6 after ovulation resulted in a 20% reduction in the total number of muscle fiber in fetus compared to fetuses from ewes fed greater than maintenance requirements at d 75 of

gestation (Quigley et al., 2005). The ratio of primary to secondary muscle fibers was reduced and there was a tendency for a reduction ion the protein to DNA ratio to be less in nutrient restricted fetuses. Maternal nutrient restriction from d 30 to 125 of gestation of cows resulted in less myofibers per muscle bundle in fetal skeletal muscle with each myofiber having a larger volume (Du et al., 2005b). The reduction in myofibers per muscle bundle was not recovered by nutritional realimentation from d 125 to 250 of gestation. Nutrient restriction from d 30 to 125 of gestation in cows increases concentrations of calpastatin in fetal muscle at 125 of gestation (Du et al., 2004). Calpastatin is an inhibitor of calpain enzymes that are responsible for degradation of myofibrillar proteins in muscle. The mammalian target of rapamycin signaling controls nutrient-stimulated protein synthesis in skeletal muscle. Nutrient restriction of cows from d 30 to 125 of gestation and ewes from d 28 to 78 of gestation results in down regulated signaling through the mammalian target of rapamycin in fetuses at 125 and 78 d (respectively) of gestation (Zhu et al., 2004; Du et al., 2005a) indicating altered nutrient stimulated protein synthesis. Ewes fed at 150% of NRC requirements for 60 d before mating to d 70 of gestation produced fetuses at 75 d of gestation with increased phosphorlated insulin receptors and decreased activity of down stream signaling molecules when compared with fetuses from ewes fed at NRC requirement (Du et al., 2007).

The effects of maternal nutrient intake on the pancreas have been identified in rats. Dietary energy restriction either throughout or during late gestation in rats resulted in a decrease in cell number, β-cell mass, islet number, insulin content in the fetal pancreas of rats and a decrease in fetal insulin concentration (Winick and Noble, 1966; Holemans et

al., 1996; Garofano et al., 1997). Protein deficient diets fed throughout gestation decreased the number of β-cells and content of insulin in the fetal pancreatic islets, and this was due to a decrease in the proliferation and an increase in apoptosis of islet cells (Berney et al., 1997; Petrik et al., 1999; Merezak et al., 2001). Vascularization of the pancreas of fetal rats decreased when dams were fed inadequate protein during gestation (Cherif et al., 1998; Cherif et al., 2001). Fourteen days of maternal hypoglycemia produced by insulin infusion by pump during late gestation results in decreased glucose and arginine-stimulated insulin secretion in the fetus (Limesand and Hay Jr, 2003).

Other fetal organs and organ systems are sensitive to maternal nutrient intake. The weight of livers of lambs was reduced at d 78 of gestation when dams were exposed to nutrient restriction from d 28 to 78 of gestation compared with lambs from dams fed required amounts of energy, protein, vitamins, and minerals (Vonnahme et al., 2003). Protein restriction of sows from d 0 to 63 of gestation resulted in decreased fetal liver weight and increased brain weight expressed on a fetal weight basis (Schoknecht et al., 1994). Protein restriction of gilts through out gestation resulted in reduced brain and liver weights of fetuses at birth (Pond et al., 1969; Atinmo et al., 1974). Decreased nutrient intake of ewes from mating until d 70 of gestation resulted in fetuses at d 130 of gestation with increased adrenal weights compared with fetus from ewes that were fed adequate diets (Steyn et al., 2001). Skeletal abnormalities at birth resulted from nutrient restriction of ewes from d 0 to 60 of gestation (Vincent et al., 1985). Fetal ovaries may also be affected by maternal nutrient intake with fewer germ cells at the resting diplotene stage of initial meioses in fetuses at d 65 of gestation and delayed follicular development indicated by development of the granulose cell layer in fetuses at d 110 of gestation (Rae

et al., 2001). When adolescent ewes were overfed during gestation, female offspring at 131 d of gestation had small, improperly-developed ovaries (De Silva et al., 2002).

**Postnatal changes in offspring after nutrient manipulation during gestation**

It is not unrealistic to believe that if fetal growth and development can be altered by maternal nutrient intake during gestation, and then these changes that occurred during fetal development may persist and influence postnatal development and physiology of tissues.

**Growth and composition of growth**

Decreased fetal growth when dams receive inadequate nutrients during gestation can have a permanent stunting effect on postnatal growth and reduce efficiency of nutrient utilization. Lambs of ewes with inadequate maternal nutrient intake during gestation had reduced postnatal growth under artificial rearing (Schinckel and Short, 1961; Villette and Theriez, 1981). Lambs with lower birth weight grew slower during their first 2 wks of life and exhibited reduced rates of efficiency of energy utilization for protein and fat deposition (Greenwood et al., 1998) and reduced rates of postnatal skeletal muscle growth (Greenwood et al., 2000). Lambs exposed to nutrient restriction from either d 0 to 30 of gestation or d 110 of gestation to parturition had similar growth rates up to one year of age (Gardner et al., 2005). Maternal protein restriction during early gestation stunted the postnatal growth and development of swine (Schoknecht et al., 1993). Runt pigs are not uncommon and are the lightest piglet of the litter and can be 33 to 50% of the birth weight of there large littermate (Widdowson, 1971). The small

intestine, liver and skeletal muscle of runt piglets are dispropionately smaller then those of larger littermates at 3 yr of age (Widdowson, 1971). Runt piglets had a reduced rate of skeletal muscle and whole body growth from birth to market weight with decreased utilization of feed for growth compared with larger littermates (Hegartly and Allen, 1978; Powell and Aberle, 1980). Progeny of gilts fed an isocaloric, protein restricted diet during gestation had reduced growth rates from farrowing to weaning (5 wk of age) and between weaning and 90 kg compared with progeny of gilts fed adequate protein (Pond et al., 1969; Atinmo et al., 1974). Nutrient restriction of cows during the last 100 d of gestation can reduce weaning weight at 7 mo of age (Corah et al., 1975; Kroker and Cummins, 1979; Boyd et al., 1987).

Overnutrition during gestation can influence postnatal growth. The progeny of sows overfed during the first 50 d of gestation had reduced growth rates from birth to weaning and from weaning to market weight; feed efficiency was reduced compared with progeny of control sows (Bee, 2004). Feeding of sows in excess of requirements for energy and protein from d 25 to 50 of gestation produced offspring with decreased postnatal ADG, muscle accretion rate and carcass weight at 104 kg of BW (Nissen et al., 2003).

Not all experiments with maternal nutrient deficiencies find reduced growth potential of offspring. Underwood et al. (2006) found that steers from cows exposed to a diet that delivered 68.1 % of NEm requirements from d 30 to 120 of gestation had greater ADG and gain to feed ratios during the feedlot finishing period and tended to have heavier BW at harvest but hot carcass weight was similar with steers from dams received 100 % of NRC requirement. However, wethers, from dams that were exposed to 50 % of

nutritional requirements from d 28-78 of gestation, were heavier at 4 and 9 mo of age then wethers whose dams were fed to meet requirements (Ford et al., 2007).

The composition of growth or the amount of fat verses muscle at a given time point may be influence by prenatal nutrient intake. Underwood et al. (2006) found similar yield grade, marbling score and percent KPH fat for steers killed at 12-13 mo of age that received 100 or 68 % of requirements from d 30-120 of gestation (Underwood et al., 2006). Nutritional restriction at different timepoints in gestation can result in differences in fat mass and HCW of lambs. Reduced nutrient intake from 60 d before mating to 7 d postmating in ewes increased perirenal fat mass of lambs at term (Edwards et al., 2005). Nutritional restriction from d 28-78 of gestation produced lambs with increased back fat at 140 d of age and increased kidney pelvic fat in grams or corrected for HCW along with increased HCW at 280 d of age (Ford et al., 2007). Decreased nutrient intake from d 110 of gestation to term in ewes resulted in lambs with increased omental and perirenal fat depots at 1 yr of age (Gardner et al., 2005). Progeny of overfed sows from d 0 to 50 of gestation have a greater content of adipose tissue at birth and at slaughter compared with offspring of underfed sows (Bee, 2004). Runt piglets at similar weight have larger quantities of intramuscular fat (Hegartly and Allen, 1978; Powell and Aberle, 1980) and lighter muscled carcasses (Powell and Aberle, 1980).

**Glucose regulation**

Nutrient intake during gestation can result in metabolic changes in the offspring's regulation of blood glucose levels. Nutrient restriction (50% of requirements for energy, protein, vitamins and minerals) from d 28 to 78 of gestation in ewes results in wethers

that had a greater area under the curve (AUC) for plasma glucose compared to control animals in response to intravenous glucose tolerance test (IVGTT) at 63 and 250 d of age (Ford et al., 2007). The AUC for plasma insulin was greater at 63 d of age than controls but was decreased for 250 d of age. Nutrient restriction from d 1 to 30 of gestation in the ewe did not alter metabolic response to feeding or to IVGTT in lambs at 11 mo of age, however nutrient restriction from d 110 to term resulted in lambs with increased AUC for plasma glucose and insulin (Gardner et al., 2005). This response to IVGTT in the late nutrient restricted lambs was associated with decreased adipose GLUT 4 glucose transported in adipose tissue but no difference was noted for GLUT 4 in muscle tissue. Calves exposed to an increased plane of nutrition from d 118 to 202 of gestation had increased plasma glucose at 7 mo of age and had a greater increase in plasma glucose with time after infusion compared with control calves (Kastner et al., 2004).

The surgical removal of a majority of the caruncles in ewes before breeding results in lambs with no difference in fasting plasma glucose at 35 d of age, however lambs had a reduced rate of plasma glucose clearance at 30 to 50 min post IVGTT (De Blasio et al., 2007b). There was no difference in total AUC for plasma glucose during the IVGTT. At 375 d of age, wethers had an increased initial rise in glucose during IVGTT and an overall decrease in glucose tolerance during IVGTT and a decreased fasting plasma insulin concentration (Owens et al., 2007). During a hyperinsulinemic euglycemic clamp (HEC) wethers had a decreased ability for insulin to dispose of plasma glucose but an increase in the ability of insulin to signal the disposal of circulating free fatty acids. Ewe lambs from either caruncle removed ewes or control ewes at 375 d of age showed no difference during the IVGTT, however during the HEC, ewe lambs from caruncle

removed dams had increased insulin sensitivity for glucose disposal compared to control ewe lambs.

Exposure of ewes to cortisol or synthetic cortisteroids during gestation can lead to altered glucose and insulin regulation in lambs. Exposure to dexamethasone for 48 h at 27 or 64 d of gestation resulted in female offspring at 4.8 yr of age that have no difference in AUC for glucose or insulin compared to control offspring following an IVGTT (Gatford et al., 2000). However the female offspring treated at 27 d of gestation had an increased response to insulin in adipose tissue during HEC. Dexamethasone exposure from d 26 to 28 of gestation produced lambs that had reduced AUC for glucose after IVGTT at 4 yr of age and an increased plasma insulin concentration for the first 30 min of the IVGTT (De Blasio et al., 2007a).

**Hypothalamic-pituitary axis and reproduction**

The effects of maternal nutrient intake on reproduction of the offspring are just now beginning to be evaluated by research. Maternal nutrient intake of ewes had a negative effect on fetal gonadal development (Rae et al., 2001). Offspring of undernourished mothers had reduced ovulation rates or numbers of offspring (mice, Meikle and Westberg 2001; sheep, Rae et al., 2002). Undernutrition of ewes during late gestation results in a reduction in reproduction rate in adult female offspring (Gunn et al., 1995; Rhind et al., 1998). Hypothalamic-pituitary functions as measured by plasma gonadotropin profiles or expression of pituitary mRNA for LHβ or FSHβ of female offspring was not affected by maternal undernutrition (Borwick et al., 2003) or overnutrition (Da Silva et al., 2003). Undernutrition of ewes from mating until d 90 of

gestation produced male offspring with greater FSH concentrations then control rams at 20 mo of age (Rae et al., 2002). Undernutrition of ewes during the second half of gestation produced newborn ram lambs with a reduced number of sertoli cells (Bielli et al., 2002). Overnourished adolescent ewes produced ram lambs with delayed onset of puberty when compared with ram lambs from ewes fed required amounts of nutrients (Da Silva et al., 2001).

**Organ and tissue changes**

Maternal nutrient intake during gestation can alter the growth and development of fetal organs and tissues. It is becoming clear that these changes that occur during gestation in organ and tissue morphology and function can persist after birth and alter the physiology of the adult offspring.

**Skeletal Muscle**

Embryonic cells from the epiblast layer of the primitive streak embryos that express a muscle specific transcript factor differentiate into skeletal muscle in culture conditions (George-Weinstein et al., 1996). These cells then are controlled by a family of myogenic regulatory factors (myoD, myf5, myogenin and MRF4) that function to regulate the development of skeletal muscle (Ludolph and Konieczny, 1995). The final step in muscle formation is biphasic. The initial wave of synchronous fusion of the myoblasts leads to a population of primary fibers, this occurs around d 14 of gestation in rats and between 6 to 8 wk in humans (Wigmore and Dunglison, 1998). The second wave of myogenesis leads to asynchronous formation of secondary fibers on the surface of the

primary fibers. This occurs between d 17 and 21 in rats and wk 8 and 18 in humans (Barbet et al., 1991; Wigmore and Dunglison, 1998). In cattle, primary myotubes appear prior to d 47 of gestation (Russel and Oteruelo 1981) and secondary muscle fibers differentiate from the population of myotubes at around d 90 of gestation (Gagniere et al., 1999). The total fiber number is fixed at d 80 to 90 in pigs (Christensen et al., 2000; Staum, 1963; Wigmore and Strickland, 1983), d 80 to 125 in sheep (Greenwood et al., 2000; McCoard et al., 2000), and d 240 of gestation in cattle (Robelin et al., 1991). Final maturation of skeletal muscle is by fiber type conversion and hypertrophy of the muscle fibers during the postnatal period. This final stage is under the control of neural and growth factors (Maltin et al., 2001). The number of primary fibers is genetically determined, whereas the number of secondary fibers is determined by local and environmental signals (Dwyer and Stickland, 1992; Dwyer et al., 1993).

The effects of prenatal nutrition on skeletal muscle development had been evaluated using several different models. A reduction to 50% of nutrient requirements from d 2 -78 of gestation in ewes resulted in offspring at 8 mo of age that had decreased numbers of myofibers (Zhu et al., 2006). Dietary restriction from d 25 to 50 of gestation of gilts resulted in decreased total and secondary fibers and a decreased secondary to primary fiber ratio in the semitendinosus muscle of 61 d old female progeny (Gatford et al., 2003).

Greenwood et al. (2000) found no difference in myofiber numbers in three different muscles from either low or high birth weight lambs. However, daily accretion of muscle was less in lambs that had a low birth weight. Lambs with a low birth weight

have skeletal muscle that contain less DNA with a greater protein to DNA ratio at any given muscle weight compared with the muscle of lambs with a high birth weigh.

Maternal nutrient intake not only affects myofibers, it also affects some of the cellular machinery of skeletal muscle. A reduction to 50% of requirements from d 2-78 of gestation increased the ratio of myosin IIb to other isoforms of myosin compared with control lambs at 8 mo of age (Zhu et al., 2006). Nutrient restricted lambs also have increased intramuscular triglyceride content and a decreased activity of carnitine palmitoyl transferase-1, an enzyme that controls fatty acid oxidation. This indicates that protein synthesis is decreased in muscle due to nutrient restriction. Protein restriction in rats throughout gestation results in offspring at 15 mo of age with reduced zeta isoform of protein kinase C (Ozanne et al., 2003). The zeta isoform of protein kinase C is involved in the glucose transport via GLUT-4 glucose transporter (Standaert et al., 1997).

Both the morphology and size of muscle fibers are affected by prenatal nutritional level. In addition the molecular pathways for insulin signaling, protein degradation and glucose transport are affected in animals that are exposed to either inadequate or excess nutrient supply in utero.

**Kidney**

Nephrogenesis begins around d 12 of gestation in rats and is completed on d 8 after birth (Larsson and Maunsbach, 1980). Bilateral uterine ligation of the pregnant rat at d 19 of gestation significantly reduced glomerule number in the full term fetal kidney (Pham et al., 2003). Uteroplacental insufficiency reduced nephron numbers by up to 30 % in rats, rabbits and pigs and the decrease in nephron numbers was associated with a

parallel drop in glomerial filtration rate (Bassan et al., 2000; Bauer et al., 2002; Merlet-Bénichou et al., 1994). Maternal protein restriction throughout pregnancy in rats produces a significant reduction in nephron number in the offspring (Langley-Evans et al., 1999; Vehaskari et al., 2001; Woods et al., 2001), and a decrease in renal function (glomerial filtration rate/kidney weight, Nwagwu et al., 2000; Woods et al., 2001) both during early postnatal life but also in adulthood. Increased concentration of Na in plasma, which is a result of a primary Na retaining state as a consequence of a shift in the pressure-natriuresis curve to the right, have resulted from protein restriction in rats (Manning and Vehaskari, 2001). Prenatal exposure of rats to dexamethasone for 2 d during late gestation (either d 15 to 16 or d17 to18 of gestation) reduced glomerular number, glomerulosclerosis, and hypertension as adults (Ortiz et al., 2001; Ortiz et al., 2003). Offspring of rats fed a low protein diet throughout gestation have hypercalciuria through a reduction in passive calcium readsorption in the proximal tubule of the kidney (Ashton et al., 2007)

Nephrogenesis is complete in sheep by d 130 of gestation (Moritz and Wintour, 1999). Ewes exposed to dexamethasone from d 26 to 28 of gestation produced lambs with significantly fewer nephrons and increased glomerular volume in adult life (Wintour et al., 2003). Fewer nephrons were associated with enlarged and dilated proximal tubules and greater accumulation of collagen in the tubular interstitium and periadventitia on the renal cortical vessels. Fetal sheep kidneys at d 26-28 and fetal rat kidneys at d 15-16 are similar in terms of stage of fetal kidney development (Dodic et al., 2002) indicating a specific developmental point when glucocorticoids can alter development of the kidney. Maternal undernutrition of ewes from d 28 to 80 of gestation results in offspring at 6 mo

of age with fewer total nephrons compared with offspring of ewes fed adequate nutrition (Gopalakrishnan et al., 2005)

**Circulatory system**

Maternal nutrient intake can affect the heart of offspring at birth and in adult life. Maternal protein restriction of rats during gestation produced pups with reduced numbers of cardiomyocytes at birth (Corstius et al., 2005), and a 15% increase in the amount of interstitial fibrosis in the left ventricle of the heart of adult offspring (Lim et al., 2006). Maternal protein restriction of rats during pregnancy produced offspring with a decreased density of β1-adrenergic receptors and the response to β agonist was altered (Fernandez-Twinn et al., 2006). Inadequate protein in the diet during gestation altered the composition of the plasma membrane and fatty acid content of the cardiomyocytes of rat pups at 21 d of age (Tappia et al., 2005).

The endothelium of the vasculature in postnatal offspring was also affected by maternal nutrition. Offspring of rats fed 70% of requirements during the first 18 d of gestation has greater blood pressure from 60 d after birth, and the maximum vasoconstriction response to phenylephrine or to norepinephrine was reduced in femoral arteries of pups at 20 d of age (Ozaki et al., 2001). Similar responses occur for offspring from rats fed a restricted diet (50%) during the second half of gestation (Holemans et al., 1999) or a low protein diet throughout pregnancy (Brawley et al., 2003). Undernutrition during gestation in rats resulted in decreased gene expression of endothelial NO synthase (eNOS) in male aorta and a reduction in eNOS activity in both male and females at 14 wk of age (Franco et al., 2002). In male offspring of rats fed a low protein diet during

pregnancy, vascular relaxation induced by acetylcholine or brabykinin (both endothelium dependent vasodilators) and sodium nitroprussid and a phosphodiesterase type 3 inhibiter (endothelium independent vasodilated) was reduced (Brawley et al., 2003). This indicates another possible reason for increased blood pressure in offspring of undernourished dams.

**Pancreas**

Bilateral uterine ligation during gestation in rats resulted in no difference in β-cell mass, islet size, or pancreas weight at either 1 or 7 wk of age between growth restricted and control rat pups, however at 15 wk of age β-cell mass was decreased by 50% compared with controls, and at 26 wk of age it was one third that of controls (Simmons et al., 2001). A protein deficient diet during gestation resulted in small irregular shaped islets with reduced amounts of β-cell in the rat (Berney et al., 1997). Basal plasma insulin and glucose concentrations are similar between progeny of protein restricted and control rats. But the insulin response to oral glucose challenge was less in protein restricted adult female offspring compared with control female offspring (Hoet and Hanson, 1999).

**Conclusion**

Nutrition of beef cows regulates postpartum reproduction. The greatest factor that limits postpartum reproduction is postpartum anestrus. Anestrus is a physiological condition when ovulation and estrous behavior are not present and this condition is normal in postpartum beef cows. Postpartum anestrus is largely controlled by nutritional

factors and suckling. Body condition score has a strong relationship with carcass fat and energy (Wagner et al., 1988). Cows that calve in a greater BCS have increased pregnancy rates compared with cows that calve in a thinner BCS (Rakestraw et al., 1986; Richards et al. 1986; Selk et al., 1988). Body condition at calving is largely effected by prepartum nutrition. The effects of postpartum nutrition are dependent on BCS of cows at calving. If cows calve with an adequate BCS then postpartum nutrition has less effect on the interval from calving to first estrus (Richards et al., 1986; Marston et al., 1995, Spitzer et al., 1995). However when cows calve with a thin BCS then the interval from calving to first estrus is shorted by feeding greater amounts of energy after calving (Wiltbank et al., 1964,; Spitzer et al., 1995).

Maternal nutritional intake can affect the placenta even before it is formed and can reduce placental weights compared with placenta from dams fed adequate nutrition (Schoknecht et al., 1994; Dandrea et al., 2001; McMullin et al., 2005). Overfeeding adolescent ewes impaired the growth of the placenta (Wallace et al., 1996; Wallace et al., 2004). Not only is the size and morphology of the placenta affected by maternal nutrient intake but many enzymes concentrations are influences. Nitric oxide synthase is decreased when dams are fed inadequate nutrients (Wu et al., 1998). Many enzymes that effect vascular development in the placenta are influenced by maternal nutrient intake. The insulin like growth factor system is affected by maternal nutrient intake (McMullen et al., 2005)

Maternal nutrient status influences nutrient partitioning of gestating animals and influences development of the fetus (Dodfrey, 2002; Wallace, 1948). Nutrient restriction of ewes during late gestation results in decreased fetal growth. Decreased nutrient intake

of sows decreased fetal growth. If nutrient restriction is severe enough to decrease maternal BW and BCS of cows during the third trimester of pregnancy, then fetal growth will be decreased (Bellows and Short 1978; Kroker and Cummins, 1979; Carstens et al., 1987). The weights of the lungs, kidneys, and liver are reduced when maternal nutrient intake is restricted in ewes. The fetal heart weight is increased when nutrients were restricted (Vonnahme et al., 2003). Skeletal muscle is affected by maternal nutrient intake. These changes in organ weight indicate an adaptive response by the fetus.

The fetal response to restricted maternal nutrient intake may influence postnatal physiology. Postnatal growth and the composition of growth may be altered by nutrient intake of the dam during gestation. Glucose regulation of the offspring is effected by maternal nutrient intake. Reproduction of the offspring as an adult may be influenced by maternal intake of nutrients (Gunn et al., 1995; Rhind et al., 1998). Organ function and morphology in the adult offspring may be influenced by nutrient restriction of the dam during gestation.

Nutrition has a major role on the profitability of a cow calf operation. It not only has a pivotal role in reproduction, it could also influence calf weights at weaning and beyond. Maternal nutrient intake can affect the composition and efficiency of gain during the growth of calves. The effects of maternal nutrient intake on reproduction and on the offspring will influence the profitability of a beef operation regardless if calves are marketed at weaning or retained through the stocker phase and into the feedlot.

# CHAPTER III

## Estrus and luteal activity of postpartum beef cows after treatment with estradiol


## ABSTRACT

Multiparous Hereford x Angus postpartum cows (n = 83) were used to determine the effects of days after calving (25 or 50 d) and BCS at calving (M ≥ 5 or T < 5) on estrus and luteal activity after treatment with estradiol cypionate (ECP). Cows were maintained on dormant native pasture during the last third of gestation and fed either 0.9 or 1.8 kg/d of a 38% CP supplement to achieve a thin (T < 5) or moderate (M ≥ 5) BCS at calving. After calving, cows were fed 1.8 kg/d of a 40% CP supplement and ad libitum hay. An estrous detection system (Heatwatch, DDX inc.) was used commencing 10 d before treatment. Cows were treated (i.m.) with 1 mg estradiol cypionate (ECP) or corn oil (C) at 25 or 50 d after calving. Progesterone was quantified in plasma samples obtained from cows twice weekly for 1 wk before treatment, daily for 7 d after treatment, and twice weekly until 4 wk after treatment, then once weekly until the second estrus or 90 d after calving. Ovaries were examined by ultrasonography at treatment and concentrations of progesterone in plasma were used to ascertain the absence of corpora lutea. Treatment of cows with ECP at 25 d after calving increased ($P < 0.01$) the incidence of estrus within 4 d after treatment in M (55 vs 11%, respectively) and T (50 vs 0 %) cows compared with

C cows. Treatment of T cows at 50 d after calving increased ($P < 0.01$) estrus within 4 d (50 vs 0 %). Only 10% of M cows were anovulatory at 50 d after calving and response to ECP could not be evaluated. Treatment with ECP did not influence the onset of ovarian luteal activity (LA, progesterone >1 ng/ml for 1 d) within 10 d after treatment ($P = 0.52$). Onset of normal LA after calving was 53 ± 4 d for M cows compared with 82 ± 4 d for thin cows ($P < 0.001$) when cows were treated at 25 d postcalving. Days to LA were greater ($P = 0.02$) for T and M cows treated with ECP at 25 d after calving (74 ± 5 d) compared with C cows (61 ± 5 d), and ECP treatment tended to increase ($P = 0.07$) days to LA for T cows treated at 50 d after calving. Body condition score of cows did not influence the incidence of estrus when cows were treated with ECP at 25 d after calving. Although ECP induced estrus in beef cows during the first 50 d after calving, normal luteal function was not initiated.

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

## INTRODUCTION

Postpartum anestrus is a major cause of infertility in beef cows (Wettemann, 1980; Short et al., 1990; Wettemann et al., 2003). Cows must be pregnant within 85 d after parturition to have a 12-month calving interval. Body condition score at calving is the major factor that controls the duration of postpartum anestrus (Richards et al., 1986; Selk et al., 1988; Spitzer et al., 1995). Treatment of prepubertal Holstein heifers with estradiol induced an LH surge without estrus (Swanson and McCarthy, 1978). Treatment of ovariectomized cows with estradiol causes release of LH (Short et al., 1973; Short et al., 1979; Forrest et al., 1981) and estrous behavior (Short et al., 1973; Nessan and King, 1981; Cook et al., 1986). However, when estradiol benzoate was administered to dairy cows an LH surge was not detected until 3 wk after calving (Zaied et al., 1981). Similarly, estradiol treatment did not cause secretion of LH in suckled beef cows during the first 10 d after calving (Nancarrow et al., 1977). Treatment of anovulatory suckled beef cows with estradiol benzoate at 3 to 6 wk after calving induced estrus (Nancarrow et al., 1977; Rubio et al., 2004). In contrast, Radford et al. (1976, 1978) observed that treatment of suckled anestrous cows with estradiol benzoate at 6 wk after calving did not induce estrus or secretion of LH. There appears to be differential effects of estrogens in the postpartum suckled beef cow. Estrogens can induce estrus without subsequent release of LH and ovulation. Therefore, the objective of this study was to determine the effects of days after calving and BCS at calving on estrous and luteal responses of suckled anestrous beef cows after treatment with estradiol.

## MATERIAL AND METHODS

### Study Design and Animals

Multiparous Hereford x Angus cows (n=83) were used to evaluate the effects of BCS at calving and days after calving on estrus and luteal activity after treatment with estradiol cypionate. During the last third of gestation, cows grazed abundant dormant native range pasture and were fed either 0.9 or 1.8 kg/d of a 38 % CP (on a DM basis) soybean meal-based supplement to result in loss or maintenance of BW. Cows that maintained BW had a BCS (1 = emaciated; 9 = obese; Wagner et al., 1988) of 5 or greater (moderate; M), and cows that lost BW had a BCS less than 5 (thin; T). Cows calved during a 56-d period in January and February. After calving, cows were fed 1.8 kg of the same protein supplement with ad libitum native grass hay. Cow weight (without access to feed and water for 15 h) and BCS were recorded 10 d before the first cow calved. The Heatwatch ® system (DDX, Denver, CO) was used to monitor estrous behavior commencing at 14 d before treatment. Onset of estrus was defined as the first of two mounts received in a 4-h period. The end of estrus was defined as the last mount received with a mount 4-h before, and with no mounts during the next 12 h. Ovaries of cows were evaluated just before treatment for the presence of corpora lutea (CL) and size of the largest follicle by transrectal ultrasonography (Aloka 500-V ultrasound equipment with a 7.5-MHz probe; Corometrics Medical Systems, Wallingford, CT). If a CL was detected by ultrasonography, or luteal activity was detected by plasma concentrations of progesterone, the cow was removed from the study. Cows were stratified by calving date and follicle size (< 10 mm or > 10 mm) and allotted to treatment. At 25 ± 6 or 50 ± 6 d

after calving, M and T cows were given 1 mg of estradiol cypionate (ECP, Sigma, St. Louis MO, in 1 ml corn oil; i.m.) and control (C) received 1 ml corn oil (i.m.).

**Blood Collection and Lab Analysis**

Blood samples were obtained via tail vein puncture twice weekly for 1 wk before treatment, daily for 1 wk following treatment, twice weekly until 4 wk after treatment, and once weekly until 90 d after calving or until two estruses were observed and confirmed with plasma progesterone > 1 ng/mL for 2 consequent samples. Blood samples were collected in blood collection tubes (BD, Franklin lakes, NJ) containing EDTA, then placed on ice and centrifuged within 4 h at 2600 x g for 15 min. Plasma was stored at – 20°C until progesterone was quantified by RIA (Vizcarra et al., 1997).

Luteal activity (LA) was defined as a concentration of progesterone in plasma ≥1 ng/mL. The onset of normal luteal activity was defined as progesterone ≥ 1 ng/mL in two or more consecutive samples for at least 8 d. Eleven ECP and 6 Control cows did not have normal luteal activity by 90 d after calving so luteal activity was assigned as 1 wk after the last sample was obtained.

**Statistical analyses**

Body weight and BCS in late gestation and percentage anovulatory were analyzed using the GLM procedure of SAS (SAS Inst., Inc, Cary NC) with treatment in the model. The experimental design was originally as 2 x 2 x 2 factorial with BCS in late gestation (T vs M), days from calving to treatment (25 or 50) and ECP or C treatment as factors. However, due to 90% of the M cows with luteal activity at 50 d after calving there was an

insufficient numbers of animals to analyze the experiment as the 2 x 2 x 2 factorial. The effects of BCS and ECP treatment on estrous response within 4 and 10 d after treatment, LA within 10 d, number of mounts, duration of estrus, and days to resumption of normal LA were tested for normality, and analyzed using the GLM procedure with two models. One model evaluated ECP treatment and BCS effects for cows treated at 25 d after calving and contained ECP treatment, BCS, and the interaction, with date of treatment as a covariable. The second model evaluated effects in T cows and included ECP treatment, days postpartum and the interaction, with date of treatment as a covariable. Concentrations of progesterone on d 3 to 7 after treatment were analyzed as repeated measures using the MIXED procedure of SAS with lab assay block in the model as a random effect. One model evaluated cows treated at 25 d after calving and contained treatment, BCS, treatment block, day after treatment, treatment x BCS, BCS x day after treatment , trt x d after treatment and treatment x days after treatment x BCS. The second model evaluated all T cows and contained treatment, treatment block, days after calving, day after calving, treatment x days after calving, days after calving x days after treatment, treatment x days after treatment, and days after calving x treatment days after treatment.

## RESULTS

### BW, BCS, and Percentage Anovulatory

Body weight and BCS were greater ($P < 0.001$) for M (597 ± 13 kg, 5.1 ± 0.1 BCS) than for T (505 ± 10 kg, 4.3 ± 0.2) cows in late gestation. Fifty-one cows were identified as anovulatory at treatment based on both the absence of a CL detected by ultrasonography and concentrations of progesterone < 1 ng/mL for 1 wk before treatment. At 25-d after calving, 22% of the T and 5% of the M cows ($P = 0.14$) had luteal activity

and were not treated. At 50-d after calving 35% of the T and 90% of the M cows ($P$ = 0.01) had luteal activity and were not treated.

**Estrous Response after Estrogen Treatment**

Percentage of cows in estrus within 4 d after treatment with ECP or oil was influenced by treatment but not by BCS of cows treated on d 25 after calving or by days after calving for T cows (Table 1).There were not any significant interactions (treatment x BCS, $P$ =0.99; treatment x d after calving, $P$ = 0.84). Fifty percent of T cows treated at 25 d after calving and none of the control cows were estrus within 4 d of treatment ($P$ < 0.01). When T cows were treated at 50 d after calving, 55% were estrus within 4 d and none of the controls were estrus ($P$ < 0.01). More M cows treated at 25 d after calving were estrus within 4 d (55%; $P$ < 0.01) compared with control cows (11%). Percentages of cows in estrus within 10 d of treatment were similar to the percentages of cows in estrus within 4 d of treatment. For estrus within 10 d of treatment, 60% of T cows treated at 25 d after calving were estrus and 33% of M control cows at 25 d postpartum were estrus. The percentage of cows in estrus at 4 d after treatment in the other treatment groups did not change when the period was extended to 10 d after treatment with ECP.

Duration of estrus and number of mounts received for cows that were estrus within 10 d of treatment are in Table 2. Thin cows treated with ECP at 50 d after calving were estrus longer ($P$ = 0.05) than T cows treated at 25 d after calving. At 25 d after calving M control and ECP cows tended ($P$ = 0.09) to be estrus longer than T ECP cows. Neither ECP treatment nor BCS ($P$ = 0.65 and $P$ = 0.35, respectively) influenced the number of mounts received at estrus. None of the T cows on the C treatment exhibited estrus

**Luteal Activity after Estrogen Administration**

There were no significant interactions for luteal activity within 10 d of treatment with ECP (ECP treatment x BCS, $P$ =0.62; ECP treatment x d after calving, $P$ = 0.88). Treatment of T cows at 25 or 50 d after calving, and M cows at 25 d after calving with ECP did not influence ($P$ = 0.53) the percentage of cows with concentrations of progesterone > 1 ng/mL in plasma within 10 d after treatment (Table 1). However, more ($P$ < 0.01) M C and ECP treated cows at 25 d after calving had increased (≥ 1 ng/mL) concentrations of progesterone in plasma within 10 d after treatment compared with T C and ECP treated cows at 25 d after calving.

Analysis of plasma progesterone concentrations from d 3 to 7 after treatment with ECP or C in cows treated at 25 d after calving had a BCS x Days after treatment interaction ( $P$ < 0.0001). Moderate cows increased plasma progesterone concentration from d 3 (0.09 ± 0.12 ng/ml) to d 7 (0.73 ± 0.12 ng/ml) while T cows Plasma progesterone concentration did not change from d 3 to 7 (0.07 ± 0.12) after treatment with C or ECP. All other main effects and interactions were non significant ($P$ > 0.35). In the T cows all main effects and interactions were nonsignificant ($P$ > 0.13) except for days after calving x days after treatment ($P$ =0.02). Cows that were 25 d after calving had no change in plasma progesterone concentration from d 3 to 7 after treatment with ECP or C (0.6 ± 0.5 ng/ml). While Cows that were treated at 50 d after calving had plasma progesterone that increased from d 3 to d 7 after treatment with ECP or C ( 0.8 ± 0.6, 0.34 ± 0.6 ng,ml; respectively).

There were no interactions for days after calving to resumption of normal luteal activity (ECP treatment x BCS, $P$ =0.94; ECP treatment x d after calving, $P$ = 0.85).

Cows with moderate BCS resumed normal luteal activity sooner after calving ($P < 0.001$) than thin cows (Table 1). Treatment of anestrous cows with ECP at 25 d after calving increased ($P = 0.03$) the interval until normal luteal activity regardless of BCS. Similarly, treatment of thin cows with ECP at 50 d after calving tended ($P = 0.07$) to increase the interval to normal luteal activity compared with controls (Table 1).

## DISCUSSION

Treatment of postpartum anestrous beef cows with ECP elicited estrus but did not result in luteinization of follicles. Treatment of dairy cows with estradiol benzoate induced estrus in 12 to 16 h (Cook et al., 1986). Amount of estrogen administered can effect the estrous response of animals in similar physiological states. When large doses ($\geq$ 2 mg) of estrogen were administered to ovarectomized cows all cows responded with estrus (Short et al., 1973; Nessan and King, 1981; Cook et al., 1987). However, when low doses (< 2 mg) of estrogen were given to ovariectomized cows either estrus was not elicited (Nessan and King, 1981) or only a small percentage of cows responded with estrus (Cook et al., 1986). When a moderate dose of estrogen was given a greater number of cows responded with estrus (Cook et al., 1986). Postpartum cows do not respond to estrogen like ovariectomized cows. Nancarrow et al. (1977) observed that cows greater than 21 d after calving responded with estrus after administration of 500 µg of estradiol benzoate. However, when the same dose of estradiol benzoate was administered to suckled cows, estrus was not initiated until 6 wk after parturition while their non-suckled counterparts were in estrus as soon as two wk after calving (Radford et al., 1976; 1978). Administration of 1 mg of ECP caused 58% of 40 d postpartum

anestrous suckled cows to respond with estrus (Rubio et al., 2004). It appears that suckled cows do not respond to estrogen administration with estrus as early after calving as non-suckled cows.

The duration of estrus in the present experiment (< 8 h) was similar to the 5.5 h reported by Ciccioli et al. (2003) in postpartum primiparous cows from the same spring calving herd. Length of estrus in this experiment was similar to those of Allrich et al. (1989) and Nessan and King (1981) who administered small doses of estradiol benzoate to ovariectomized dairy heifers and cows. The duration of estrus after estradiol administration may be dependent on the dose of estrogen administered (Reames et al., 2005). Similarly, the duration of estrus increased when larger doses of estradiol benzoate were given to ovariectomized dairy cows (Allrich et al., 1989; Nessan and King, 1981). However, Cook et al. (1986) observed no difference in duration of estrus after small or moderate doses (125, 250 or 500 μg) of estradiol benzoate were administered to ovariectomized dairy cows.

The number of mounts per estrus (< 10) in the current experiment are less than those observed in primiparous cows at the first estrus after calving (Ciccioli et al., 2003). Nessan and King (1981) and Allrich (1989) reported between13and 16 mounts received per estrus when dairy cows were treated with 200 μg of estradiol benzoate. However, when 0.5 mg of estradiol benzoate was administered the number of mounts received per estrus was increased (Allrich, 1989). There are two possible reasons for the reduced number of mounts per estrus in this study when compared with other estrogen induced estrus or normal postpartum estrus within the same herd as our study. The first could be that only a few cows were in estrus at a time and this limited behavior and the number of

mounts received per estrus. More animals in estrus at a time results in a greater number of mounts that are received by estrous cows (Helmer and Britt, 1985; Floyd et al., 2001). The behavioral differences observed between our study and the previous reports could be due to differences in concentrations of estrogen in plasma. As dosage of estradiol benzoate increases so does concentration of estrogen in plasma (O'Rourke et al., 2000). The type of estrogen administered can also affect the concentration of estrogen in plasma. Estradiol benzoate results in a quicker initial increase in concentration of estradiol in plasma when compared with ECP, which results in a slower release of estradiol over a longer period of time in dairy cows (Vynckier et al., 1990; Souza et al., 2006),

Body condition score at parturition had a significant effect on luteal activity within 10 d of treatment, while estrogen treatment had no effect. Body condition at parturition is the major factor effecting postpartum pregnancy rate (Selk et al., 1988; Spitzer et al., 1995). Perpartum BCS has a major influence on postpartum luteal activity (Spitzer at al., 1995). Estrogens can elicit the release of a LH surge in ovariectomized cows (Short et al., 1973; Forrest et al., 1981) and prepubertal Holstein heifers (Swanson and McCarthy, 1978). However, in postpartum suckled cows it is not established as to when the hypothalamus becomes sensitive to estrogen and results in the release of LH from the pituitary. Estrogen treatment of beef cows at 2 wk after calving results in LH release (Short et al., 1979; Nancarrow et al., 1977) and in dairy cows estrogen treatment between 2 and 4 wk results in LH release (Zaied et al., 1981). However, administration of estrogen did not induce an LH surge for at least 6 wk postpartum in suckled beef cows (Radford et al., 1976; 1978). Similarly, anestrous suckled beef cows at 40 d postpartum

did not respond with luteal activity after a 1 mg dose of ECP (Rubio et al., 2004). It appears that estrogen is unable to cause the release of GnRH that preceded a LH surge in postpartum anestrous suckled beef cows. The pituitary glands of 40 to 45 d postpartum suckled beef cows are able to release LH when exogenous GnRH is administered and respond with ovulation, or luteinization, of a follicle (Troxel et al., 1980; Wettemann et al., 1982), indicating the lack of response to estrogen is not a pituitary disorder.

The increase in the interval from calving to normal luteal activity associated with estrogen administration, compared with C cows, in the present study has been reported for dairy cows (Haughian et al., 2002). The postpartum anovulatory period was increased when dairy cows were treated with 10 mg of ECP on 7 d postpartum, and this was associated with a decrease in plasma concentrations of FSH (Haughian et al., 2002). Exogenous estrogen suppressed plasma FSH concentrations in ovariectomized heifers (Bolt et al., 1990) and cyclic heifers (Price and Webb, 1988; Barnes et al., 1990). Suppression of FSH and LH secretion by exogenous estrogen may be dose dependent elevation of plasma estrogen caused by large doses of exogenous estrogens suppressing FSH and LH and lower doses having no effect (Wolfe et al., 1992). However, Day et al. (1990) found that a continuous elevation of estradiol in beef cows from growth promoting implant during the first 40 d after calving did not influence days from parturition to first normal estrous cycle. The negative effect of estrogen on plasma concentration of FSH and LH may have caused the increase in interval from calving to normal luteal activity in the present experiment. Another possible cause for the increased interval from calving to normal luteal activity is that ECP regressed the dominant follicle (Engelhardt et al., 1998; Rajamahendran and Walton, 1990). The difference in the

interval from calving to luteal activity for ECP treated and C cows (13 to 10 d) was similar to the duration between follicular waves.

In two breeds of sheep with a propensity for either single or multiple ovulations, it was determined that each breed had different estrogen requirements to induce estrus and the GnRH/LH surge within the brain (Ben Said et al., 2007). The dose of estrogen required to elicit estrus was consistently less than the dose of estrogen required to cause a GnRH/LH surge in sheep. This difference in the concentration of estrogen necessary to elicit estrus and a GnRH/LH surge may be related to the induction of estrus without ovulation when postpartum cows are treated with moderate doses (0.5 to 1 mg) of estrogen.

Treatment of suckled anestrous beef cows with ECP increased the percentage of cows in estrus at 25 or 50 d after calving. However, the percentage of cows with luteal activity by 10 d after treatment was not effected by ECP treatment. If cows had a greater prepartum BCS, a greater percentage had luteal activity within 10 d after treatment. Treatment of anestrous beef cows with ECP resulted in increased days from parturition to normal luteal activity. In postpartum anestrous suckled beef cows estrogen can elicit estrus behavior but not a GnRH/LH surge indicating a hypothalamic pituitary dysfunction. This dysfunction must be resolved for normal reproduction to occur.

Table 1. Influence of BCS at calving and days after calving on estrus and luteal responses to treatment of anovulatory postpartum cows with estradiol cypionate (ECP)

| | 25 d | | | | 50 d | |
|---|---|---|---|---|---|---|
| | Thin | | Mod | | Thin | |
| | C | ECP | C | ECP | C | ECP |
| Cows, n | 8 | 10 | 9 | 9 | 7 | 6 |
| Estrus within 4 d, % | 0 [ac] | 50 [bd] | 11 [a] | 55 [b] | 0 [c] | 50 [d] |
| Estrus within 10 d, % | 0 [ac] | 60 [bd] | 33 [a] | 55 [b] | 0 [c] | 50 [d] |
| Progesterone > 1 ng/ml with 10 d, % | 0 [a] | 0 [a] | 33 [b] | 22 [b] | 29 | 17 |
| Calving to normal luteal activity, d | 75 ± 6 [a e i] | 88 ± 5 [a f j] | 47 ± 3 [b g] | 59 ± 6 [b h] | 81 ± 6 [i] | 91 ± 6 [j] |

[a, b] Means differ within cows treated at 25 d after calving ($P < 0.01$)

[c,d] Means differ within Thin cows ($P < 0.01$)

[e, f,g,h] Means differ within cows treated at 25 d after calving ($P < 0.05$)

[i,j] Means differ within Thin cows ($P < 0.09$)

Table 2. Influence of BCS at calving and days after calving on duration of estrus and number of mounts received by postpartum anestrous cows that were estrus within 10 d after treatment with estradiol cypionate (ECP) or controls (C)

| Items | 25 d | | | | 50 d | |
|---|---|---|---|---|---|---|
| | Thin | | Mod | | Thin | |
| | C | ECP | C | ECP | C | ECP |
| Cows, n | 0 | 6 | 3 | 5 | 0 | 6 |
| Duration of estrus ,h | - | 3.3 ± 0.6 [ac] | 6.0 ± 3.1 [d] | 7.4 ± 1.4 [d] | - | 7.7 ± 1.9 [b] |
| Mounts received, no. | - | 4.7 ± 1.3 | 8.7 ± 2.7 | 9.6 ± 3.6 | - | 4.6 ± 1.3 |

[a, b] Means differ within Thin cows ($P < 0.05$)

[c, d] Means differ within 25 d after calving( $P = 0.09$)

CHAPTER IV

# Effects of nutrient restriction during early gestation on postnatal growth, carcass and organ weights of beef steers, and regulation of plasma glucose in yearling calves


## Abstract

Angus x Hereford heifers were used to evaluate the effect of prenatal nutritional restriction on postnatal growth and development. Heifers (15 mo of age) were inseminated with semen from an Angus bull. At 32.0 ± 0.5 d of gestation heifers were stratified by BW and BCS and allotted to low (L, fed 55% of NRC 1996 requirements, n = 10 and 7 in yr 1 and 2, respectively,) or moderate nutrition (M, fed 110% NRC requirements, n = 10 and 7). After 83 d of feeding, heifers were commingled and received a diet in excess of NRC requirements. Bulls were castrated at birth and calves were weaned at 230 ± 7 d of age in yr 1 and 227 ± 7d of age in yr 2 and maintained as a group. At 16 mo of age, L (n = 5) and M (n = 5) steers in yr 1 had access to a high-concentrate diet ad libitum to a BW of 575 ± 12 kg. Steers were harvested and weights of the empty body, heart, lungs and trachea, spleen, kidney, liver, pancreas, and the gastrointestinal tract were recorded. Samples of heart, kidney, liver, pancreas, muscle (complexus), KPH and subcutaneous fat were stored at -80ºC and DNA in tissues was quantified using Hoechst H33258 dye. In yr 2, calves (14 mo) were subjected to an intravenous glucose tolerance test and intravenous insulin challenge. Growth data were analyzed with the

GLM procedure of SAS and amounts of DNA per tissue were analyzed with the PROC MIXED procedure of SAS. Plasma concentrations of glucose and insulin were analyzed as repeated measurements using the PROC MIXED procedure of SAS and areas under the response curves were calculated using Sigma Plot software. Body weight and BCS were similar ($P < 0.31$) for L and M heifers at the beginning of the experiment in both years. At the end of restriction, L heifers weighed less ($P < 0.001$ and $P = 0.01$ in yr 1 and 2, respectively) and had a lower BCS ($P < 0.001$ in yr1 and 2) compared with M heifers. Length of gestation was 274 ± 2 d for L heifers and 278 ± 2 d ($P = 0.05$) for M heifers in yr 1 and was not influenced by restriction in yr 2. Nutrient restriction in early gestation did not influence birth weight or postnatal growth of calves in either year. Lungs and trachea of steers exposed to L weighed less ($P = 0.05$, 5.30 ± 0.55 kg) compared with M steers (6.35 ± 0.78 kg); weights of other tissues were not influenced by treatment. Muscle from L steers had a greater ($P = 0.04$) concentration of DNA than for M steers (0.58 ± 0.02 vs 0.52 ± 0.1 mg/g, respectively). Muscle fiber area of the complexus muscle was greater ($P = 0.04$) in L steers compared with M steers. Concentrations of glucose and insulin in plasma after administration of intravenous glucose or insulin were similar for L and M calves. Expression of genes regulating fat metabolism and glucose uptake in subcutaneous fat were not effected by nutritional treatment. However, fatty acid binding protein 4, fatty acid translocase and GLUT 4 were down regulated in KPH of L steers compared with M steers. Nutritional restriction of heifers during early gestation did not alter postnatal growth rate or regulation of plasma concentrations glucose in calves. However, concentrations of DNA in muscle tissue of

steers at maturity were greater in animals from dams exposed to restricted nutrient intake during early gestation.

Keywords: Carcass, Growth, Organ Composition, Prenatal Nutrition

## Introduction

The thrifty phenotype hypothesis is that when fetal environment is poor, there is an adaptive fetal response which optimizes growth of key body organs and tissues at the expense of other less important tissues (Hales and Barker, 1992). This adaptation can lead to altered postnatal metabolism, which may give the animal a greater chance of survival when nutrients are limited (Hales and Barker, 2001). However, when nutrients are abundant in the postnatal period, this altered metabolism can be detrimental in rodents (Hales and Barker, 1992; 2001).

Inadequate nutrition of beef cows during gestation can be caused by environmental conditions, such as drought or a season decline in forage quality and quantity. The effect of inadequate nutrition on prenatal livestock can occur at multiple stages in development. Restricted nutrient intake during early pregnancy (d 30 to 125 of gestation) decreased fetal weight of calves compared with controls at 125 d of gestation (Ford et al., 2005). Inadequate nutrient intake during late gestation (d 145 to 259 of gestation) increased bovine placenta weight and decreased fructose in amniotic fluid (Rasby et al., 1990). Inadequate nutrition of cows from d 30 to 125 of gestation increased caruncular surface density at 250 d of gestation and decreased cotyledonary surface density compared with cows fed adequate nutrition (Vonnahme at al., 2007). The number of myofibers were reduced and diameter of muscle fibers were increased by early prenatal nutrition

restriction in cattle and sheep (Du et al., 2005b; Zhu et al., 2006). Exposure of lambs to low nutrition from 28 through 78 d of gestation increased growth rate postnatally, increased area under the curve for plasma glucose and decreased area under curve for plasma insulin during i.v. glucose tolerance test (Ford et al., 2007). Similarly, average daily gains and feed efficiency of steers were increased if they were exposed to inadequate nutrient intake during 31 to 120 d of gestation (Underwood et al., 2006). Nutrient restriction during gestation of women results in offspring with decreased glucose tolerance and increased plasma insulin concentrations at 50 yr of age (de Rooij et al., 2006).

Nutrient restriction from d 1 to 30 of gestation in the ewes decreased adipose GLUT 4 glucose transporter but had no effect on GLUT 4 in muscle tissue (Gardner et al., 2005). There are other genes that control substrate passage and utilization in adipocytes and the effects of prenatal nutrition have not been evaluated on genes controlling fatty acid transport in cattle. Fatty acid binding protein 4 binds to and activates hormone sensitive lipase (Jenkins-Kruchten et al., 2003) and has been associated with NEFA crossing the plasma membrane into the adipocytes and the utilization of glucose by adipocytes (Baar et al., 2005). Fatty acid translocase (CD36) is a transported of fatty acids into adipocytes and other tissues and has a function in regulating fatty acid esterification and oxidation during insulin stimulation (Bonen et al., 2007). The objectives of these experiments were to determine the effect of nutrient restriction during early gestation on postnatal growth, glucose regulation, expression of genes associated with fat synthesis, and concentrations of DNA and protein in tissues of steers.

## MATERIALS AND METHODS

### Animal model

During two spring breeding season, Angus x Hereford heifers at 15 mo of age were inseminated with semen from an Angus sire. At 32.0 ± 0.5 d after AI, pregnancy was diagnosed by transrectal ultrasonography (Aloka 500-V with a 7.5-MHz probe; Corometrics Medical Systems, Wallingford, CT). Pregnant heifers were stratified by BW and BCS and allotted to either low nutrition (L, 55% NRC, 1996,fed 3.71 Mcal of NEm·heifer$^{-1}$·d$^{-1}$ composed of 3.17 kg of prairie hay and 0.45 kg of a 38% CP soybean based protein supplement in a drylot; n = 10 and 7in yr 1 and 2, respectively) or moderate nutrition [M, excess of 100%NRC, 1996, ad libitum native grass pasture (*Bothriocloa caucasica* and *Sorghastrum nutans*); n = 10 and 7 in yr 1 and 2, respectively]. After 83 d in yr 1 and 84 d in yr 2, heifers were commingled on native grass pasture and supplemented with protein (38% CP) and grass hay to exceed NRC (1996) requirements.

Heifers calved during a 31-d period beginning on January 31, 2005 (yr 1) and during a 26-d period commencing on January 25, 2006 in yr 2. Bull calves were castrated at birth and all calves were weaned at 230 ± 7 d of age (yr 1) or 227 ± 7 d of age (yr 2). The L and M calves were maintained as a group after weaning. Body weight was recorded after 18 h without feed and water at weaning and throughout the postweaning period.

**Experiment 1**

Calves born in year 1 were maintained on dormant native range with 2.27 kg of a 20% CP supplement after weaning until April 15, 2006 when supplementation was stopped. Calves were maintained on the same pastures until 16 mo of age. Steers at approximately 16 mo of age (L, n= 5; M, n= 5; 341 ± 5 kg ) were transported 15 km and L and M steers were placed into two adjacent 12 x 30 m open pens for 135 d at the Willard Sparks Beef Research Center, Stillwater, OK. Steers were adapted to a finishing diet over a 28-d period and implanted upon arrival and at d 58 on feed with Revalor-S (120 mg of trenbolone acetate and 24 mg of estradiol, Intervet, Millsboro, DE). The finishing ration was corn and corn distiller's grain based and contained 1.87 Mcal/ kg NEm, 1.23 Mcal/kg NEg, 12.13 % CP and was in excess of NRC (1996) requirements. Body weight without feed and water was recorded at entry into the feedlot and then once monthly. Steers were harvested at 88 ± 1 wk of age and 1.0 ± 0.3 cm of back fat.

**Slaughter and sample collection**

Steers were harvested in 3 groups within a 5 d period with equal numbers of steers per treatment each day. Steers were transported to the Oklahoma Food and Agriculture Products Research and Technology Center abattoir 12 h before slaughter. All steers were stunned with a captive bolt, exsanguinated, and viscera removed. Weights of blood, feet and ears, hide, head, hot carcass weight, lungs and trachea, rinsed heart, esophagus, kidney, liver, pancreas, and spleen were recorded. The reticulo-rumen was cut at the reticulo-omasal orifice and fat was removed from the forestomach. The reticulo-rumen was inverted and rinsed with warm water, excess rinse water removed, and weight was

recorded. The omasum was removed from the abomasum at the omasal-abomasal orifice, its contents were rinsed out with warm water, allowed to drip, and weight was recorded. The abomasum was removed from the small intestine at the pyloric sphincter. The abomasum was rinsed with warm water, excess rinse water removes, and weight was recorded. The small intestine and large intestine were separated from the mesentery. The small intestine was separated from the large intestine at the ileocecal junction. Contents of the small intestine were squeezed out and the small intestine was looped without tension across two stationary boards, with pegs 1 m apart to measure small intestine length, and weight was recorded. Contents of the large intestine were squeezed out and weight was recorded. The mesentery weight was recorded.

Four samples were taken from adjacent tissue from the heart (left ventricle), kidney, liver, pancreas, muscle (complexus), KPH, and subcutaneous fat. All tissues were collected within 2 h after stunning. Cross sections of kidney tissue contained both cortex and medulla. Tissue samples were taken from the right lobe of the liver and random places in the pancreas. Samples of subcutaneous fat were collected from over the pecteralasis muscle and from around the pelvis. Muscle samples were obtained from the complexus muscle on the interior of the neck, on the right side of the carcass after the carcass was split. All tissue samples were snap frozen in liquid nitrogen and stored at -80ºC. Two additional 1.0 x 1.0 cm samples of complexus muscle were fixed in 4 % paraformaldehyde in phosphate buffer for 24 h then stored in 70 % EtOH at room temperature for 4 mo for histological analysis.

**Histology**

Tissue was removed from 70 % EtOH and embedded in paraffin. Then two cross sections (10 μm) of muscle were taken 5 sections apart and placed on slides. Sections were stained with haematoxylin and eosin and evaluated with a microscope (Olympus BX 51, Center Valley, PA). Images were recorded at 40 x magnification using a BP 71 digital camera (Olympus). Fields were chosen so that the field was composed mostly of regular shaped muscle fibers with a minimum of other structures. Muscle fiber area was determined in 10 fields per animal utilizing the Image J software (National Institute of Health, Bethesda, MD).Muscle fiber area was averaged across the 10 fields to give an average muscle fiber area per animal.

**Chemical analyses and DNA quantification of tissue samples**

Tissue DM was determined on 0.5 g samples of tissue by AOAC (1990). Protein concentration was determined in 0.2 g tissue samples by Leco Nitrogen Determinator (model FP-428; Leco Corp., St. Joseph, MI) in accordance with AOAC (1990). Concentrations of DNA were determined by Hoechst Dye 33258 (Argos Organics; Trenton, NJ) in a flourospectrometer (ND-3300, Nanodrop Technologies, Wilmington, DE wavelength excitation 365 nm and emission 450 nm; LaBarca and Paigen, 1980). Five milliliters of ice cold 1 x TNE buffer (100 mM Tris (pH 7.4), 150 mM NaCl, and 0.2 mM EDTA) was added to two 0.5 g samples of tissue and homogenized on ice using a tissue homogenizer (PRO 200; Pro Scientific; Monroe, CT). Homogenates were diluted using ice cold 1 x TNE buffer. One hundred μL of diluted homogenate were added to an amber colored 1.5 mL microcentrifuge tube (Axygen Scientific, Union City, CA.) and

100 µL of a 0.2 µg/mL solution of Hoechst 33258 (dissolved in 1 x TEN buffer) was added to each tube, vortexed 5 sec, and incubated for 45 min at room temperature. Two microliters of diluted homogenate Hoechst 33258 mixture were placed on the pedestal of the Nanodrop 3300 and a relative frequency unit was measured. Calf thymic DNA (Rockland labs, Gilbertsville, PA) was used to develop a standard curve (0, 100, 200, 300, 400, 500, and 700 µg/µL).

**Gene expression**

Tissue from both fat depots (0.5 g) were homogenized on ice in TRIzol (Invitrogen, Carlsbad, CA) using a tissue homogenizer (PRO 200; Pro Scientific; Monroe, CT). Homogenizer was wiped clean using tissue and 95% ETOH and then ran for 5 sec each, twice in 95 %ETOH and again for 5 seconds each twice in distiller H2O before samples were homogenized and between samples. Then RNA was extracted from tissue using the TRIzol procedures. Tissue [0.5 g of muscle and 1 g of adipose tissue] was taken from the freezer and 5 ml of TRIzol was added and tissue was homogenized for 30 sec on ice. The homogenate was centrifuged at 6750 x g for 10 minutes and then the fat layer that formed on top of the trizol was carefully penetrated and trizol was extracted and placed equally in three 2 ml microcentrifuge tube (Axygen Scientific, Union City, CA.). Chloroform was added at a rate of 0.2 mL per ml of trizol used (0.33 ml per microcentrifuge tube), vortex and incubated at room temperature for 3 min. The homogenate was centrifuged at 6750 x g for 30 min at 4°C. The upper aqueous phase was transferred and divided between three 1.5 ml microcentrifuge tube (Axygen Scientific) and 0.835 ml of isopropyl alcohol was added to each tube. The 1.5 mL tubes were

centrifuged at 11,200 x G for 10 min at 4°C. Supernatant was discarded and 1 mL of O° C 75% ethanol was added to each tube and vortex to break pellet away from tube. Tubes were centrifuged at 11, 200 x g for 5 min at 4°C. Ethanol was decanted and the pellet was allowed to air-dry and was dissolved in 50 μL of DEPC water. Quality of RNA was determined by ND-1000 (Nanodrop Technologies, Wilmington, DE) and also by denaturing agrose gel electrophoresis using 1.5% ethidium bromide-stained agarose gel. Fluorescent real time quantitative PCR (RT-PCR) was used to determine mRNA expression for fatty acid binding protein 4 (AP2), fatty acid translocate (CD36), and C/EBP alpha (C/EBP α) in both fat depots and GLUT 4 in both fat depots and muscle (complexus). Expression was quantified using SYBR Green (Qiagen Inc, Valencia, CA). Primer sequences are given in Table 1. Primers were validated for optimum performance and the single RT- PCR product was sequenced to assure the amplification of the desired product. Quantification of gene expression for the four genes was accomplished using the comparative threshold cycle (Ct) method (Hetting et al., 2001; Ross et al., 2003; and Santiago et al., 2005) with 18 S ribosomal RNA (18 S Ribosonal RNA kit, Eurogentec, Philadelphia, PA) used as the house keeping gene for normalization.

**Experiment 2**

Calves born in yr 2 (n = 14) were maintained on dormant native grass pasture with 2.27 kg of a 20 % CP supplement after weaning until May 15, 2007, when calves were transported to the Nutrition Physiology Research Center and maintained in pens with 2 or 3 calves per pen (2.3 x 4.8 m). Calves were fed 2 kg of chopped prairie hay and 5 kg of a supplement (37.6% wheat midds, 25% alfalfa pellets, 15% cottonseed hulls, 15%

soybean hulls and 3% soybean meal; 1.34 Mcal/kg NEm, 0.73 Mcal/kg NEg and 14.4% CP) in the morning and orts measured daily. After 2 wk adaptation, a polyvinyl cannula (vinyl tubing size 11(Bolab, Lake Havasu City, AZ; using a 11 gauge needle) was inserted into each jugular vein with sodium citrate as an anticoagulant and calves were confined in metabolism stalls (1.2 x 2.1 m). On the second morning following cannulation, 1 h after feeding at 0800, blood samples were collected at -15 and 0 min before calves were given a bolus i.v. infusion of glucose (intravenous glucose tolerance test (IVGTT), sterile 50 % glucose solution at 0.3 g of glucose/kg of BW). Blood samples were obtained from the contralateral cannula every 15 min for 2 h after infusion. The day after IVGTT, calves were given an insulin challenge (IC) beginning 2 h after feeding. Bovine insulin (Sigma, St. Louis MO, 18.6 U/mg) was dissolved in phosphate buffered saline (0.15 M, 7.0 pH) with 10 ml of 0.1 1 molar NaOH added to dissolve the insulin. The insulin solution was infused at a rate of 0.2 U of insulin/kg of BW in a single bolus and cannula were flushed with 15 mL of phosphate buffered saline. Blood samples were collected at the same times as for the IVGTT. All blood samples were collected in sodium fluoride/oxalate tubes, placed on ice for 3 h or less, centrifuged at 2,600 x g for 15 min and plasma was removed and stored at – 20 ° C. Plasma insulin and glucose concentrations were quantified in laboratory blocks (each lab block with an equal number of animals from both treatments) using a RIA (Bossis et al. 1999) and a colorimetric procedure (Affinity glucose regent, Thermal Electron Corp.) respectively. Glucose assay intraassay CV was 1.8 % and interassay CV was 3.9 %. Insulin assay intra- and interassay CV were, 10% and 15% respectively

**Statistical analyses**

For Exp. 1 and 2, maternal BW changes were analyzed using the GLM procedure of SAS (SAS Inst., Inc, Cary, NC) with treatment in the model. Birth weight, weaning and postweaning growth, and gestation length in exp. 1 and 2 were analyzed using the Mixed procedure of SAS with treatment, sex and the interaction in the model. For exp. 1, weight of the organs at slaughter, carcass characteristics, empty body weight, proximate analysis of tissues, muscle fiber area and gene expression were analyzed using the GLM procedure of SAS with treatment in the model. Concentrations of DNA and total DNA per organ were analyzed using the Mixed procedure of SAS with treatment in the model and laboratory assay block as a random variable.

For exp. 2, concentrations of glucose and insulin in plasma for the period from -15 min to 120 min relative to infusion for both the IVGTT and IC were analyzed as a repeated measure using the Mixed procedure of SAS with lab assay block as a random variable and prenatal nutritional treatment and time in the model. Sex effects were cofounded with lab block and could not be determined. Plasma insulin and glucose for both IVGTT and IC were plotted and area under the curve (AUC) was determined for insulin and glucose using the trapezoidal rule with Sigma Plot software (SPSS Inc., Chicago, IL) and analyzed using the Mixed procedure of SAS. For the AUC -15 and 0 samples were averaged and this was used as a basal concentration called 0 for calculating AUC. For the IVGTT the 0 to 90 min relative to infusion of glucose was used to calculate AUC for plasma glucose and for plasma insulin 0 to 60 min was used. For the IC 0 to 120 min was used for plasma glucose and 0 to 60 for plasma insulin. To evaluate the clearance of insulin and glucose following IVGTT and IC exponential decay was

determined using linear regression (prog Reg of SAS) for the log of plasma insulin and glucose concentration. Plasma glucose concentrations were analyzed from 15 to 120 min after IVGTT and from 45 to 120 min after IC. Plasma insulin concentrations were analyzed from 15 to 120 min after IVGTT and 15 to 60 min after IC. The regression coefficients for each regression were analyzed using GLM procedures of SAS with treatment in the model.

## Results

Maternal BW and BCS from initiation of nutritional treatment until precalving for yr 1 and 2 are presented in Table 2. Body weight ($P = 0.31$) and BCS ($P = 0.33$) were similar for L and M heifers at the start of treatment in yr 1. Body weight ($P < 0.001$ and $P = 0.05$ for yr 1 and 2, respectively) and BCS ($P = 0.008$ and $P = 0.007$ for yr 1and 2, respectively) were less in L compared with M heifers after 1 mo of nutritional treatment. At the end of treatment L heifers in yr 1 had lost 63 ± 2 kg while M heifers had gained 43 ± 2 kg ($P < 0.001$). Low heifers lost 26 ± 4 kg in yr 2 while M heifers gained 55 ± 5 kg. Low heifers at the end of nutritional restriction had a BCS of 4.3 ± 0.1 in yr 1 and 4.6 ± 0.1 in yr 2 while M heifers had a BCS of 5.5 ± 0.1 in yr 1 and 5.3 ± 0.1 in yr 2 ($P < 0.001$ and $P < 0.001$ for yr 1and 2, respectively). Precalving BW and BCS were less ($P = 0.006$ and $P < 0.001$, respectively) for L heifers compared with M heifers in yr 1. Precalving BW was not affected ($P = 0.63$) by nutritional treatment in yr 2 but precalving BCS was less ($P = 0.04$) for L heifers compared with M heifers in yr 2. Gestation length was

decreased ($P = 0.05$, Table 2) by 4 d in yr 1 in L compared with M heifers. Gestation length was similar ($P = 0.52$) for L and M heifers in yr 2.

Birth weights were not influenced by treatment in yr 1 ($P = 0.31$, Table 3) or yr 2 ($P = 0.94$, Table 4). In yr 2, there was a treatment x sex effect ($P = 0.05$) on birth weight. Bull calves from L heifers were heavier than L heifer calves and M bull calves (Table 4); L and M heifers' calves had similar birth weight. Steers were heavier ($P = 0.05$, Table 3) than heifers at weaning (10/6/2005) regardless of prenatal nutrition in yr 1. Average daily gain from birth to weaning in yr 1 tended ($P = 0.08$) to be greater for steers than heifers. Neither sex nor nutritional treatment influenced ($P \geq 0.26$) weaning weight (9/19/2006( or ADG from birth to weaning ($P \geq 0.39$; Table 4) in yr 1. Postweaning growth in yr 1 was not influenced by treatment ($P = 0.08$ to 0.33) except on January 6, 2006 when BW for L calves was heavier ($P = 0.05$) than for M calves (Table 2). Treatment did not influence BW during the postweaning period ($P = 0.70$ to 0.99; Table 4) in yr 2. Prenatal nutritional had no effect ($P \geq 0.14$) on average daily gain for yr 1 or 2 (Tables 2 and 3).

*Experiment 1*

Low steers were heavier ($P = 0.04$) than M steers when started in the feedlot on July 3, 2006 (Table 3), on August 30, 2006 ($P = 0.007$), and tended to be heaver at harvest ($P = 0.07$, October 19, 2006). Although L steers were heavier upon entry into the feedlot and at harvest, prenatal nutrition did not influence ($P = 0.40$) ADG during the feeding period (Table 3).

Hot carcass weight, empty body weight, weight of organs, and weight of organs expressed as g of organ / kg of empty body weight are in Table 5. Hot carcass weight and

empty body weight were not influenced ($P \geq 0.20$) by prenatal nutritional treatment. The lungs and trachea were lighter ($P = 0.05$, Table 5) when expressed as absolute weight or corrected for empty body weight (P = 0.05) in steers exposed to low nutrition during d 32 to 115 of gestation. Prenatal nutritional treatment did not affect ($P = 0.16$ to 0.96) the weight of all other organs measured (either absolute or on an empty body weight basis, Table 5). Twelfth rib fat thickness , dressing percentage, yield and quality grade (Table 6) for L and M steers were not influenced ($P \geq 0.24$) by nutritional treatment. Similarly, LM area and percentage KPH were similar ($P \geq 0.18$) for L and M steers.

Concentrations of DNA and protein, DNA to protein ratio, DM and lipid in tissues are summarized in Table 7. Complexus muscle from L steers had more ($P = 0.02$) DNA per gram of tissue compared with M steers. The protein to DNA ratio tended to be decreased ($P = 0.10$) in complexus muscle of L steers compared with M steers while the concentration of protein and lipid along with DM did not differ between prenatal treatment groups. Amounts of DNA, protein and lipid, and the protein to DNA ratio in heart and kidney were not influenced ($P \geq 0.17$) by nutritional treatment. Dry matter of the kidney was unaffected by prenatal nutrient restriction, while the DM was decreased ($P = 0.04$) in hearts from L steers compared with M steers. Livers from L steers had greater ($P = 0.01$) concentrations of protein compared with M steers, but DNA concentration, protein to DNA ratio, DM, and lipid were unaffected by nutritional treatment ($P \geq 0.31$). Protein and DNA concentrations along with DM in the pancreas was not influenced by prenatal nutrition ($P \geq 0.31$), but the amount of total DNA in the pancreas of L steers was greater ($P = 0.05$) compared with M steers. The lipid content of the pancreas tended to be decreased ($P = 0.07$) in pancreases from L steers compared

with M steers. The content of DNA, DM and lipid of subcutaneous fat was not influenced ($P \geq 0.69$) by prenatal nutrition. However, there tended to be a greater concentration ($P = 0.09$) of DNA in KPH from L steers then M steers. Dry matter and lipid of KPH were not influenced ($P \geq 0.19$) by treatment.

Area of individual fibers in the complexus muscle of the neck was greater ($P = 0.04$) in steers exposed to low nutrition from d 32-110 of gestation compared with steers exposed to adequate nutrition (Figure 1).

Gene expression for fatty acid binding protein 4, fatty acid translocase, C/EBP α and GLUT 4 are in Table 8. Abundance of target gene mRNA in subcutaneous fat was not affected by nutritional treatment. However expression of mRNA for fatty acid binding protein 4, fatty acid translocase and GLUT 4 were decreased ($P = 0.0003$, 0.006, and 0.009, respectively) in KPH of L steers compared with KPH of M steers. Expression of GLUT 4 tended to be increased ($P = 0.08$) in the complexus muscle from L steers compared with M steers

*Experiment 2*

Nutritional treatment did not effect BW of calves before IVGTT ($P = 0.79$, Table 4). There was a tendency ($P = 0.09$) for M calves to have greater concentrations of glucose in plasma after glucose infusion (Figure 2a). However AUC from 0 to 90 min after infusion for plasma glucose was similar ($P = 0.31$) for L and M calves during IVGTT (Figure 2b). Concentrations of insulin in plasma during IVGTT were similar (P= 0.55) for L and M calves (Figure 2c) and the AUC (0 to 60 min postinfusion) for concentrations of insulin in plasma were not influenced by treatment ($P = 0.43$; Figure

2d). The regression of log transformation of plasma glucose concentration during 15 to 120 min after IVGTT indicated greater ($P = 0.05$) clearance rate of glucose from plasma after glucose infusion of L calves compared with M calves (-0.0035 ± 0.0002, -0.0029 ± 0.0002; L and M respectively). Regression coefficients of log transformed plasma concentrations of insulin during 15 to 120 min after IVGTT were not influenced by prenatal restriction ($P = 0.30$, 0.0075 ± 0.0008, -0.0058 ± 0.0013; L and M respectively)

Concentrations of glucose in plasma after i.v. administration of bovine insulin were similar ($P = 0.68$) for L and M calves (Figure 3b). The increase in plasma insulin following i.v. administration of insulin was not affected by nutritional treatment ($P = 0.55$; Figure 3a). The AUC for plasma glucose and insulin after infusion of insulin were similar (P = 0.92 and 0.52 respectively) for L and M calves (Figure 3c and 3d respectively). Log transformation of plasma concentrations of glucose during 45 to 120 min after IC had similar regression coefficients ($P = 0.99$; 0.0037 ± 0.004 for both L and M calves). Regression of log transformed plasma concentrations of insulin 15 to 60 min after IC were not influenced by prenatal nutrient restriction ($P = 0.66$, -0.021 ± 0.002, -0.020 ± 0.00; L M respectively).

## Discussion

In yr 1, heifers had BCS at the end of nutritional treatment of 4.3 ± 0.1 and 5.5 ± 0.1 (L and M, respectively). In yr 2 L heifers had a BCS of 4.6 ± 0.1 while M heifers had a BCS of 5.3 ± 0.1.In yr 1 L heifers lost 63 ± 2 kg while M heifers gained 43 ± 2 kg. While in yr 2 L heifers lost 26 ± 4 kg while M heifers only gained 55 ± 5 kg. Nutritional restriction of heifers in yr 1 resulted in a greater loss of BW and BCS than in yr 2 even

thought the same diet was fed both years. The differences in BCS and BW changes could be due to the availability of grass before the start of nutritional restriction due to differences in rainfall in yr 1 and 2. Gestation length was reduced by prenatal nutrient intake by 4 d in yr 1 and was not effected in yr 2. Birth weights were similar for L and M calves in both years. A shortened gestation length of 8 d was reported for cows that received a protein deficient diet during 5 to 9 mo of gestation (Waldhalm et al., 1979). However, no difference in gestation length or calf birth weight were reported for heifers fed a protein deficient diet for the last90 or 140 d of gestation (Carstens et al., 1987; Martin et al., 1997). Decreased caloric intake during the last 90 d of gestation or before decreased calf birth weight in cows (Wiltbank et al., 1962, Houghton et al., 1990, Spitzer et al., 1995) and in beef heifers (Corah et al., 1975; Bellows and Short, 1978; Kroker and Cumming 1979) and reduced gestation length (Hafez et al., 1968; Warrington et al., 1988). In all the above references the dietary manipulation that resulted in either decreased gestation length or reduced calf weight occurred before and during the third trimester of pregnancy. However, others have observed that restriction of dietary energy starting before the last trimester of pregnancy had no effect on birth weight or gestation length (Doornbos at al., 1984; Goehring et al., 1989; Hough et al., 1990).

Postnatal growth was not affected by prenatal nutrition in the first trimester in yr 1 or 2. Growth during the period from birth to weaning and postweaning was not influenced by prenatal nutrition. The ADG for the feedlot period was not different between treatment groups. Even though average daily gains were similar between treatment groups, L steers were heavier at the start of feeding and were 34 kg heavier at harvest than M steers. Steers from cows fed 68% of NEm requirements from d 30 to 120

of gestation had greater ADG and gain to feed ratios and tended to have heavier BW at harvest than steers from cows fed 100% of requirements (Underwood et al., 2006). However, hot carcass weights were similar for the restricted and control groups. Lambs exposed to restricted nutrition from d 0 to 30 of gestation or d 110 of gestation to term had similar growth rates at 1 yr of age (Gardner at al., 2005). However, wethers from ewes exposed to 50% of nutritional requirements from d 28 to 78 of gestation were heavier at 4 and 9 mo of age compared with wethers from ewes fed at nutrient requirements (Ford et al., 2007).

Carcass measurements of steers were not influences by prenatal nutritional treatment in this study. Underwood et al. (2006) reported similar yield grade, marbling score and percentage KPH fat for steers harvested at 12 to 13 mo of age whose dams received either 100 or 68% of requirements from d 30 to 120 of gestation. Nutritional restriction of lambs from 60 d before mating to 7-d postmating increased perirenal fat mass in lambs at term (Edwards et al., 2005). Nutritional restriction from 28 to 78 d of gestation produced lambs with increased back fat at 140 d of age and increased perirenal fat and HCW at 280 d of age (Ford et al., 2007). Nutrient restriction from d 110 of gestation to term resulted in lambs with increased omental and perirenal fat depots at 1 yr of age (Gardner et al., 2005).

Lungs and trachea were lighter in steers exposed to low prenatal nutrition during the first trimester of pregnancy with no differences in weights of the other organs that were measured. Martin et al (1997) found lighter lungs and trachea in calves at birth from dams exposed to a protein restricted diet from d 140 of gestation until term. Ewes that were exposed to energy and protein restriction from d 28 to 78 of gestation had fetuses

with smaller livers, lungs and kidneys at d 78 of gestation, however, the weight of left and right ventricles of the heart, as a percentage of fetal weight, were increased compared with fetuses from dams fed to meet requirements (Vonnahme et al., 2003). The liver, lungs, kidney and heart weight differences were at mid gestation (148 d on the ewe), and therefore differences in organ weight may not be observed at parturition or later in the postnatal life.

The similar weights of the digestive tract and liver in our study are probably due to the same diet being fed during the postweaning and feedlot period of the L and M steers. Gastrointestinal tract mass in relation to BW is influenced by DMI or energy density of the diet (Hersom et al., 2004). Physical form and fiber content of the diet appears to affect the forestomach through both physical and chemical stimulation (Sainz and Bentley, 1997). Liver mass is dependent on nutrient load and responds with increased weight as DMI and energy density of the diet increases (Jones et al., 1985; Sainz and Bentley, 1997; Hersom et al., 2004). An increase in liver mass may be due to increases in liver cell size (Burrin et al., 1992; Sainz and Bentley, 1997). Small intestine mass is dependent on nutrient load and physical form of the diet and increases via increased number of cells (Sainz and Bentley, 1997; McLeod and Baldwin, 2000).

Concentration of DNA in tissue can be used as an index of hyperplasia (Enesco and Labland, 1962). Protein per unit of DNA in tissue can be used as an indication of hypertrophy (Allen et al., 1979). DNA concentration was increased in the complexus muscle of steers of L cows. Muscle is a multinucleated tissue so concentration of DNA does not indicate the number of cells. The protein to DNA ratio tended to be reduced in steers exposed to low nutrition during the first trimester of gestation. Steers from L cows

had larger muscle fibers with greater numbers of nuclei. Cell size or number of cells in heart, liver, and kidney were not effected by prenatal nutrition, as indicated by DNA concentrations and protein to DNA ratio. Pancreas of L steers had greater total DNA in the whole organ then the pancreas of M steers. This is related to L steers having a tendency for a heavier pancreas, and indicates that the pancreases of L steers had more cells then the pancreases of M steers. Number of cells per g of subcutaneous fat, indicted by DNA concentrations, were not influenced by prenatal nutrition. However, the concentration of DNA/g of KPH tended to be greater in L steers compared with M steers. This indicates a greater number of adipocytes in the KPH of L steers or less filling of adipocytes.

Abundance of mRNA for genes affecting fat metabolism and glucose uptake were decreased in KPH of steers exposed to L nutrition during early gestation. Fatty acid binding protein 4 binds to and activates hormone sensitive lipase (Jenkins-Kruchten et al., 2003). Fatty acid binding protein 4 has also been associated with NEFA crossing the plasma membrane into the adipocytes and in the utilization of glucose by adipocytes (Baar et al., 2005). Fatty acid translocase is a transporter of fatty acids into adipocytes and other tissues and has a function in regulating fatty acid esterification and oxidation during insulin stimulation (Bonen et al., 2007). GLUT 4 is the major insulin dependent sodium linked glucose transporter in tissue. Decreased mRNA abundance in KPH of L steers for both fatty acid and glucose transporters, and proteins that influence the activity of hormone sensitive lipase, indicate a decreased ability to utilize both NEFA and glucose as substrates for fatty acid synthesis in L steers compared with M steers. The down regulation of nutrient transporters support the hypothesis that less filling of adipocytes is

the cause for the increased DNA concentration in KPH from L steers compared with M steers. The trend towards upregulation of GLUT4 in complexus muscle in L steers compared with M steers is opposite the down regulation of fat and glucose transports and associated molecules in KPH.

Prenatal nutrient availability influences the ability of calves to regulate plasma concentrations of glucose at 15 mo of age. Calves exposed to L prenatal nutrition, cleared glucose from plasma more rapidly after infusion of glucose compared with M steers. Kastner et al. (2004) observed that calves from cows exposed to a high plane of nutrition from d 78 to 174 of gestation, had greater concentrations of plasma glucose before and during IVGTT at weaning and postweaning compared with calves exposed to moderate prenatal nutrition. Energy and protein restriction from d 28 to 78 of gestation in ewes resulted in wethers that had a greater AUC for plasma glucose compared to control animals in response to (IVGTT) at 63 and 250 d of age (Ford et al., 2007), indicating an inability to move plasma glucose into body tissue via insulin dependent mechanisms. The AUC for plasma insulin was greater at 63 d of age in nutrient restricted wethers than controls but was decreased at 250 d of age indicating a lower insulin production after stimulation by elevated plasma glucose concentrations. Nutrient restriction of ewes from d 1 to 30 of gestation did not alter metabolic response to feeding or to IVGTT in lambs at 11 mo of age; however, nutrient restriction from d 110 to term resulted in lambs with increased AUC for plasma glucose and insulin (Gardner et al., 2005). This response to IVGTT in late gestation nutrient restricted lambs was associated with decreased adipose GLUT 4 glucose transported but no difference in GLUT 4 in muscle tissue.

In summary, Nutritional restriction of beef heifers to 55% of NRC recommendations from d 32 to 110 of gestation shortened gestation length in yr 1 but not in yr 2 and did not affect birth weight in either year. Restricted nutrient intake of heifers during early gestation did not influence postnatal growth of calves. Carcass traits of steers were not affected by prenatal nutritional intake of dams. The lungs and trachea were smaller in steers from dams fed 55 % of NRC requirements from d 32 to 115 of gestation. Concentrations of DNA were increased and protein: DNA tended to be decreased in muscle when dams received 55% of NRC recommendations during early gestation. Muscle fiber area was increased in L steers compared to M steers. Gene expression for fatty acid binding protein 4, fatty acid translocase, and GLUT 4 were decreased in KPH of L steers compared to M steers at 22 mo of age. Glucose regulation at 15 mo of age was affected by prenatal nutritional intake of dams. Calves from L cows had a greater rate of glucose disappearance from plasma after IVGTT compared with calves from M cows.

This experiment clearly links BCS and BW changes of pregnant heifers fed a restricted diet to changes in gestation length, organ and tissue composition and gene expression of KPH. Nutritional restriction during the first trimester of pregnancy altered muscle formation in 22 mo old steers as indicated by muscle fiber area and concentrations of DNA and DNA to protein ratio in the adult animal. Gene expression was altered in KPH from L steers compared with M steers indicating possible metabolic changes in the KPH fat. The change in gene expression and DNA concentrations in KPH support a lack of filling of adipocytes in KPH from L steers compared with M steers. However, growth and carcass composition at 22 mo of age was not influenced by prenatal nutrition. Glucose regulation at 15 mo of age was influenced by prenatal

nutrition. This indicates that some effects of maternal undernutrition from d 32 to 115 of gestation may start to be observed at 15 mo of age but may develop further later in life.

Table 1. Sequence of primers[1] used for RT PCR gene expression evaluation in tissues from steers exposed to Low or Moderate nutrition during early gestation.

| Gene | Sequence | Temp | Accession # |
|---|---|---|---|
| C/EBP α | FWD primer 5' ATCTGCGAACACGAGACGTCCATC | 60°C | 281677 |
| | REV primer 5' GTAGTCAAAGTCGTTGCCGCCT | | |
| CD 36 | FWD primer 5' CAATGGAAAGGACGACATAAG | 60°C | 281052 |
| | REV primer 5' TGGAAATGAGGCTGCATCTGT | | |
| AP2 | FWD primer 5' AAGCTGCACTTCTTTCTCACC | 60°C | 281759 |
| | REV primer 5' GACCACACCCCCATTCAAAC | | |
| Glut 4 | FWD primer 5' TCACCTTAGTCTCGGTGTTCTTGG | 60°C | 282359 |
| | REV primer 5' AGATGGCCACAATGGAGACATAGC | | |

[1] All primers were made by IDT (San Diego, CA

Table 2. Body weight (kg), body condition score, and gestation length of heifers exposed to Low or Moderate nutrition during d 32 to 115 of gestation in yr1 and 2

| | Trt | | | |
|---|---|---|---|---|
| Variable | Low | Moderate | SE | *P* value |
| Yr 1 | n = 10 | n = 10 | | |
| BW at start of treatment (6/9/04) | 390 | 381 | 12 | 0.42 |
| BW on 7/14/04 | 347 | 405 | 13 | < 0.001 |
| BW on 8/31/04 | 328 | 424 | 12 | < 0.001 |
| BW change during treatment | -63 | 43 | 4 | < 0.001 |
| BW on 12/3/04 | 401 | 445 | 15 | < 0.01 |
| BW on 1/27/05 | 418 | 459 | 15 | < 0.01 |
| BCS at start of treatment (6/9/04) | 5 | 5.1 | 0.1 | 0.33 |
| BCS on 7/14/04 | 4.7 | 5.3 | 0.2 | < 0.01 |
| BCS on 8/31/04 | 4.3 | 5.5 | 0.2 | < 0.001 |
| BCS on 12/3/04 | 4.5 | 5.1 | 0.2 | < 0.001 |
| BCS on 1/27/05 | 4.4 | 4.9 | 0.2 | < 0.001 |
| Gestation, d | 274 | 278 | 1 | 0.05 |
| Yr 2 | n = 8 | n = 8 | | |
| BW at start of treatment (6/7/05) | 386 | 365 | 22 | 0.31 |
| BW on 7/14/05 | 363 | 405 | 22 | 0.05 |
| BW on 8/17/05 | 360 | 420 | 23 | 0.012 |
| BW change during treatment[1] | 25 | 57 | 2 | < 0.001 |
| BW on 10/4/05 | 383 | 442 | 20 | < 0.01 |
| BW on 1/23/06 | 500 | 511 | 27 | 0.63 |
| BCS on 7/14/05 | 4.9 | 5.4 | 0.1 | < 0.01 |
| BCS on 8/17/05 | 4.6 | 5.3 | 0.01 | < 0.001 |
| BCS on 10/4/05 | 4.7 | 5.3 | 0.1 | < 0.01 |
| BCS on 1/24/06 | 4.9 | 5.2 | 0.1 | 0.04 |
| Gestation, d | 273 | 274 | 1 | 0.52 |

[1] BW were measures at d 71 of restriction

Table 3. Birth weight, weaning and postweaning weight and ADG of calves from Low or Moderate prenatal nutrition in yr 1

| | Treatment | | | | *P* value | | |
|---|---|---|---|---|---|---|---|
| | Low | | Moderate | | | | |
| Date | Steers n = 5 | Heifers n = 5 | Steers n = 5 | Heifers n = 5 | trt | Sex | trt*sex |
| Birth weight, kg | 31.4 ± 1.1 | 31.3 ± 1.1 | 32.1 ± 1.3 | 33.1 ± 1.3 | 0.31 | 0.73 | 0.64 |
| 10/6/05 (weaning) | 223 ± 12 | 210 ± 8 | 218 ± 3 | 199 ± 6 | 0.32 | 0.05 | 0.70 |
| 1/6/06 | 267 ± 8 | 258 ± 5 | 256 ± 5 | 246 ± 3 | 0.05 | 0.09 | 0.89 |
| 3/9/06 | 285 ± 6 | 278 ± 5 | 277 ± 3 | 273 ± 9 | 0.33 | 0.40 | 0.77 |
| 4/13/06 | 308 ± 6 | 290 ± 4 | 300 ± 4 | 281 ± 4 | 0.08 | 0.001 | 0.93 |
| ADG, kg/d | | | | | | | |
| Birth to 10/6/05 | 0.81 ± 0.04 | 0.75 ± 0.04 | 0.84 ± 0.02 | 0.79 ± 0.02 | 0.14 | 0.08 | 0.9 |
| 10/6/05-1/6/06 | 0.46 ± 0.04 | 0.25 ± 0.05 | 0.41 ± 0.04 | 0.51 ± 0.07 | 0.55 | 0.18 | 0.73 |
| 1/6/06-3/9/06 | 0.30 ± 0.04 | 0.32 ± 0.03 | 0.34 ± 0.03 | 0.48 ± 0.17 | 0.28 | 0.33 | 0.49 |
| 3/9/06-4/13/06 | 0.07 ± 0.01 | 0.04 ± 0.01 | 0.07 ± 0.01 | 0.01 ± 0.03 | 0.52 | 0.03 | 0.53 |
| 10/6/05-4/13/06 | 0.68 ± 0.24 | 0.43 ± 0.03 | 0.44 ± 0.03 | 0.44 ± 0.04 | 0.33 | 0.30 | 0.30 |
| BW of steers in the feedlot, kg | | | | | | | |
| 7/3/2006 | 349 ± 5 | | 332 ± 5 | | 0.04 | | |
| 7/31/2006 | 410 ± 5 | | 400 ± 6 | | 0.31 | | |
| 8/30/2006 | 485 ± 6 | | 450 ± 7 | | 0.007 | | |
| 10/19/2006 | 594 ± 12 | | 560 ± 11 | | 0.07 | | |
| ADG, kg/d | | | | | | | |
| 7/03-7/31 | 2.15 ± 0.30 | | 2.44 ± 0.06 | | 0.32 | | |
| 7/31-8/30 | 2.53 ± 0.09 | | 1.69 ± 0.17 | | 0.002 | | |
| 7/03-8-30 | 2.34 ± 0.14 | | 2.04 ± 0.08 | | 0.10 | | |
| 8/30-10/19 | 2.16 ± 0.11 | | 2.19 ± 0.18 | | 0.89 | | |
| 7/03-10/19 | 2.26 ± 0.13 | | 2.11 ± 0.11 | | 0.40 | | |

Table 4. Birth, weaning and postweaning BW and ADG of calves from low or moderate prenatal nutrition in yr 2

| | Treatment | | | | *P* value | | |
|---|---|---|---|---|---|---|---|
| | Low | | Moderate | | | | |
| Date | Steers n = 5 | Heifers n = 2 | Steers n = 2 | Heifers n = 5 | trt | Sex | trt*sex |
| birth wt, kg | 37.5 ± 1.6 | 34.5 ± 0.9 | 30.6 ± 0.7 | 33.9 ± 0.6 | 0.94 | 0.03 | 0.05 |
| 9/19/2006(weaning) | 241 ± 13 | 228 ± 2 | 210 ± 7 | 228 ± 8 | 0.86 | 0.26 | 0.25 |
| 10/30/2006 | 282 ± 15 | 263 ± 5 | 246 ± 7 | 270 ± 12 | 0.89 | 0.39 | 0.22 |
| 12/5/2006 | 291 ± 14 | 267 ± 1 | 251 ± 8 | 277 ± 9 | 0.94 | 0.32 | 0.11 |
| 4/4/2007 | 324 ± 12 | 301 ± 9 | 281 ± 2 | 303 ± 10 | 0.99 | 0.15 | 0.12 |
| 5/15/2007 | 355 ± 10 | 328 ± 13 | 333 ± 15 | 329 ± 10 | 0.7 | 0.11 | 0.09 |
| 5/22/2007 | 355 ± 9 | 340 ± 14 | 311 ± 14 | 333 ± 9 | 0.79 | 0.05 | 0.13 |
| ADG, kg/d | | | | | | | |
| birth to weaning | 0.90 ± 0.06 | 0.85 ± 0.03 | 0.78 ± 0.03 | 0.87 ± 0.03 | 0.39 | 0.79 | 0.23 |
| 9/19/06-10/30/06 | 1.00 ± 0.06 | 0.86 ± 0.06 | 0.86 ± 0.01 | 1.00 ± 0.12 | 0.98 | 0.98 | 0.22 |
| 10/30/06-12/5/06 | 0.24 ± 0.07 | 0.11 ± 0.17 | 0.14 ± 0.02 | 0.21 ± 0.10 | 0.97 | 0.77 | 0.38 |
| 9/19/06-12/5/06 | 0.62 ± 0.05 | 0.66 ± 0.08 | 0.61 ± 0.02 | 0.60 ± 0.04 | 0.6 | 0.77 | 0.67 |
| 12/5/06-4/4/07 | 0.23 ± 0.02 | 0.29 ± 0.08 | 0.25 ± 0.05 | 0.22 ± 0.02 | 0.95 | 0.36 | 0.33 |

Table 5. Influence of low or moderate prenatal nutrition from d 32 to 115 of gestation on carcass weight, empty body weight and weight of the organs in kg and on a empty body weight basis

| | Treatment [a] | | P | Treatment [b] | | |
|---|---|---|---|---|---|---|
| Measurement | Low | Moderate | value | Low | moderate | P value |
| Animal, no | 5 | 5 | | 5 | 5 | |
| Hot carcass | 346 ± 22 | 335 ± 23 | 0.49 | | | |
| Empty Body | 514 ± 27 | 493 ± 22 | 0.20 | | | |
| Hide | 44 ± 1 | 39 ± 1 | 0.02 | 86 ± 3 | 80 ± 3 | 0.14 |
| head | 16.5 ± 0.3 | 16.2 ± 0.3 | 0.57 | 32 ± 1 | 33 ± 1 | 0.46 |
| blood | 19 ± 1 | 17 ± 1 | 0.12 | 37 ± 2 | 35 ± 2 | 0.48 |
| feet and ears | 15 ± 1 | 13 ± 1 | 0.32 | 29 ± 3 | 26 ± 3 | 0.52 |
| Lung and trachea | 4.3 ± 0.5 | 6.3 ± 0.8 | 0.05 | 10.5 ± 0.6 | 13.0 ± 0.9 | 0.05 |
| Heart | 2.5 ± 0.3 | 2.4 ± 0.2 | 0.59 | 4.8 ± 0.4 | 4.8 ± 0.4 | 0.89 |
| Esophagus | 0.4 ± 0.1 | 0.4 ± 0.1 | 0.44 | 0.8 ± 0.2 | 0.8 ± 0.2 | 0.65 |
| Reticulo-rumen | 13.7 ± 1.9 | 13.3 ± 2.0 | 0.74 | 26.6 ± 2.9 | 27.1 ± 4.4 | 0.86 |
| Omasum | 4.2 ± 1.3 | 4.3 ± 1.1 | 0.96 | 8.1 ± 2.2 | 8.6 ± 2.3 | 0.74 |
| Abomasum | 2.7 ± 1.4 | 2.2 ± 0.7 | 0.49 | 5.2 ± 2.9 | 4.4 ± 1.4 | 0.59 |
| Small intestine | 6.8 ± 0.9 | 6.2 ± 1.2 | 0.35 | 13.3 ± 1.6 | 12.6 ± 2.3 | 0.56 |
| Large intestine | 6.6 ± 1.3 | 5.2 ± 1.7 | 0.16 | 12.9 ± 2.2 | 10.5 ± 3.4 | 0.22 |
| Kidney | 1.3 ± 0.2 | 1.2 ± 0.1 | 0.19 | 2.5 ± 0.3 | 2.4 ± 0.3 | 0.45 |
| Liver | 8.7 ± 1.0 | 8.6 ± 0.5 | 0.78 | 17.0 ± 1.2 | 17.5 ± 0.5 | 0.43 |
| Pancreas | 0.6 ± 0.2 | 0.4 ± 0.1 | 0.17 | 1.2 ± 0.5 | 0.8 ± 0.2 | 0.19 |
| Spleen | 1.0 ± 0.1 | 1.5 ± 0.9 | 0.31 | 2.1 ± 0.3 | 3.0 ± 1.8 | 0.28 |
| Mesentary | 18.9 ± 4.5 | 19.2 ± 1.4 | 0.91 | 36.9 ± 8.5 | 39.1 ± 3.8 | 0.6 |
| Small intestine length, m | 39.0 ± 1.5 | 39.3 ± 3.0 | 0.84 | | | |

[a] = wt expresses as kg

[b] = wt expressed as g of tissue / kg of EBW

Table 6. Influence of low or moderate prenatal nutrition on carcass characteristics of steers at 580 kg

| | treatment | | |
|---|---|---|---|
| | Low | Moderate | |
| Carcass measurements | n = 5 | n = 5 | *P* value |
| Dressing percent | 60.0 ± 1.7 | 61.7 ± 2.9 | 0.28 |
| Yield grade | 2.5 ± 0.4 | 2.7 ± 0.3 | 0.28 |
| Marbling score | 11.6 ±0.2 | 11.2 ± 0.2 | 0.24 |
| LM area ,$cm^2$ | 86.8 ± 3.1 | 82.3± 6.1 | 0.18 |
| Fat thickness at 12th rib, cm | 1.0 ± 0.3 | 1.0 ± 0.2 | 1.00 |
| KPH, % | 1.3 ± 0.5 | 1.6 ± 0.2 | 0.22 |

Table 7. Concentration of DNA, protein, protein to DNA ratio, dry matter, and lipid of tissues collected from steers at 88 wk of age exposed to moderate or low nutrition from d 32-110 of gestation

| | Treatment | | |
|---|---|---|---|
| | Low n = 5 | Moderate n = 5 | *P* values |
| Muscle | | | |
| DNA, mg/g | 0.58 ± 0.02 | 0.52 ± 0.02 | 0.02 |
| Protein, mg/g | 189.9 ± 3.24 | 190.6 ± 4.02 | 0.89 |
| Protein:DNA | 331 ± 15 | 370 ± 15 | 0.10 |
| DM, % | 21.7 ± 0.8 | 21.3 ± 0.7 | 0.71 |
| Lipid, % | 3.10 ± 1.02 | 1.87 ± 0.54 | 0.32 |
| Heart | | | |
| DNA, mg/g | 0.51 ± 0.03 | 0.49 ± 0.03 | 0.34 |
| DNA in whole organ, g | 1.26 ± 0.91 | 1.16 ± 0.91 | 0.33 |
| Protein, mg/g | 177.7 ± 0.95 | 177.2 ± 2.09 | 0.82 |
| Protein:DNA | 347 ± 27 | 367 ± 27 | 0.69 |
| DM, % | 17.0 ± 0.2 | 18.3 ± 0.7 | 0.04 |
| Lipid, % | 0.53 ± 0.21 | 0.48 ± 0.15 | 0.84 |
| Liver | | | |
| DNA, mg/g | 3.07 ± 0.39 | 3.25 ± 0.39 | 0.52 |
| DNA in whole organ, g | 30.44 ± 2.20 | 27.77 ± 2.20 | 0.31 |
| Protein, mg/g | 197.7 ± 1.92 | 184.0 ± 3.47 | 0.01 |
| Protein:DNA | 53.4 ± 7.0 | 49.3 ± 7.0 | 0.48 |
| DM, % | 24.8 ± 0.7 | 24.4 ± 0.8 | 0.74 |
| Lipid, % | 1.87 ± 0.57 | 1.82 ± 0.44 | 0.95 |
| Kidney | | | |
| DNA, mg/g | 6.20 ± 0.51 | 6.39 ± 0.51 | 0.77 |
| DNA in whole organ, g | 8.05 ± 0.80 | 7.41 ± 0.80 | 0.62 |
| Protein, mg/g | 156.6 ± 1.21 | 154.2 ± 4.01 | 0.59 |
| Protein:DNA | 32.8 ± 2.6 | 29.2 ± 2.6 | 0.39 |
| DM, % | 17.7 ± 0.6 | 16.8 ± 1.3 | 0.55 |
| Lipid, % | 1.47 ± 0.20 | 1.04 ± 0.20 | 0.17 |
| Pancreas | | | |
| DNA, mg/g | 3.79 ± 0.40 | 4.24 ± 0.40 | 0.34 |
| DNA in whole organ, g | 2.41 ± 0.51 | 1.64 ± 0.51 | 0.05 |
| Protein, mg/g | 167.3 ± 4.10 | 159.3 ± 6.22 | 0.31 |
| Protein:DNA | 46.3 ± 5.1 | 39.1 ± 5.1 | 0.29 |
| DM, % | 25.4 ± 5.2 | 26.1 ± 3.0 | 0.91 |
| Lipid, % | 5.72 ± 1.07 | 12.62 ± 3.04 | 0.07 |
| Subcutaneous fat | | | |
| DNA, mg/g | 0.56 ± 0.10 | 0.50 ± 0.10 | 0.72 |
| DM, % | 69.3 ± 5.1 | 72.1 ± 4.5 | 0.69 |
| Lipid, % | 92.5 ± 2.1 | 93.4 ± 1.4 | 0.72 |
| KPH fat | | | |
| DNA, mg/g | 0.38 ± 0.05 | 0.32 ± 0.05 | 0.09 |
| DM, % | 92.8 ± 1.0 | 92.3 ± 0.9 | 0.69 |
| Lipid, % | 99.1 ± 0.3 | 99.4 ± 0.01 | 0.19 |

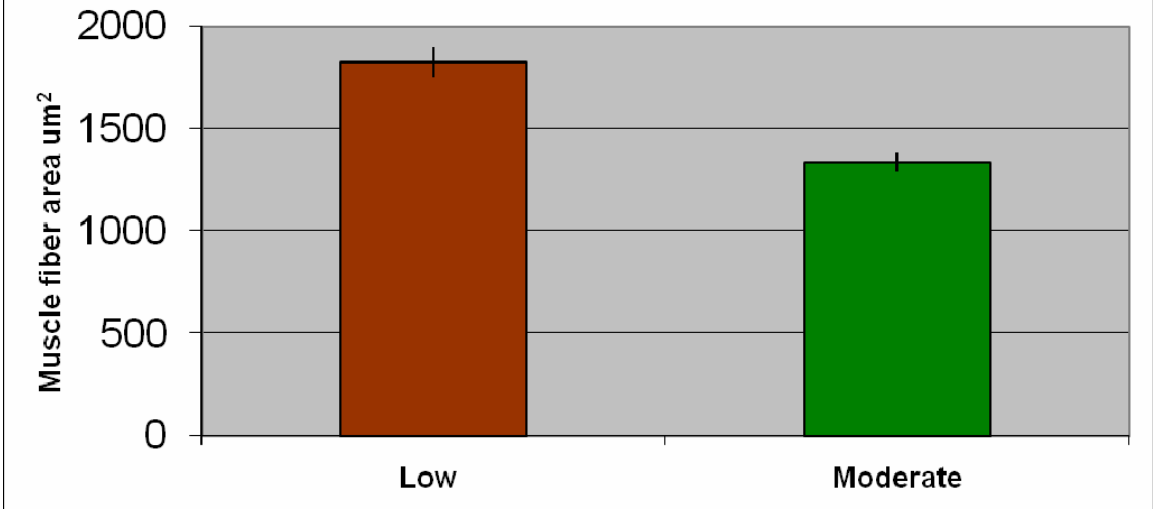
Table 8. Quantitative RT-PCR analysis of gene expression for fatty acid binding protein 4 (AP2), fatty acid translocase (CD36), C/EBP α and GLUT 4 in subcutaneous and pelvic fat from steers at 22 mo of age exposed to either low or moderate prenatal nutrition

| Tissue | Gene | Treatment | n | ΔCt [1] | Fold Difference [2] | P value |
|---|---|---|---|---|---|---|
| KPH | AP2 | Low | 4 | 6.67 ± 0.39 | -5.48 ± 0.39 | 0.0003 |
| | | Mod | 5 | 4.21 ± 0.13 | 1.00 ± 0.13 | |
| | CD36 | Low | 4 | 11.19 ± 0.43 | -3.85 ± 0.43 | 0.006 |
| | | Mod | 5 | 9.55 ± 0.29 | 1.00 ± 0.29 | |
| | C/EBP α | Low | 4 | 18.59 ± 1.27 | -1.88 ± 1.27 | 0.16 |
| | | Mod | 5 | 16.72 ± 0.38 | 1.00 ± 0.38 | |
| | GLUT 4 | Low | 4 | 20.57 ± 0.33 | -2.89 ± 0.33 | 0.009 |
| | | Mod | 5 | 19.04 ± 0.25 | 1.00 ± 0.25 | |
| Subcutaneous Fat | AP2 | Low | 5 | 6.47 ± 0.75 | -1.33 ± 0.75 | 0.69 |
| | | Mod | 4 | 6.05 ± 0.60 | 1.00 ± 0.60 | |
| | CD36 | Low | 5 | 11.65 ± 0.56 | -1.12 ± 0.56 | 0.85 |
| | | Mod | 4 | 11.49 ± 0.65 | 1.00 ± 0.65 | |
| | C/EBP α | Low | 4 | 16.88 ± 0.21 | -1.56 ± 0.21 | 0.60 |
| | | Mod | 4 | 17.10 ± 0.34 | 1.00 ± 0.34 | |
| | GLUT 4 | Low | 5 | 18.94 ± 0.34 | -1.18 ± 0.34 | 0.80 |
| | | Mod | 4 | 18.70 ± 0.98 | 1.00 ± 0.98 | |
| Muscle | GLUT 4 | Low | 4 | 13.30 ± 0.38 | 2.56 ± 0.38 | 0.08 |
| | | Mod | 4 | 14.43 ± 0.38 | 1.00±0.38 | |

[1] ΔCt = Ct for target gene - Ct for normalization control, 18S

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

Figure 1. Area of complexus muscle fibers of 88 wk old steers that were exposed to low or moderate nutrition from d 32 to 115 of gestation (P = 0.04).



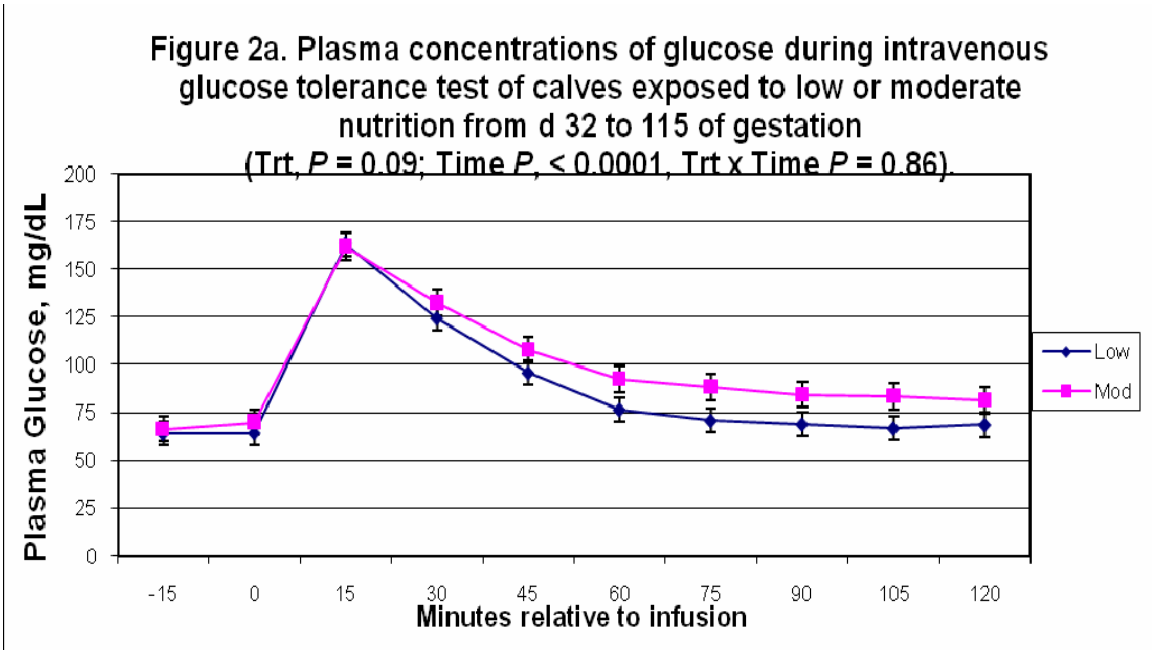


Figure 2a. Plasma concentrations of glucose during intravenous glucose tolerance test of calves exposed to low or moderate nutrition from d 32 to 115 of gestation
(Trt, P = 0.09; Time P, < 0.0001, Trt x Time P = 0.86).
Plasma Glucose, mg/dL
200
175
150
125
100
75
50
25
0
-15
0
15
30
45
60
75
90
105
120
Minutes relative to infusion
Low
Mod

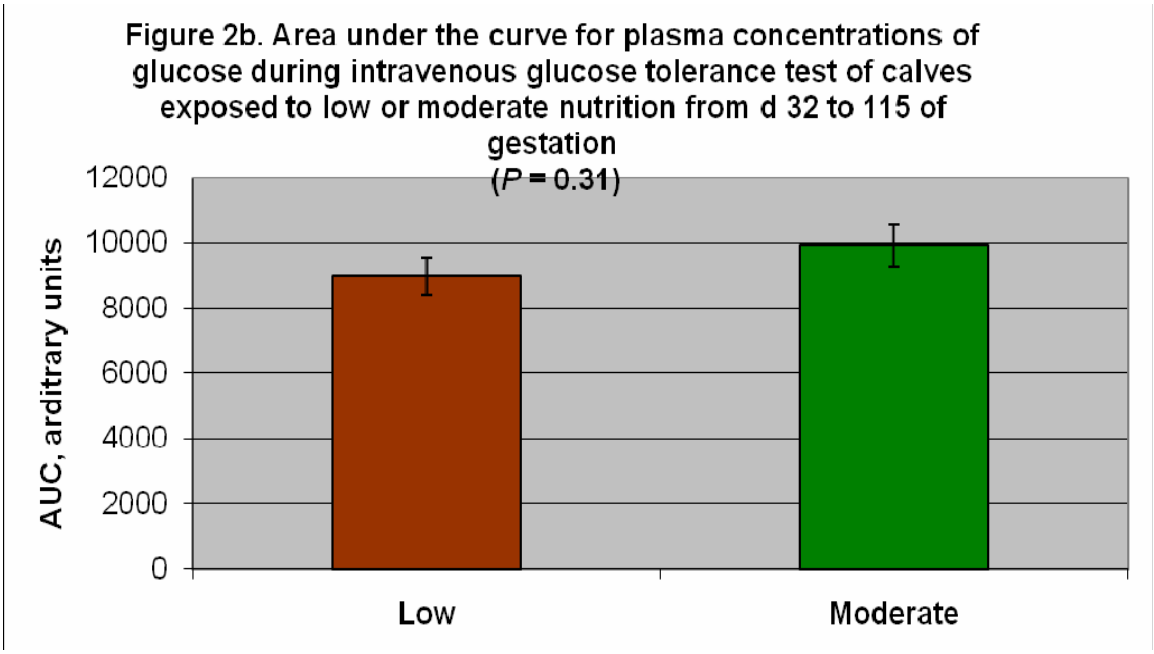


Figure 2b. Area under the curve for plasma concentrations of glucose during intravenous glucose tolerance test of calves exposed to low or moderate nutrition from d 32 to 115 of gestation
(P = 0.31)
AUC, arditrary units
12000
10000
8000
6000
4000
2000
0
Low
Moderate

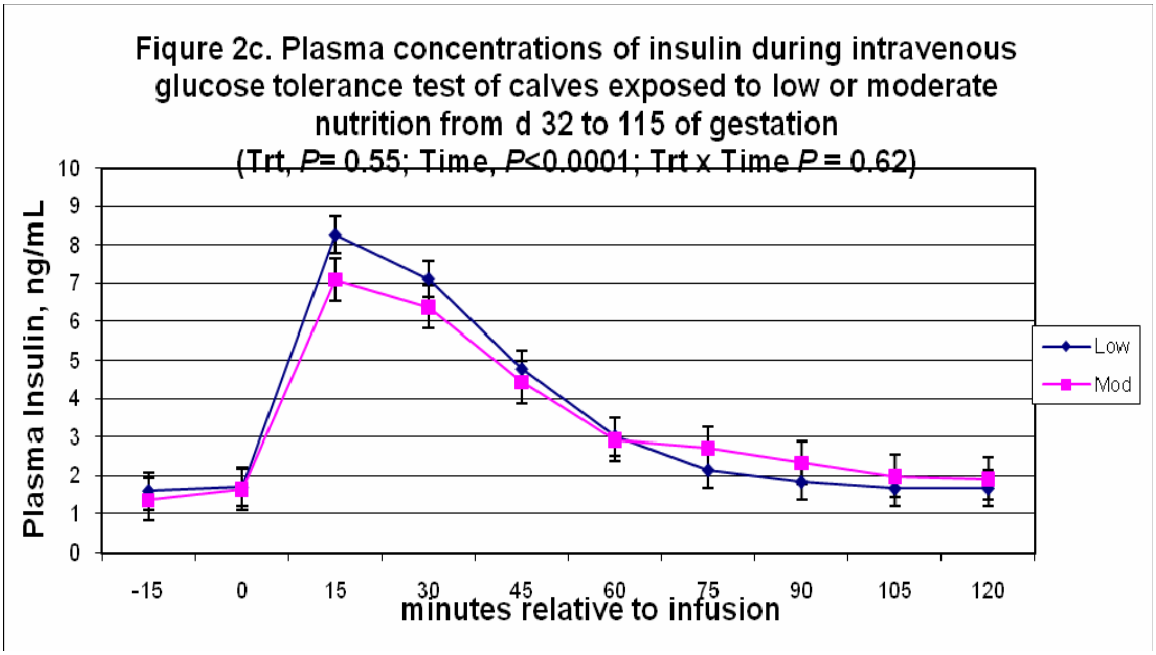
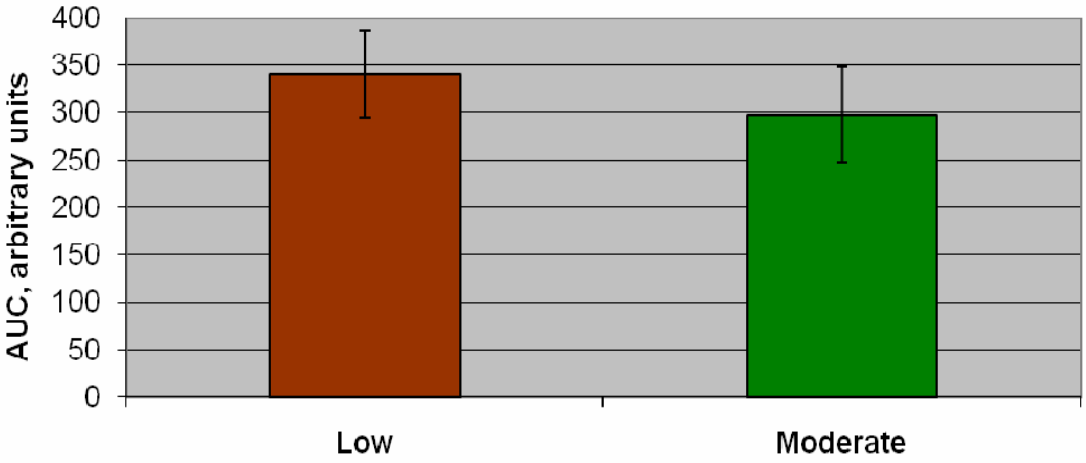
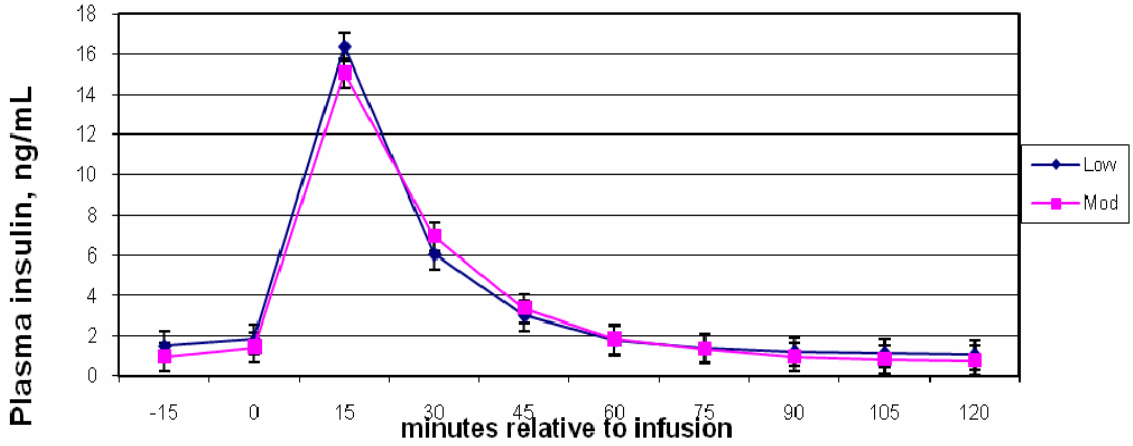

Fiqure 2c. Plasma concentrations of insulin during intravenous glucose tolerance test of calves exposed to low or moderate nutrition from d 32 to 115 of gestation
(Trt, P= 0.55; Time, P<0.0001; Trt x Time P = 0.62)
Plasma Insulin, ng/mL
0
1
2
3
4
5
6
7
8
9
10
-15
0
15
30
45
60
75
90
105
120
minutes relative to infusion
Low
Mod

Figure 2d. Area under the curve for plasma concentrations of insulin during intravenous glucose tolerance test of calves exposed to low or moderate nutrition from d 32 to 115 of gestation ($P = 0.43$)



Fiqure 3a. Plasma concentrations of insulin during intervenous insulin challenge of calves exposed to low or moderate nutrition from d 32 to 115 of gestation
(Trt, *P* = 0.55; Time, *P* <0.0001; Trt x Time *P* = 0.45)



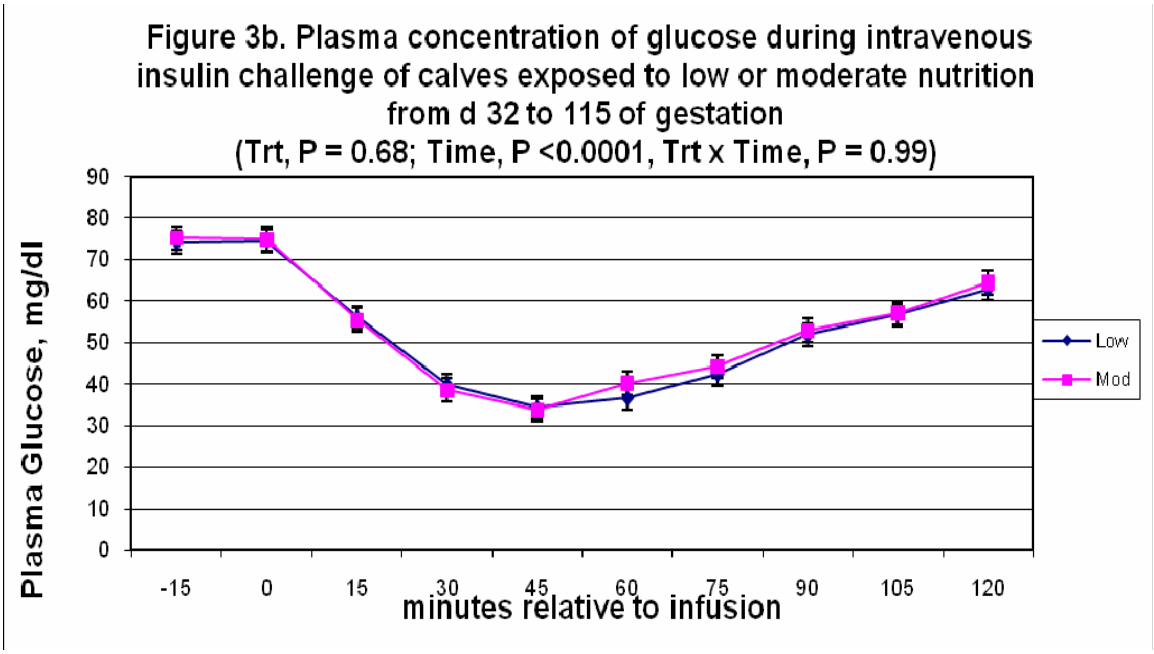
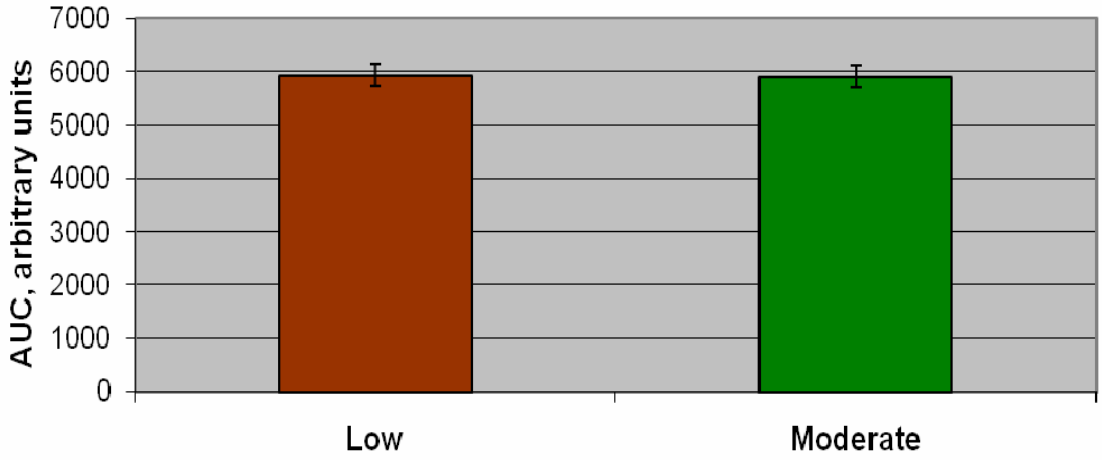


Figure 3b. Plasma concentration of glucose during intravenous insulin challenge of calves exposed to low or moderate nutrition from d 32 to 115 of gestation
(Trt, P = 0.68; Time, P <0.0001, Trt x Time, P = 0.99)
Plasma Glucose, mg/dl
90
80
70
60
50
40
30
20
10
0
-15
0
15
30
45
60
75
90
105
120
minutes relative to infusion
Low
Mod

Figure 3c. Area under the curve for plasma concentration of glucose during insulin challenge of calves exposed to low or moderate nutrition from d 32 to 115 of gestation (*P* = 0.92)



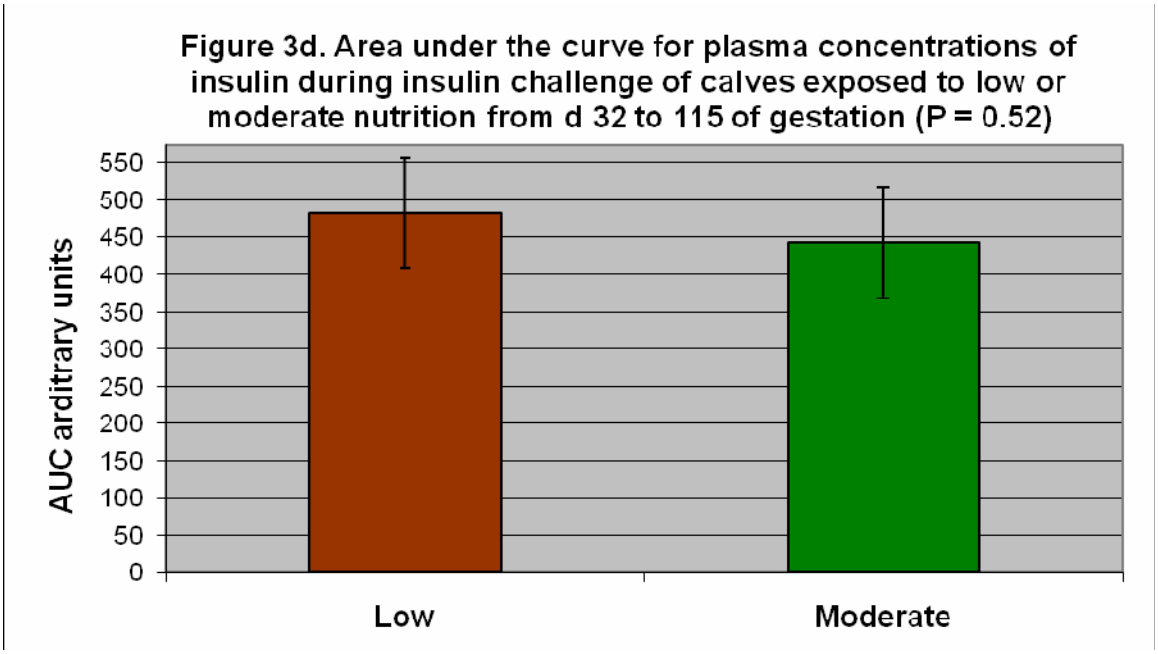


Figure 3d. Area under the curve for plasma concentrations of insulin during insulin challenge of calves exposed to low or moderate nutrition from d 32 to 115 of gestation (P = 0.52)
AUC arditrary units
550
500
450
400
350
300
250
200
150
100
50
0
Low
Moderate

# CHAPTER V

# CONCLUSION

Treatment of suckled postpartum anestrous beef cows with estradiol cypionate at either 25 or 50 d postpartum increased the percentage of cows in estrus. However, the percentage of cows with luteal activity by 10 d after treatment with estradiol was not affected by treatment. A greater percentage of cows that calved with a BCS $\geq$ 5 had luteal activity within 10 d of treatment with estrogen and were in estrus longer than cows that calved with a lower BCS. Cows that calved with a greater BCS had a shorter interval from calving to resumption of normal luteal activity. Treatment of anestrous beef cows with estradiol cypionate increased the days from parturition to normal luteal activity. Treatment of postpartum anestrous cow with estradiol resulted in estrus behavior without subsequent luteal activity. This indicates that within the hypothalamus estrogen is not able to stimulate a surge of GnRH that will produce the preovulatory LH surge. This malfunction of the hypothalamus may be partially responsible for an extended interval from calving to first ovulation.

Maternal nutrient restriction (55% of NRC recommendations for energy) from d 32 to 115 of gestation decreased maternal BW and BCS in heifers. Birth weight of calves was not influenced by nutritional treatment in either yr of a 2-yr experiment. Gestation length was reduced by 4 d in yr 1 but was not effected by nutritional treatment in yr 2. Weaning weights, postweaning weight, and ADG from birth to weaning or during the

postweaning period were not influenced by prenatal nutritional treatment in both yr 1and yr 2. Low steers were heavier when started in the feedlot and tended to be heavier at harvest compared with M steers; however, ADG during the feeding period was not affected by prenatal nutrient intake of steers.

Hot carcass and empty body weight were similar for L and M steers when harvested at 88 wk of age with 1 cm of fat thickness at the 12$^{th}$ rib. Lungs and trachea of L steers were lighter then lungs and trachea of M steers. All other organs measured were similar for L and M steers. Carcass characteristics were not influenced by prenatal nutritional treatment. The complexus muscle from L steers had a greater concentration of DNA and the DNA to protein ratio tended to be less compared with M steers. Total DNA in the pancreas was greater in L steers compared with M steers. There tended to be more DNA in KPH fat from L steers then M steers. Muscle fiber area of the complexus was greater in steers exposed to L nutrition from d 32 to 115 of gestation compared with steers exposed to adequate nutrition. Abundance of mRNA for fatty acid binding protein 4, fatty acid translocase, C/EBP α and GLUT 4 in subcutaneous fat was not influenced by nutritional treatment. However, abundance of fatty acid binding protein 4, fatty acid translocase and GLUT 4 were decreased in KPH of L steers compared with M steers

Low and M calves had similar area under the curve for plasma glucose and insulin during intravenous glucose tolerance test (IVGTT). However L calves had a greater clearance rate of glucose from plasma compared with M calves. This was supported by the tendency for M calves to have greater concentrations of glucose in plasma during after IVGTT. Concentrations of insulin in plasma during IVGTT were similar for L and M calves and area under the curve for plasma insulin concentration were not influenced

by prenatal treatment. The decrease in plasma glucose after i.v. administration of bovine insulin was similar for L and M calves. The increase in plasma insulin following i.v. administration of insulin was not affected by prenatal nutritional treatment. The area under the curve for plasma glucose and insulin during i.v. insulin administration were similar for L and M calves.

Nutritional restriction during the first trimester of pregnancy altered muscle formation as indicated by increased muscle fiber area, greater concentrations of DNA, and reduced DNA to protein ratio in the adult animal. Kidney, pelvic, and heart fat gene expression was altered in L steers compared with M steers indicating possible metabolic changes in the KPH fat. However, growth and carcass composition at 22 mo of age was not influenced by prenatal nutrition, indicating the full effects of maternal undernutrition from d 32 to 115 of gestation may not be observed until later in life. This is important for producers that calve during the fall. In Oklahoma fall calving cows are rebreed beginning in December. After breeding cows are allowed to lose BCS and BW until the emergence of adequate pasture in the spring. This reduction in BCS and BW may effect the calves growth and final carcass composition depending on postnatal management and environment.

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VITA

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Title of Study: THE EFFECTS OF NUTRIENT INTAKE DURING GESTATION ON ESTRUS OF BEEF COWS AND POSTNATAL GROWTH AND DEVELOPMENT OF CALVES

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Major Field: Animal Breeding and Reproduction

Scope and Method of Study: Effects of treatment of anestrous beef cows with estradiol cypionate at 25 of 50 d after calving on onset of estrus and luteal activity was evaluated in cows that calved with a BCS $< 5$ or $\geq 5$. Effects of nutrient restriction from d 32 to 115 of gestation on the growth, carcass characteristics, and organ composition of calves were evaluated over 2 yr. Heifers (15 mo of age) were AI to a common sire, confirmed pregnant with transrectal ultrasonography stratified by BW and BCS and allotted to either low (L; 56 % of NRC recommended NEm) or moderate (M; fed in excess of NRC requirements) nutritional treatment. Birth weight, weaning weight and postweaning growth of calves were recorded. Feedlot growth, carcass composition and organ composition were determined for L and M steers in yr 1. In yr 2 plasma glucose regulation was evaluated using intravenous glucose tolerance test and also response to intravenous injection of bovine insulin.

Findings and Conclusions: Treatment of suckled postpartum anestrous beef cows with estradiol at either 25 or 50 d postpartum increased the percentage of cows in estrus, however, the percentage of cows with luteal activity by 10 d after treatment was not influenced. Cows that calved with a greater BCS have a shorter period from calving to resumption of normal luteal activity. Treatment of anestrous beef cows with estradiol cypionate increased the days from parturition to normal luteal activity. Maternal nutrient restriction from d 32 to 115 of gestation decreased maternal BW and BCS of heifers. Gestation length was reduced in yr 1 but was not effected by nutritional treatment in yr 2. Weaning weights, postweaning weights, and ADG were not influenced by prenatal nutritional treatment. Lungs and trachea of L steers were lighter then the lungs and trachea of M steers. The complexus muscle from L steers had increased concentration of DNA per gram of muscle, the DNA to protein ratio tended to be reduced and increased muscle fiber area was increased compared with muscle from M steers. Abundance of fatty acid binding protein 4, fatty acid translocase and GLUT 4 mRNA were decreased in kidney pelvic fat of L steers compared with KPH of M steers. Low and M calves responded similar to intravenous glucose tolerance test. Nutrient restriction during early gestation changes muscle development and metabolism of KPH in steers at 22 mo of age.

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