Beef cattle production: Understanding the effect of heifer development system, late gestation protein supplementation, and ovarian steroidogenic environment on productivity, reproduction, and longevity

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BEEF CATTLE PRODUCTION: UNDERSTANDING THE EFFECT OF HEIFER DEVELOPMENT SYSTEM, LATE GESTATION PROTEIN SUPPLEMENTATION, AND OVARIAN STEROIDOGENIC ENVIRONMENT ON PRODUCTIVITY, REPRODUCTION, AND LONGEVITY

by

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BEEF CATTLE PRODUCTION: UNDERSTANDING THE EFFECT OF HEIFER DEVELOPMENT SYSTEM, LATE GESTATION PROTEIN SUPPLEMENTATION, AND OVARIAN STEROIDOGENIC ENVIRONMENT ON PRODUCTIVITY, REPRODUCTION, AND LONGEVITY

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Beef cattle longevity is related to reproductive success, which can be influenced by development system and maternal environment. Three experiments were conducted to determine the impact of development system, maternal protein supplementation, and ovarian steroidogenic environment on beef cattle reproduction. Experiment 1 evaluated the effect of heifer development system on heifer performance and reproduction. Heifers developed on corn residue (CR) had reduced BW from prebreeding through pregnancy diagnosis compared with drylot (DL) developed heifers, but there was no difference in reproductive performance. A subset DL and CR heifers were placed in an individual feeding system to determine feed efficiency during late gestation. Drylot heifers tended to have increased final BW and ADG. In Exp. 2, primiparous heifers were individually fed grass hay and received either no supplement (CON), a distillers based supplement (HIGH), or a dried corn gluten feed (LOW) based supplement during late gestation. Weaning calf BW tended to be greater for steers born to HIGH supplemented dams compared with CON. Similarly, carcass characteristics were altered in calves based on maternal nutrition. The objective of Exp. 3 was to identify the effect of ovarian and
follicular environment on oocyte molecular phenotype. Ovariectomy was performed on beef cows approximately 36 h after CIDR removal and PGF administration. Cows were classified as high (HGE) or low (LGE) based on granulosa cell efficiency. Cows classified LGE had increased androgen secretion and expression of steroidogenic pathway enzymes in theca cells. Furthermore, mRNA abundance of ZAR1 was reduced in LGE oocytes. In summary, these experiments support the following findings: (1) developing heifers on CR does not reduce reproductive performance or impact late gestation feed efficiency compared with DL-developed heifers; (2) primiparous heifer protein supplementation improved steer calf weaning BW and altered progeny carcass characteristics; (3) increased androgen production in LGE animals increases steroidogenic enzyme gene expression and/or mRNA stability during oocyte growth and maturation.
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CHAPTER I: Review of the literature

INTRODUCTION

There are several characteristics reported that make the “ideal” beef cow. First, she calves at 2 yr of age, doesn’t require human intervention to calve or assistance in nursing her calf, maintains a 365-d calving interval and weans a marketable calf each yr. Furthermore, this animal must remain structurally sound, be able to graze the forages provided in her area and be tolerant of environmental stressors and disease (Hohenboken, 1988). Profitability of beef cattle producers is tied directly to the productive life span of mature cows. Heifer development costs are recovered through subsequent calf crops. Reproductive failure represents a major reason females leave the herd, impacting producers’ ability to recoup heifer development costs. Nutrition plays a major role in all aspects of beef cattle productivity. Furthermore, it is suggested the fetus is rarely able to completely express its full genetic potential for growth owing to insults caused by maternal environment (Gluckman and Liggins, 1984).

The main factors influencing nutrient partitioning between the dam and fetus include age of the dam, number of fetuses, production demand, and environmental stress (Reynolds et al., 2010). These factors play a role in programming the fetus for its future environment and available resources. Also, fetal programming reportedly impacts neonatal mortality and morbidity, postnatal growth rate, body composition, health, and reproduction (Wu et al., 2006).
EPIGENETIC MODIFICATIONS

Epigenetics is defined as heritable changes in gene expression resulting from alterations in chromatin structure but not in DNA sequence. Three main mechanisms cause epigenetic changes to the genome: DNA methylation, histone modification, and noncoding micro-RNAs (Canini et al., 2011). These processes regulate both the intensity and timing of gene expression during cell differentiation (Zeisel, 2009; McKay and Mathers, 2011). Current understanding of these genomic modifications has led to the hypothesis that epigenetics is a key mechanism allowing for phenotypic plasticity with regard to a fixed genotype (Mathers and McKay, 2009).

Human epidemiological studies report associations between low birth BW and adult disease. Researchers propose a fetal programming mechanism occurs in which environment stimuli in utero impact fetal growth and health, not only during gestation but also later in life (Barker, 1995; 1999). Animal models that report intrauterine growth retardation due to maternal undernutrition indicate altered organ and tissue development in utero (Vonnahme et al., 2003; Ford et al., 2009). These studies suggest modification of the growing fetus to allow environmental adaptation. Epigenetic modifications can result from internal as well as external stimuli (Mathers and McKay, 2009), thus allowing gene expression in the fetus to best fit with environmental stimulation.

To help explain the main events and processes linking dietary exposures to epigenetic marks and, later, health outcomes, Mathers & McKay (2009) developed the four Rs of nutritional epigenomics (Figure 1). From this model, we learn nutrition stimuli and other exposures are (a) received and (b) recorded by the genome. Exposures are also
(c) remembered across successive cell generations and, finally, (d) revealed in altered gene expression, cell function, and overall health (Mathers and McKay, 2009).

**DNA methylation**

Most mammalian DNA, including exons, intergenic DNA, and transposons, is methylated. Methylation sites are located at cytosine bases followed by a guanosine (CpG) (Holliday, 1993). Although most CpG sites are methylated, specific CpG-rich areas of the genome, known as CpG islands, are not methylated (Figure 2). These regions span the 5’ end of the regulatory region of a gene (Ziesel, 2009). The pattern of CpG island DNA methylation varies based on tissue type, and this variation likely results in the differing expression of genes in various tissues (Suzuki and Bird, 2008).

**Figure 1.** The four ‘Rs’ of nutritional epigenomics. Environmental (i.e., maternal nutrition) factors occurring at select times of gestation can result in alterations of the genome that are recorded, remembered, and revealed (adapted from Maters and McKay 2009).
Figure 2. Epigenetic marks alter gene expression. Normally transcription factors (TF) bind to the promoter regions of DNA and induce gene expression producing messenger RNA (mRNA). However, when specific CpG islands in the promoter are methylated, capping proteins that prevent access of the transcription factor to DNA are attracted and gene expression is repressed (adapted from Zeisel, 2009).

The DNA methyltransferase (Dnmt) family of enzymes plays an important role in DNA methylation and, ultimately, embryonic development and survival. DNA methyltransferase1, Dnmt3a, and Dnmt3b catalyze cytosine methylation. Furthermore, Dnmt3a and Dnmt3b can establish methylation patterns on unmodified DNA, whereas Dnmt1 maintains these patterns (Cheng and Blumenthal, 2008) when DNA is duplicated prior to cell division. Dnmt-null mice die in early gestation (Lei et al., 1996), and methyltransferase mutations can cause not only abnormal fetal growth but also immunodeficiency and brain abnormalities in humans (Clouaire and Stancheva, 2008).

Methyltransferases utilize S-adenosylmethionine (SAM) as a methyl donor, and SAM can be directly influenced by diet. Methyl donors for SAM include choline, methionine, and methyl-tetrahydrofolate, which are related metabolically at the point homocystine is converted to methionine (Ziesel, 2009). Owing to the close relation and interaction among pathways, if the metabolism of any of these methyl donors is altered, compensatory changes will occur in the other methyl donors (Figure 3). Offspring of
pregnant mice fed choline- and methionine-deficient diets reportedly had diminished methylation in CpG islands of genes controlling brain development (Niculesca et al., 2006). Additionally, if diets are choline deficient in late pregnancy, offspring have decreased methylation of genes inhibiting cell cycling, which results in decreased progenitor cell proliferation, increased apoptosis in the fetal hippocampus, and reduced visuospatial and auditory memory (Williams et al., 1998; Albright et al., 1999; Niculescu et al., 2004).

A metabolite of one-carbon metabolism, SAM acts as a methyl donor for methylation reactions, whereas S-adenosylhomocysteine (SAH) acts as a product inhibitor for methyltransferases in DNA and histone methylation. Thus, alterations of folate, vitamin B12, methionine, choline, and betaine through nutrition can alter one-carbon metabolism and disrupt the availability of methyl donor groups (Choi and Friso, 2010). Restriction of folate, vitamin B12, and methionine from the periconceptional diet of ewes resulted in offspring that were obese as adults and had impaired immune function (Sinclair, 2007). Furthermore, studies in rats indicate DNA methylation of the liver increased 14% for individuals provided a low-folate diet from weaning to puberty (Kotsopoulos et al., 2008). These reports indicate reducing dietary methyl nutrients may alter methylation patterns and potentially increase disease susceptibility later in life.
Figure 3. Summary diagram of the main pathways involved in cellular one-carbon metabolism including the production of S-adenosylmethionine (SAM) for DNA methylation. Nutrients and other dietary components that have the potential to modulate epigenetic marks through altering SAM production are illustrated in red (adapted from McKay and Mathers, 2011).

**Histone modifications**

Extensive wrapping of the DNA during packaging occurs in the nucleus, forming chromatin. The fundamental unit of chromatin is the nucleosome which consists of an octamer of the four core histones (H3, H4, H2A, H2B), wrapped with 147 base pairs of DNA (Kouzarides, 2007). Histone tails protrude from the globular core and allow for further epigenetic modification via posttranslational modifications of specific amino acid residues (Kouzarides, 2007; McKay and Mathers, 2011). Histones can undergo more than 100 distinct posttranslational modifications (Kouzarides, 2007). For example, similar to
DNA, histones can be modified by methylation. However, unlike DNA, histones can also be modified through several types of N-terminal tail modifications, including acetylation, phosphorylation, biotinylation, ubiquitination, and ADP ribosylation (Canini et al., 2011) and these modifications can lead to further compaction of DNA and alter access of transcription factors (Faulk and Dolinoy, 2011). DNA methyltransferase-1 and Dnmt3a reportedly can also act with histone deacetylases to repress transcription (Burgers, et al., 2002).

Protein restriction in maternal diets has resulted in reduced DNA methylation and histone modification in rat offspring as both juveniles and adults (Lillycrop et al., 2007; 2008). However, high-protein maternal diets during gestation and lactation result in sex-specific differences in progeny. Male rat pups from mothers fed a high-protein diet had higher blood pressure compared with male pups from control-fed rats. Female pups born to high–protein intake rats had greater fat pad and body mass compared with females from control-fed rats (Thone-Reineke et al., 2006).

**MicroRNAs and epigenetics**

Hundreds of microRNA (miRNA), usually 22 nucleotides in length, are encoded in the genome and can impact gene expression. MicroRNA bind to target mRNA and impact stability and/or translation; miRNA binding with complete complementarity can lead to degradation of the target mRNA (Esquela-Kerscher and Slack, 2006; Chaung and Jones, 2007). After transcription and packaging, mature miRNA are able to regulate gene expression at the posttranscriptional level (Iori et al., 2010). MicroRNA bind through partial complementarity to the 3’ untranslated region of target mRNA and disrupt translation through either mRNA degradation or translation inhibition (Iori et al., 2010).
Owing to its ability to regulate gene expression, miRNA is involved in many cellular processes, including development, differentiation, and metabolism (Iori et al., 2010).

MicroRNA can play an important role in regulating epigenetic changes (Choi and Friso, 2010). Furthermore, miRNA reportedly control DNA methylation and histone modifications; promoter methylation and histone modification also affect miRNA expression (Choi and Friso, 2010). Much understanding of miRNA functions has been determined through cancer research. Abnormal expression of miRNA can alter cell proliferation and apoptosis, which leads to progression of cancer in humans (Iori et al., 2010).

**Intergenerational effects of fetal programming**

Programming of the genome can have lasting effects on future generations through intergenerational influences, described as factors, conditions, exposures, and environments in one generation that impact the health, growth, and development of subsequent generations (Emanuel, 1986). Drake & Walker (2004) suggested three possible explanations for the occurrence of intergenerational effects: genetic attributes may manifest themselves similarly in mother and offspring; adverse extrinsic environmental conditions may persist across generations; and adverse in utero experiences may permanently affect maternal growth and development, altering the mother’s metabolism in a way that provides an adverse environment for her fetus (Drake and Walker, 2004). In a recent review, Ford and Long (2012) report data from human, rat, and sheep studies indicating an intergenerational effect of maternal nutrition on offspring. Zamenhof et al. (1971) reported reduced birth BW in rats born two generations after protein restriction. In addition, Susser & Stein (1994) reported women
undernourished during late pregnancy had babies with reduced birth BW, who subsequently had lower birth BW babies in the next generation.

Stewart et al. (1980a) reported rats maintained on a control diet for 12 generations had greater first-generation birth BW compared with individuals born to rats fed a diet marginally deficient in protein. Epigenetic adaptations also occurred approximately halfway through the experiment when a more unpalatable diet was supplied accidentally to rats on both diets. The subsequent generation of rats had more reduced birth BW for the protein-deficient colony as well as a slight reduction in birth BW for pups born in the control colony. Following readministration of a more palatable diet, birth BW in the control-born pups increased, but colony birth BW did not return to the baseline level for approximately three generations despite adequate maternal nutrition (Stewart et al., 1980a).

**IMPACT OF EARLY GESTATIONAL NUTRITION**

Robinson et al. (1977) reported 75% of ruminant fetal growth occurs during the last two months of gestation (Figure 4). Owing to the minimal nutrient requirement during early gestation, inadequate nutrition during this time was thought to have little significance. However, Rhind et al. (1989) indicated maternal undernutrition impacted the conceptus as early as gestation d 11 in sheep, prior to implantation; the conceptus from ewes fed 50% maintenance requirement was nearly one-third the size of the conceptus from ewes fed 1.5 times their nutrient requirements. During the early phase of fetal development, critical events for normal conceptus development occur, including differentiation, vascularization, fetal organogenesis, and placental development (Funston et al., 2010a).
Maternal nutrition and placental development

The ability to alter placental development in domesticated livestock through maternal nutrition has been reviewed (Redmer et al., 2004; Reynolds et al., 2006, 2010; Vonnahme and Lemley, 2012). Establishment of functional uteroplacental and fetal circulation is one of the earliest events of embryonic and placental development (Patten, 1964; Ramsey 1982). This allows for transportation of all respiratory gas, nutrient, and waste exchanges between the maternal and fetal systems (Reynolds and Redmer, 1995, 2001). In the ruminant this involves the development of the placentome, the physiological exchange site between the mother and fetus. The placentome is comprised of two components- cotyledons (fetal portion) and caruncles (maternal portion), which are located on the uterine wall and bind together to allow nutrient exchange. Nutrient transport efficiency through the placentome is related to uteroplacental blood flow (Reynolds and Redmer, 1995). Under normal conditions, placental weights and fetal weights are highly correlated; furthermore, fetal weight is correlated to mortality (Moule, 1956). Reports indicating reduced fetal growth rates also suggest reduced placental blood flow and nutrient uptake (Wootton et al., 1977; Reynolds et al., 1990). Placental growth occurs throughout gestation, though the greatest amount of growth occurs during the first two-thirds of gestation (Reynolds et al., 1990). Although placental growth slows during late gestation (Figure 4), blood flow to the placenta increases 4.5-fold during the last half of gestation to support the exponential rate of fetal growth occurring at the same time (Reynolds et al., 1986; Reynolds and Redmer, 2001; Reynolds et al., 2006).
Figure 4. Sheep conceptus growth and development during gestation and periods of specific organ development that can be influenced by maternal nutrition. Placental growth occurs rapidly during the first half of gestation while fetal growth is minimal through the first half of gestation, and rapid during the final 2 months of gestation. CV = cardiovascular system (adapted from Symonds et al., 2007).

Restricting beef cow nutrient intake for 90 d during early to mid-gestation results in altered placental vascularity as well as function (Vonnahme et al., 2004a, 2004b). Zhu et al. (2007) reported nutrient restriction (NR) of beef cows from d 30–125 of gestation resulted in reduced \( P < 0.05 \) caruncular and cotyledonary weights compared with control cows. Fetal weights from NR cows also tended to be reduced \( P = 0.12 \) compared with control cows. Following realimentation during d 125–250 of gestation, caruncular and cotyledonary weights in NR cows were still reduced; however, fetal weight was not different. Vonnahme et al. (2007), using the same cows, reported increased placental angiogenesis as well as angiogenic factor mRNA abundance in the caruncular and cotyledonary tissues at the end of the NR period. Capillary vascularity from d 30 to d 125 of gestation was not different; however, from d 125–250, significant
differences existed when comparing control (CON) and NR cows, which suggests capillary areas, numbers, and surface densities had been hindered upon realimentation (Vonnahme et al., 2007).

In a recent review, Vonnahme & Lemley (2012) reported providing protein supplementation to cows beginning on d 190 of gestation resulted in a doubling of uterine blood flow when compared with non-supplemented cows. It is hypothesized that increased uterine blood flow may increase progeny performance, discussed later in this review, owing to increased nutrient transfer to the fetus. Sullivan et al. (2009a) fed composite beef heifers in a 2×2 factorial design in which heifers were assigned a high– or low–protein and dietary energy diet during the first trimester of gestation. During the second trimester of gestation, half of each treatment group was offered the opposite diet, leading to four treatment groups: high/high (HH), high/low (HL), low/high (LowH), and low/low (LL). Cotyledonary weight was dependent upon both first- and second-trimester diets; pregnant heifers fed the HH diet had the most cotyledons present, and heifers fed the LL diet had the fewest cotyledons in the expelled placenta. Caruncles are present in the bovine uterus as early as month 4 of gestation, and thus the number of caruncles may be determined prior to birth (Atkinson et al., 1984). Interestingly, altering maternal dietary protein level during the first or second trimester did not influence the number of caruncles present in non-pregnant adult progeny uterus (Sullivan et al., 2009a). This would suggest maternal NR impacts the number of cotyledons developed on the placenta and available to bind to the maternal caruncle.
Maternal nutrition and fetal organ development

Fetal organ formation occurs simultaneously with placental development, and onset of limb development occurs as early as d 25 of gestation. Following limb development is the sequential development of organs, including the pancreas, liver, adrenal glands, lungs, thyroid, spleen, brain, thymus, and kidneys (Hubbert et al., 1972). Testicle development begins by d 45 in male calves, and by d 50–60, ovarian development begins in female calves. Maternal nutrient status can impact fetal organ development. Long et al. (2009) reported enlarged hearts and brains in fetuses from NR cows from d 30–125 of gestation compared with fetuses from control cows. However, after realimentation of NR cows to achieve similar weight and body condition score (BCS) to those of control cows by d 220 of gestation, brain or heart weights did not differ among groups by d 245 of gestation. Meyer et al. (2010) reported no differences in fetal visceral organ weights at d 125 of gestation, using the same fetuses reported by Long et al. (2009). However, at d 245 of gestation, fetuses from NR cows had greater reticular mass and total intestinal vascularity compared with fetuses from control cows (Meyer et al., 2010). Other organs impacted by maternal nutrition include liver (Da Silva et al., 2002), lung (Gnanalingham et al., 2005), pancreas (Limesand et al., 2006), kidney (Gilbert et al., 2007), perirenal fat (McMillin et al., 2004; Matsuaki et al., 2006), and small intestine (Greenwood and Bell, 2003).

Primordial follicle assembly

Cow longevity is related to the ability to annually produce a live calf. Subsequently, reproductive failure (inability to maintain pregnancy) is one of the main reasons cows leave the production herd (Renquist et al., 2006; Cushman et al., 2009). Furthermore, fertility is closely related to ovarian characteristics (Sullivan et al., 2009b).
Follicle assembly is the developmental process by which individual oocytes develop from oocyte nests and assemble into primordial follicles (Figure 5). Although this process has been a topic of interest to scientists for more than a century, much of the complex processes involved are poorly understood (Juengel et al., 2002). Follicle assembly results from groups or ‘nests’ of oocytes, after completing mitotic proliferation and entering into meiosis, separating to form individual primordial follicles (Pepling, 2006; Nilsson and Skinner, 2009). Each species undergoes the transition from oocyte nest to assembled primordial follicle at different stages with rodents undergoing the transition shortly after birth whereas other mammals such as cattle (Sakai, 1955; Tanaka et al., 2001), sheep (Mauleon, 1974; Sawyer et al., 2002), and humans (Hirshfield, 1991) undergo this transition during fetal development. Primordial follicle assembly is initiated in cattle around gestational d 80 and most primordial follicles are formed by d 143 (Nilsson and Skinner, 2009).

Primordial follicle numbers are highly variable at birth in cattle (Erikson, 1966a) ranging from an estimated 14,000 to 250,000 (Erikson, 1966a; Erickson, 1966b). Interestingly, over the course of the cow’s lifetime, nearly 99% of all follicles within the ovarian reserve will become atretic (Ireland, 1987). While the majority of the ovarian reserve is made up of primary follicles, the number of different follicle types varies greatly among individuals. It is unclear by what mechanisms high ovarian reserve impacts ovarian function, oocyte quality, and fertility (Ireland et al., 2009). Furthermore, ovarian reserve can be correlated to birth BW and altered by maternal nutritional status (Da Silva et al., 2002).
Figure 5. Breakdown of germ cell nests and primordial follicle assembly. Prior to d 80 of gestation cow germ cells are found in cell nests. Activation of primordial follicle assembly results in increased oocyte nest breakdown and primordial follicle development (adapted from Barnett et al., 2006).

Primordial follicles remain arrested at the diplotene stage of the first meiotic division until hormonal regulation stimulates the primordial to primary follicle transition (Nilsson and Skinner, 2009). Transition of follicles to the primary stage is an important process allowing follicle growth and ovulation; however, it is also irreversible. Follicles stimulated to the primary stage will either proliferate and ovulate, or undergo atresia (Kezele and Skinner, 2003).

In studying the regulation of primordial follicle assembly in rats, Kezele and Skinner (2003) reported little breakdown of oocyte nests to follicles at birth, as expected. However, after 6 d in vivo numbers of primordial follicles rose 75% and the number of primary follicles increased to 25% of all follicles within 3 d of postnatal development. In vitro and in vivo results were similar with regard to primordial follicle assembly; however, after a 6 d culture period, 75% of the follicles had transitioned to the primary
follicle stage while only 25% remained as primordial follicles. Increased levels of primordial to primary follicle transition were attributed to the absence of estrogen and progesterone in the *in vitro* culture, inhibiting follicle development.

When neonatal rat pup ovaries were cultured in the presence of estrogen only, progesterone only, or both estrogen and progesterone, there was a 20% increase in the number of unassembled oocytes in both the progesterone only, and estrogen and progesterone treated ovaries, indicating an inhibitory effect of progesterone on primordial follicle assembly. However, all three treatments decreased primordial to primary follicle transition (Kezele and Skinner, 2003) and oocyte apoptosis decreased during follicle assembly when progesterone is present (Chen et al. 2009, Kezele and Skinner 2003).

Due to the inhibition of female sex steroids on follicle assembly in rats and mice, follicle assembly does not occur until after birth. These results suggest an endocrine model of follicle assembly (Kezele and Skinner, 2003). The proponents of this model are that exposure to high maternal progesterone during late gestation inhibits embryo follicle assembly until right after birth at which time progesterone concentration is dramatically reduced and follicle assembly commences.

Follicle stimulating hormone (FSH) is important for folliculogenesis, allowing for growth and differentiation of antral follicles during follicular waves (Ireland et al., 2009). However, concentration of FSH has a negative relationship with the number of healthy follicles and oocytes reported during follicular waves (Singh et al., 2004). Ovaries are the primary source of androgen production in the female. High variability in the number of growing follicles within individuals is hypothesized to be related to androgen production by individual follicles, and total follicle pool, which is highly variable (Jimenez-Krassel
et al., 2009). Conversely, anti-Müllerian hormone (AMH) concentrations have a positive relationship with the number of healthy follicles (Ireland et al., 2008; Ireland et al., 2009).

**Antral follicle counts**

One method of predicting ovarian reserve is to measure antral follicle counts (AFC) on the ovary via ultrasonography. Classifications are made based on number of antral follicles identified by ultrasonography with animals having ≤ 15 being classified as low, 16-24 moderate, and ≥ 25 classified as high follicle count groups (Ireland et al., 2008). These follicles, ≥ 3 mm in size, represent a sub-population of total ovarian reserve. Typically AFC are conducted in heifers prior to their first breeding season (13-15 mo old) and ~15 to 20% of the herd will be classified as low or high AFC while the remaining 80-85% will be classified as moderate AFC (reviewed in Ireland et al., 2012). Research indicates that, although follicle number variation is present among individuals, the peak number of antral follicles growing during a follicular wave is highly repeatable within individuals when ovarian ultrasonography is utilized (Burns et al., 2005; Ireland et al., 2007). Low AFC heifers had lower pregnancy rates and reduced numbers of morphologically healthy oocytes and follicles (Ireland et al., 2008; Cushman et al., 2009; Mossa et al., 2012) when compared with high AFC heifers. Furthermore, Mossa et al. (2012) reported high AFC dairy cows had a 3.34 times greater ($P < 0.05$) odds ratio of being pregnant at the end of the breeding season when compared with low AFC animals. Mossa et al. (2012) also reported a tendency for high AFC animals to return to estrous 21 d after calving compared with low AFC cows and a reduced duration from calving to conception. The decreased timing from calving to conception could be due to an increase
in the number of estrous cycles experienced by high AFC cows earlier in the breeding season. Byerley et al. (1983), observed increased pregnancy rates for heifers bred on third estrus compared with those bred on pubertal estrus, and perhaps similar improvements in fertility can be associated with first estrous after calving and later estrous cycles.

Cow reproductive longevity has been associated with AFC. Cows remaining in the herd ≥ 6 years have numerically higher AFC compared with cows that leave the herd at ≤ 2 years having never produced a calf (Cupp et al., 2011). Mossa et al. (2012) reported dairy cows with high AFC had an average of 2.6 lactations compared to 1.9 lactations for low AFC, also suggesting increased longevity of cows with greater AFC. Furthermore, Mossa et al. (2012) reported a trend ($P = 0.09$) for decreased median calving to conception period for High (100 d) compared with low AFC cows (114 d). High AFC heifers also have larger ($P \leq 0.07$) ovaries (Burns et al., 2005; Ireland et al., 2008; Cushman et al., 2009; Mossa et al., 2012), likely due to the greater density and number of follicles present in the ovary, and high AFC heifers are 3 kg heavier ($P = 0.003$) at birth compared with low AFC heifers (Cushman et al., 2009).

Reports of differences in hormone profiles between high and low AFC heifers can also be found in the literature. Ireland et al. (2009) reported a 2-fold increase ($P = 0.02$) in follicular fluid estrogen concentration, as well as increased FSH concentration, when comparing low AFC to high AFC animals. Furthermore, heifers classified as low AFC have increased gonadotropin secretion, and reduced P4 levels compared with high AFC heifers (Ireland et al., 2012). Decreased progesterone levels have been reported to increase embryonic mortality in cattle (Inskeep, 2004; Ireland et al., 2011) and could
possibly play a role in the decreased pregnancy rates reported in low AFC heifers (Ireland et al., 2008; Cushman et al., 2009; Mossa et al., 2012).

**Impact of maternal nutrition on progeny follicle development**

Rae et al. (2001) reported maternal undernutrition in sheep during the first 110 d of gestation results in reduced ovulation rates, most likely through a direct effect on folliculogenesis. Furthermore, limiting the duration of undernutrition to 1–2 mo during the first 110 d of gestation also reduces the number of follicles that develop beyond the primordial stage (Rae et al., 2001). Maternal nutrient restriction (NR) can also have long-term influences on plasma P4 levels in progeny (Long et al., 2010a; Nurmanat et al., 2011). In a small group of heifers, NR and NR with protein supplementation resulted in reduced \( P \leq 0.10 \) wet ovarian weight and decreased \( P \leq 0.05 \) luteal tissue mass compared with heifers born to control-fed cows (Long et al., 2012).

Mossa et al. (2009) reported a 60% reduction in AFC for heifers born to dams fed 60% of their energy requirements compared with heifers from control-fed dams. However, Da Silva (2002) reported reduced ovarian follicles from progeny of ewe lambs fed to achieve rapid maternal growth rates throughout pregnancy compared with progeny from ewe lambs fed to gain 50–75 g/d through the first 100 d of gestation. Sullivan et al. (2009b) also reported a negative influence of high protein and dietary energy fed to heifers during the second trimester on primordial and primary follicle density \( (n \text{ per } 100 \text{ mm}^2) \). Progeny born to heifers fed the HH or LowH diets had lower \( (P = 0.07) \) primordial and primary follicle densities compared with progeny from heifers fed the LL or HL diets; however, total AFC density was not different (Sullivan et al., 2009b). Reduced follicle densities in the LowH and HH groups coincided with high maternal plasma urea.
nitrogen (PUN) concentration (Sullivan et al., 2009b). The effect of PUN on fetal ovarian follicle development is unknown; however, it has been well established that high PUN impacts reproductive characteristics and fertility with reports indicating increased interval from calving to first ovulation, altering uterine pH, and decreasing pregnancy rates (Jordan and Swanson, 1979; Canfield et al., 1990; Elrod et al., 1993; Sinclair et al., 2000; Moellam et al., 2001).

**Maternal nutrition and muscle development**

Heritability for carcass weight, marbling, and ribeye area are all high (> 0.39; Bertrand et al., 2004). Therefore, improving maternal skeletal muscle traits may be beneficial for subsequent male progeny. Muscle fiber numbers do not increase after birth, which makes the fetal stage crucial to skeletal muscle development (Stickland, 1978; Zhu et al., 2004). Skeletal muscle is particularly vulnerable to deficiencies because it is a lower priority in nutrient partitioning compared with the brain, heart, or other organ systems (Bauman et al, 1982; Close and Pettigrew, 1990). Thus, decreasing nutrient availability to the dam during gestation can result in fewer muscle fibers, which reduces muscle mass and impacts animal performance. Both muscle fiber number and intramuscular adipocytes, which provide the sites for intramuscular fat accumulation or marbling formation, are influenced during fetal development (Tong et al., 2008; Du et al., 2010).

Maternal nutrition impacts fetal skeletal muscle formation at several important control points (Figure 6; Du et al., 2010). During the second to eighth month of gestation, the majority of muscle fibers form; therefore, reduction of muscle fiber formation during this stage through any source of stimuli (e.g., maternal nutrition) has long-lasting, irreversible consequences to the offspring (Du et al., 2010). The prospect of nutritional
management’s altering marbling may be greatest for the fetal stage, due to its importance in adipocyte formation, followed by the neonatal stage, early weaning stage (i.e., 150–250 d of age), and finally, weaning and older stages (Du et al., 2010).

Larson et al. (2009) reported increased progeny birth BW from protein-supplemented dams, which suggests a potential alteration in fetal muscle growth. Greenwood et al. (89) reported steers from cows nutritionally restricted during gestation had reduced body BW and HCW at 30 months of age compared with steers from adequately fed cows. Both Larson et al. (2009) and Greenwood et al. (2004) reported that retail yield on a carcass-weight basis was greater in steers from NR cows, which indicates an increased propensity for carcass fatness is not a consequence of NR in utero.

Although fetal adipocyte development begins early in gestation, the majority of fetal adipose tissue is not deposited until the final few weeks of gestation (Symonds et al., 2007). Adipose tissue growth occurs through preadipocyte proliferation, impacting formation of new mature adipocytes (hyperplasia) and increasing size and lipid storage capacity of mature adipocytes (hypertrophy). Beef cattle were fed one of three diets from d 60–180 of gestation: 100% of nutrient requirements, 70% of requirements, or 70% of requirements plus a rumen bypass protein supplement. Steer progeny from dams fed 70% of nutrient requirements plus supplement had numerical decreases in marbling scores when compared with steers from dams fed 100% of requirements (Long et al., 2012). Underwood et al. (2010) also reported increased tenderness in steers from dams grazed on improved pasture compared with steers from dams grazed on native range during mid-gestation.
Figure 6. Effects of maternal nutrition on bovine fetal skeletal muscle development. Dates are estimated mainly based on data from studies in sheep, rodents, and humans and represent progression through the various developmental stages. Nutrient restriction during midgestation reduces muscle fiber numbers, whereas restriction during late gestation reduces both muscle fiber sizes and the formation of intramuscular adipocytes (adapted from Du et al., 2010).

**IMPACT OF LATE GESTATION NUTRITION ON PROGENY PERFORMANCE**

Studies of maternal nutrition’s influence on progeny performance in domesticated livestock typically involve maternal undernutrition, overnutrition, or supplementation. The majority of these studies investigate maternal nutrition effects on male progeny growth and carcass characteristics. These studies have reported increased weaning BW, improved carcass characteristics, and improved health (Greenwood et al., 2004; Stalker et al., 2007; Larson et al., 2009; Underwood et al., 2010; Endecott et al., 2011) in steer progeny from improved dam nutrition. Although evidence for the influence of maternal
nutrition on female progeny growth, efficiency, and reproductive performance is limited, literature addressing this topic is increasing.

**Impact of maternal nutrition on male progeny performance**

As previously mentioned, studies have reported improved muscle development in steers from adequately fed dams when compared with progeny from nutrient restricted dams. Underwood et al. (2010) reported increased BW gains, final BW, and HCW in steers from cows grazing improved pasture from d 120 to 180 of gestation when compared with progeny from cows grazing native range during that same time (Table 1). Steers from cows grazing improved pasture had increased back fat and tended to have improved marbling scores compared with steers from cows grazing native range.

<table>
<thead>
<tr>
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<tbody>
<tr>
<td></td>
<td>NR</td>
<td>IP</td>
<td>Hay</td>
</tr>
<tr>
<td>Birth BW, kg</td>
<td>38.5</td>
<td>36.7</td>
<td>39&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weaning BW, kg</td>
<td>242&lt;sup&gt;a&lt;/sup&gt;</td>
<td>256&lt;sup&gt;b&lt;/sup&gt;</td>
<td>263&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.49&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.53</td>
</tr>
<tr>
<td>HCW, kg</td>
<td>329&lt;sup&gt;a&lt;/sup&gt;</td>
<td>348&lt;sup&gt;b&lt;/sup&gt;</td>
<td>312</td>
</tr>
<tr>
<td>12-th rib fat, cm</td>
<td>1.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.22</td>
</tr>
<tr>
<td>Marblng score&lt;sup&gt;3&lt;/sup&gt;</td>
<td>420</td>
<td>455</td>
<td>549&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a,b</sup>Means within a study with different superscripts differ (P ≤ 0.05).

<sup>1</sup>NR = dams grazed native range from day 120 to 180 of gestation; IP = dams grazed improved pasture from day 120 to 180 of gestation.

<sup>2</sup>Hay= dams offered a diet of grass hay beginning on day 209 of gestation; Corn = dams offered limit fed diet of corn beginning on day 209 of gestation; DDGS = cows offered a limit-fed diet of distillers grains with solubles beginning on day 209 of gestation.

<sup>3</sup>Where 400 = Small<sup>0</sup>.
To determine the effect dietary energy source had on progeny calf performance, Radunz (2009) offered cows 1 of 3 diets during gestation beginning on approximately d 209: hay (fiber), corn (starch), or distillers grains with solubles (fiber plus fat). Corn and distillers grains diets were limit fed to ensure isocaloric intake among treatments. Results indicated reduced birth BW for calves from dams fed grass hay when compared with calves from the other two groups (Table 1), with an increase \( P \leq 0.05 \) in calf BW reported through weaning when comparing calves from corn fed dams to hay fed dams. Feedlot performance among treatments was not different; however, calves from hay fed dams required 8 and 10 more d on feed to reach a similar fat thickness when compared with calves from distillers and corn fed dams, respectively.

<table>
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<tbody>
<tr>
<td></td>
<td>NS</td>
<td>SUP</td>
<td>NS</td>
<td>SUP</td>
</tr>
<tr>
<td>Weaning BW, kg</td>
<td>210(^a)</td>
<td>222(^b)</td>
<td>210(^a)</td>
<td>210(^a)</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>11.15(^a)</td>
<td>12.05(^b)</td>
<td>8.48</td>
<td>8.53</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>1.60(^x)</td>
<td>1.68(^y)</td>
<td>1.57</td>
<td>1.56</td>
</tr>
<tr>
<td>Feed:gain</td>
<td>6.97</td>
<td>7.19</td>
<td>5.41</td>
<td>5.46</td>
</tr>
<tr>
<td>HCW, kg</td>
<td>347(^a)</td>
<td>365(^b)</td>
<td>363</td>
<td>369</td>
</tr>
<tr>
<td>Choice, %</td>
<td>-</td>
<td>-</td>
<td>85</td>
<td>96</td>
</tr>
<tr>
<td>Marbling score(^3)</td>
<td>449</td>
<td>461</td>
<td>467</td>
<td>479</td>
</tr>
</tbody>
</table>

\(^{a,b}\)Means within a study with different superscripts differ \( P \leq 0.05 \).

\(^{x,y}\)Means within a study with different superscripts differ \( P \leq 0.10 \).

\(^1\)NS = dams did not receive protein supplement while grazing dormant Sandhills range during the last third of gestation; SUP = dams were supplement 3 times per week with the equivalent of 0.45 kg/d of 42% CP cube (DM basis) while grazing dormant Sandhills range during the last third of gestation.

\(^2\)NS = dams did not receive protein supplement while grazing dormant Sandhills range or corn residue during the last third of gestation; SUP = dams were supplemented 3 times per week with the equivalent of 0.45 lb/d of a 28% CP cube (DM basis) while grazing dormant Sandhills range or corn residue during the last third of gestation.

\(^3\)Where 400 = Small\(^0\).
Radunz (2009) reported calves born to cows limit fed distillers grains had reduced net return ($80.29/calf) compared with hay ($106.83/calf) and corn ($110.67/calf) fed cows at the end of the feedlot phase. The increase return per calf for the hay and corn diets can be attributed to the increased HCW for calves from those treatments, resulting in increased total carcass value of $37.66/calf for hay fed cows and $31.07/calf for corn fed cows. Calf purchase cost and feed cost for the cow during the treatment period were similar among groups. Furthermore, other costs, which included yardage, calf feed costs, and treatment costs were similar among treatments. However, the carcass value for calves from hay fed cows was greater compared with calves for corn and distillers fed cows (Radunz, 2009).

Stalker et al. (2006, 2007) reported steer progeny from dams supplemented the equivalent of 0.45 kg/d (42% CP, DM basis) cube during late gestation had no difference in calf birth weight when compared with steers from non-supplemented dams. Conversely, Larson et al. (2009) using the same cow herd, reported an increase in calf birth weight when comparing calves born to dams supplemented the equivalent of 0.45 kg/d (28% CP, DM basis) cube during late gestation to calves from non-supplemented dams. In the study reported by Stalker et al. (2006) cows were utilized in a switchback design whereas cows utilized by Larson et al. (2009) remained on the same treatment over the 3 year study.

Protein supplementation during late gestation increased weaning BW, ADG to weaning, and proportion of calves weaned when comparing calves from supplemented to non-supplemented dams grazing dormant winter range (Stalker et al., 2006, 2007; Larson et al., 2009; Table 2). Stalker et al. (2006) reported no differences in steer progeny
feedlot performance and carcass characteristics when comparing progeny from supplemented and non-supplemented dams. However Larson et al. (2009) reported increased ADG, HCW, and marbling scores in steers from supplemented dams. Furthermore, a greater proportion of steers from supplemented dams graded USDA Choice and USDA Choice or greater when compared with steers from non-supplemented dams. Non-supplemented cows in Larson et al. (2009) may have been under greater nutritional stress than Stalker et al. (2006) as average weaning date was approximately one month later and possibly had greater impact on fetal development.

Table 3. Effect of maternal weaning date and winter grazing treatment on progeny performancea.

<table>
<thead>
<tr>
<th>Item</th>
<th>Wean date</th>
<th>Winter grazing treatmentb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>October</td>
<td>December</td>
</tr>
<tr>
<td></td>
<td>WR0</td>
<td>WR1</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td>October weight, kg</td>
<td>213c</td>
<td>206f</td>
</tr>
<tr>
<td>Steer progeny</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>237</td>
<td>232</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.4</td>
<td>11.0</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>1.61</td>
<td>1.66</td>
</tr>
<tr>
<td>G:Ff</td>
<td>0.154</td>
<td>0.152</td>
</tr>
<tr>
<td>Heifer progeny</td>
<td></td>
<td></td>
</tr>
<tr>
<td>December weight, kg</td>
<td>228c</td>
<td>209f</td>
</tr>
<tr>
<td>Prebreeding weight, kg</td>
<td>297c</td>
<td>270f</td>
</tr>
<tr>
<td>Cycling rate, %</td>
<td>45.6</td>
<td>33.2</td>
</tr>
<tr>
<td>Pregnancy rate, %</td>
<td>69.0</td>
<td>71.3</td>
</tr>
</tbody>
</table>

aAdapted from Rolfe et al. (2011).
bWinter grazing treatments: WR0 = dams grazed winter range without supplement; WR1 = dams grazed winter range and received 0.45 kg/day 32% CP (DM Basis) supplement; WR2 = dams grazed winter range and received 0.91 kg/day 32% CP (dry matter basis) supplement; CR = dams grazed corn residue without supplement.
c,dWean date × Winter grazing treatment interaction (P < 0.05).
d,e,fConsidered cycling if blood serum progesterone concentrations were > 1 ng/mL.
e,fMeans with different superscripts differ within wean date (P < 0.05).
g,hMeans with different superscripts differ within winter grazing treatment (P < 0.05).
Rolfe et al. (2011) evaluated the effects of weaning date and pre-partum plane of nutrition on cow-calf productivity utilizing a 2×4 factorial design. Beef cows were assigned to graze either (CR) or dormant winter range (WR) during the last trimester of gestation. Cows grazing WR were assigned one of three supplement (28% CP, DM Basis) levels: no supplement (WR0), 0.45 kg/d (WR1), or 0.91 kg/d (WR2). Cows from each of the treatments were also assigned to either early (OCT) or late (DEC) weaning treatments. Rolfe et al. (2011) reported no difference in BW and performance of steer calves while in the feedlot among treatments (Table 3). There was no difference in carcass characteristics for steers based on maternal supplement level (Rolfe et al., 2011). However, Summers et al. (2011) reported increasing maternal nutrition during late gestation improves steer calf feedlot performance and carcass characteristics. Cows were offered either 0.95 kg/d or 0.37 kg/d (31.6% CP, DM Basis) distillers based supplement from December through February for high and low nutrition grouped cows, respectively. During calving (March through April) high nutrition cows received 5.2 kg/d (DM Basis) meadow hay and low nutrition cows received 4.99 kg/d (DM Basis) meadow hay.

Supplement and hay were offered based on manager discretion each of the 2 yr. There was a yr × treatment interaction, with steers from high nutrition cows in yr 1 having greater BW at reimplant and slaughter compared with steers from low nutrition cows in yr 1 or 2, and high nutrition cows in yr 2 (Summers et al., 2011). There was also an improvement in carcass characteristics for steers from high nutrition cows compared with low nutrition cows. Steers from high nutrition cows had increased marbling scores compared with steers from low nutrition cows. Furthermore, there was a greater proportion of steer calves from high nutrition yr 2 grading USDA modest or greater
compared with steers from low nutrition cows in yr 1 (Summers et al., 2011). This study utilized only a small subset of the steer calves available, along with the differences in yr 1 and 2 treatment levels due to manager discretion likely caused the yr to yr variation. It is possible protein supplementation at 0.45 kg/d may not be capturing all the potential gains for steer progeny from supplemented cows. However, Rolfe et al. (2011) did not see any increase in calf performance by increasing maternal supplementation level in the first year of their study. Thus research is still needed to determine the adequate amount of maternal protein supplementation to maximize cow and calf production but not hinder profitability.

**Economic impact of maternal protein supplementation**

Stalker et al. (2007) reported a $7.72/cow increase in net revenue for non-supplemented compared with supplemented cows at weaning. This increase in net revenue is due to the increased feed costs of supplement cows owning to supplement and delivery of the supplement. These results differ from data reported by Stalker et al. (2006) reporting a $25.38/calf increase in calf value if dams were supplemented protein. This increase was due to an increase in the number of calves weaned for supplemented compared with non-supplemented dams, as well as a 6 kg increase in weaning BW for calves from supplemented cows. Larson et al. (2009) also reported increased net returns at weaning if calves were born from supplemented dams. Net returns at slaughter were reported to be $25.60-45.76/calf greater, for calves from protein supplemented calves compared with steers born to non-supplemented dams (Stalker et al., 2006, 2007; Larson et al., 2009). Furthermore, Summers et al. (2011) reported an $11.88 increase in net profit for steer calves from high nutrition cows compared with low nutrition cows. The
improvement in net returns in these four studies can be attributed to increased HCW, improved USDA quality grade resulting in greater premiums for the carcass, or due to increased number of carcasses resulting from increased number of calves weaned from protein supplemented dams.

**Impact of maternal undernutrition on female progeny performance**

The majority of studies evaluating the effect of maternal nutrition on heifer progeny performance are limited to first-calf-heifer data. However, Roberts et al. (2009a) conducted a study over a seven-year period monitoring the production of composite (1/2 Red Angus, 1/4 Charolais, 1/4 Tarentaise) cows from dams fed either marginal or adequate levels of harvested feeds from December to March (Table 4). Groups of cows were placed in separate pastures, and a supplement of alfalfa cake or hay, depending on yr, was provided either daily or every other d at an average of 1.8 kg/d for adequate cows or 1 kg/d for marginal cows. During the winter supplementation period, pasture forage was generally accessible for grazing. On d when snow limited pasture availability, cows were fed at a rate of 10.9 and 9.1 kg/d for adequate and marginal cows, respectively. Each year at weaning, heifer calves were assigned to a pen for a 140-d feeding period and offered either a control diet, fed to appetite, or a restricted diet, offered at 80% of the amount consumed by heifers on the control diet adjusted to a common BW. Heifers were managed similarly after the feeding period through the breeding season and fall. Control heifers were then provided the adequate level of feed during each subsequent winter, and restricted heifers were provided marginal levels. Heifers born to marginal dams had greater BW later in life compared with heifers of adequate dams (Roberts et al., 2011). This difference may be caused in part by the increased BCS of the cows from marginal
dams. Furthermore, calves born to restricted dams and marginal granddams were lighter ($P < 0.01$) at both birth and weaning compared with calves born to control dams and adequate granddams; however, pregnancy rates for bred heifers did not differ (Roberts et al., 2009a).

### Table 4. Effects of level of feed input provided to dam and to progeny on progeny performance$^a$.

<table>
<thead>
<tr>
<th>Level of winter supplement to dam$^b$</th>
<th>Marginal</th>
<th>Adequate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Restricted</td>
<td>Control</td>
</tr>
<tr>
<td>Heifer development and treatment$^c$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Five year weight$^d$, kg</td>
<td>515</td>
<td>530</td>
</tr>
<tr>
<td>BCS at 5 yr$^e$</td>
<td>4.9</td>
<td>5.1</td>
</tr>
<tr>
<td>Retention at 5 yr, %</td>
<td>48</td>
<td>46</td>
</tr>
<tr>
<td>Calf birth weight, kg</td>
<td>33.6$^f$</td>
<td>35</td>
</tr>
<tr>
<td>Calf weaning weight$^g$, kg</td>
<td>196$^f$</td>
<td>201</td>
</tr>
</tbody>
</table>

$^a$ Data from Roberts et al. (2009, 2011).

$^b$ Level of supplementation provided to cows from December to March. Marginal= equivalent of 1.1 kg/d; Adequate= equivalent of 1.8 kg/d.

$^c$ Dietary level offered to heifers during 140-day postweaning development period. Restricted= 80% of feed provided to control and 1.1 kg/d supplement each subsequent winter. Control= fed *ad libitum* during postweaning and 1.8 kg/d supplement each winter.

$^d$ $P < 0.01$ for effect of dam treatment and heifer development treatment.

$^e$ $P < 0.001$ for interaction of dam treatment and heifer development treatment.

$^f$ Differs from others in same row.

### Effect of late gestation protein supplementation on heifer progeny performance

Rolfe et al. (2011) reported birth BW was affected by previous maternal weaning date and grazing treatment. Progeny of OCT-weaned dams receiving 0.91 kg/d protein supplement while grazing WR (WR2) had greater ($P < 0.01$) birth BW than calves from non-supplemented dams grazing WR (WR0) (Table 3). Calves from DEC-weaned WR0 dams had reduced ($P < 0.01$) birth BW compared with all other groups except DEC-weaned calves from dams grazing WR and receiving 0.45 kg/d protein supplement.
October body BW was reduced 15-23 (±3 kg) \((P < 0.01)\) in calves born to WR0 dams compared with all other groups. Dam winter treatment also affected December and prebreeding BW; WR0-born calves had reduced \((P < 0.03)\) BW compared with other winter treatment groups (Rolfe et al., 2011).

Martin et al. (2007a) conducted a study with cows grazing WR during late gestation. One group received a 42% CP (DM Basis) cube offered three times weekly at the equivalent of 0.45 kg/d, and another group received no supplement. After calving, pairs were offered cool-season grass hay or placed on sub-irrigated meadows during early lactation. Calf birth BW was not different between heifer progeny from supplemented and non-supplemented dams; however, heifer progeny from supplemented cows had increased adjusted 205-d weaning BW (226 vs. 218 ± 7 kg), prebreeding BW (276 vs. 266 ± 9 kg), BW at pregnancy diagnosis (400 vs. 386 ± 31 kg), and improved pregnancy rates (93 vs. 80%) compared with heifers from non-supplemented dams (Table 5). Martin et al. (2007a) also reported after a subset of these heifers were placed in a Calan gate individual feeding system, DMI, ADG, and residual feed intake (RFI) did not differ between heifer progeny from supplemented and non-supplemented dams.

Using the same cow herd (Table 5), Funston et al. (2010b) offered a distillers-based supplement (28% CP; DM Basis) three times weekly at the equivalent of 0.45 kg/d or no supplement during late gestation to cows grazing either WR or CR. Calf weaning BW was greater \((P = 0.04)\) for heifers from protein-supplemented dams (232 vs. 225 ± 6 kg; Funston et al., 2009b). Martin et al. (2007a) also reported, although not significant \((P = 0.12)\), increased weaning BW for heifers from protein-supplemented dams. Funston et al. (2010b) also reported heifers from protein-supplemented cows attained puberty 14 d
earlier than heifers from non-supplemented dams ($P = 0.09$). Furthermore, there was a trend ($P = 0.13$) for higher pregnancy rates when comparing heifers from protein-supplemented dams with heifers from non-supplemented dams, possibly related to decreased age at puberty. Both studies conducted by Martin et al. (2007a) and Funston et al. (2009b) utilized 2×2 factorial designed experiments. The slight differences reported between these authors may be explained by differences in study design and animal allotment to treatment. Both treatment factors studied by Funston et al. (2009b) were applied to the cow prepartum, and cows in this study remained on the same treatment all 3 yr. In contrast, Martin et al. (2007a) studied the effect of protein supplementation or no supplementation during late gestation, but also the effect of grazing cow/calf pairs on sub-irrigated meadow or provided meadow hay during early lactation. The researchers in this experiment utilized a cross over design in the first two yr of the study and randomly assigned cows to a treatment group during the third yr of the experiment. Also Martin et al. (2007a) utilized a 42% crude protein supplement consisting of 50% sunflower meal and 47% cottonseed meal, whereas Funston et al (2010b) provided a 28% crude protein supplement consisting of mostly dried distillers grains with solubles. Utilizing NRC (2000) feed values, the supplement fed by Martin et al. (2007a) contained approximately 33% rumen undegradable protein (RUP), whereas the supplement provide by Funston et al. (2010b) provided approximately 48% of the protein as RUP. Increased levels of RUP would provide increased levels of diet supplied amino acids to the intestine for utilization by the animal, compared with protein degraded in the rumen and utilized by rumen microbes.
Table 5. Effect of maternal protein supplementation on heifer progeny performance.

<table>
<thead>
<tr>
<th>Item</th>
<th>Dietary treatment</th>
<th>Martin et al. (2007)</th>
<th>Funston et al. (2010b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>SUP</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>35</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Weaning weight, kg</td>
<td>207</td>
<td>212</td>
<td>225d</td>
</tr>
<tr>
<td>Adj. 205-day weight, kg</td>
<td>218d</td>
<td>226e</td>
<td>213</td>
</tr>
<tr>
<td>Dry matter intake, kg/d</td>
<td>6.50</td>
<td>6.75</td>
<td>9.48</td>
</tr>
<tr>
<td>Average daily gain, kg/d</td>
<td>0.41</td>
<td>0.40</td>
<td>0.85</td>
</tr>
<tr>
<td>Residual feed intake</td>
<td>-0.12</td>
<td>0.07</td>
<td>0.08</td>
</tr>
<tr>
<td>Final BWc, kg</td>
<td>290x</td>
<td>304y</td>
<td>—</td>
</tr>
<tr>
<td>Age at puberty, d</td>
<td>334</td>
<td>339</td>
<td>366x</td>
</tr>
<tr>
<td>Prebreeding weight, kg</td>
<td>266d</td>
<td>276e</td>
<td>317</td>
</tr>
<tr>
<td>Pregnancy diagnosis weight, kg</td>
<td>386d</td>
<td>400e</td>
<td>364x</td>
</tr>
<tr>
<td>Pregnant, %</td>
<td>80d</td>
<td>93e</td>
<td>80</td>
</tr>
<tr>
<td>Calved in first 21 d, %</td>
<td>49d</td>
<td>77e</td>
<td>85</td>
</tr>
</tbody>
</table>

aNS = dams did not receive protein supplement while grazing dormant Sandhills range during the last third of gestation; SUP = dams were supplemented three times per week with the equivalent of 0.45 kg/d of 42% CP cube (dry matter basis) while grazing dormant Sandhills range during the last third of gestation.
bNS = dams did not receive protein supplement while grazing dormant Sandhills range or corn residue during the last third of gestation; SUP = dams were supplemented three times per week with the equivalent of 0.45 kg/d of a 28% CP cube (dry matter basis) while grazing dormant Sandhills range or corn residue during the last third of gestation.
cFinal weight of heifers after 84-day individual feeding period.
d,e Means within a study with different superscripts differ ($P \leq 0.05$).
x,y Means within a study with different superscripts differ ($P \leq 0.10$).

Warner et al. (2011) reported no differences in pregnancy rates for heifers from dams grazing CR and receiving protein supplement compared with dams grazing CR and receiving no supplement during late gestation. These results coincide with data presented by Funston et al. (2010b) in which pregnancy rates for heifers from protein-supplemented cows were impacted by dam winter grazing treatment, with pregnancy rates being similar for heifers born to cows grazing CR regardless of protein-supplementation treatment (Funston et al., 2010b). Corah et al. (1975) reported heifers born to primiparous heifers fed 100% of their dietary energy requirement during the last 90 d of gestation were
pubertal 19 d earlier than heifers born to primiparous heifers fed 65% of their dietary energy requirement, although this difference was not significant. Rolfe et al. (2011) reported a similar proportion of heifers cycling, and overall pregnancy rates did not differ ($P > 0.31$) based on maternal weaning treatment or winter treatment when analyzing the first year of data. However, the authors did report a numeric increase in pregnancy rates in all groups compared with WR0, similar to data reported by Funston et al. (2010b) in which heifers born to supplemented dams had greater pregnancy rates compared with heifers of non-supplemented dams.

Funston et al. (2010b) reported no differences in heifer BW at prebreeding and no differences in calf birth BW, calf production, or second-calf rebreeding when comparing heifer progeny from supplemented and non-supplemented cows. Gunn et al. (1995) reported a decrease in the proportion of singleton births and an increase in the proportion of multiple births over three parities in progeny born to ewes offered a protein supplement while grazing native pastures during the last 100 d of gestation, compared with progeny from non-supplemented ewes. Late-gestation supplementation did not alter the proportion of barren ewe progeny; however, supplementation during lactation did (Gunn et al., 1995). Martin et al. (2007a) reported a 28% increase in the proportion of heifers that calved in the first 21-d of the calving season from protein-supplemented dams compared with heifers from non-supplemented dams.

**Impact of maternal body reserves on progeny performance**

Body condition score (BCS) is an estimate of energy reserves available to help the animal with maintenance and production requirements. Pryce et al. (2002) reported no difference in progeny heifer reproductive performance considering dairy cow maternal
nutritional status, determined by BCS, DMI, and milk yield of fat and protein. Rolfe et al. (2011) reported similar cow BCS and BW at the October weaning; however, by December, BCS and BW for DEC cows was reduced \( (P < 0.01) \) compared with OCT-weaned cows. Cow BCS precalving remained greater \( (P = 0.02) \) for OCT-weaned cows compared with DEC-weaned cows, but BCS did not differ for weaning treatment by prebreeding (Rolfe et al., 2011). The difference in BCS and BW can be attributed to the added requirements of lactation during the two-month difference in weaning treatment. In October, calves from OCT-weaned dams had greater \( (P = 0.02) \) body BW compared with calves from DEC-weaned dams. Body weight was greater for heifers born to OCT-weaned calves at the December weaning, and greater BW continued to the breeding season (Rolfe et al., 2011). Although heifer BW was different for OCT- compared with DEC-weaned heifers, steer progeny BW, feedlot performance, and carcass characteristics were similar \( (P \geq 0.22) \) among weaning treatments.

**Impact of maternal overnutrition on progeny performance**

Although producers typically manage animals in such a way that there is no overabundance of nutrients, researchers have studied the effects of overnutrition on progeny performance. Overnutrition in cattle has been reported to increase BW and skeletal size, decrease milk production, and decrease reproductive life and longevity (Hansen and Steensberg, 1950; Hansson, 1956; Pinney, 1962; Arnett et al., 1971). Interestingly, maternal overnutrition has been reported to decrease birth BW of lambs (Wallace et al., 1996; Da Silva et al., 2002). Furthermore, reproductive phenotypes were altered in sheep born to overnourished dams, with female progeny having reduced ovarian size and male progeny increased age at puberty (Da Silva et al., 2001, 2002).
In many reports in human and livestock species, it appears maternal overnutrition results in progeny phenotypes similar to progeny from undernourished females (reviewed in Ford and Long, 2012). The authors hypothesize the mechanism involving the similarities in phenotypes is due in part to the reduction of placental vascularity and nutrient transporter in animals fed 150% maintenance requirements from 60 d prior to conception through gestation (Ma et al., 2010). Ewes fed 50% maintenance requirements from early to mid gestation and then 100% requirement until lambing had fetus BW approximately 60% below fetal BW from overnourished ewes at mid gestation (Ma et al., 2010). However, after nutrient realignment in nutrient restricted ewes (late gestation) placental vascularity and nutrient transporter activity increased, unlike in the overnourished model, giving rise to lambs born from each treatment with similar birth BW (Ma et al., 2010).

*Effect of maternal age, milk production, and birth date on progeny performance*

Cows becoming pregnant at a young age must not only provide energy for developing fetal growth but also supply energy for their own growth requirements. In fact, Caton et al. (2007) reported primiparous heifers require an additional 1.46 Mcal/d of net energy during the last trimester of gestation compared with mature cows. This increase in daily energy requirements is associated directly with the growth requirement of the heifer. Increasing cow longevity is advantageous to producers because it allows them to spread heifer development costs across more units of output (calves) or a greater number of lactations, in dairy cattle.

One potential side effect for the developing fetuses of older dams could be a possible increase in chromosome abnormalities owing to reduced oocyte competence
Extremes in age, both old and young, resulted in mice pups with reduced BW and delayed puberty (Wang and von Saal, 2000). Old cows (13–16 years old) recovered fewer embryos and had a greater proportion of unfertilized oocytes compared with their younger daughters (3–6 years old; Malhi et al., 2007). Fuerst-Waltl et al. (2004) analyzed records of approximately 217,000 Austrian dual-purpose Simmental cows and reported energy-correct milk yield, milk fat, and protein yield decrease as maternal age increases through the first three lactations.

Although lactation coincides with fetal growth and development, thus competing for energy, it appears maternal milk production has no effect on progeny milking performance (Banos et al., 2007; Berry et al., 2008). Banos et al. (2007) reported heifers born to young cows (18–23 months old) had greater daily milk yield and BCS and calved three d earlier than heifers born to older cows (30–36 months old). However, heifers born to younger dams had reduced fertility, requiring 7% more inseminations per conception compared with heifers from older cows (Banos et al., 2007). Cows born to young, second-calving dams (36–41 months old) had improved productivity with increased fertility and daily milk yield during first lactation compared with cows from dams who were older (47–55 months old) at second calving (Banos et al., 2007).

Endecott et al. (2011) reported bull calves born to dams 4 yr or older had greater final BW compared with bull calves born to dams 2 or 3 yr old. Furthermore, bull calves born to older dams had increased back fat thickness compared with bull calves born to 3 yr old dams when measured during the postweaning phase and at slaughter. Ribeye area was also greater for bull calves born to cows 4 yrs or older compared with calves born to 2 or 3 yr old dams (Endecott et al., 2011). Mature BW of a cow is not reached until
approximately 5 yr of age. It is likely bull calves born to 2 and 3 yr old cows had to compete for nutrients during gestation with the dam for her maintenance and growth requirements. Also lactation is a time of great energy demand in the cow and nutrient demand for the growing cow would also be competing with the demands of lactation which could potentially reduce milk yield in these younger animals.

Thirteen years of spring calving records at the University of Nebraska’s Gudmundsen Sandhills Laboratory (GSL) were reviewed to examine the impact of heifer birth date on productivity (Funston et al., 2012). Heifers were classified as being born in the first, second, or third 21-d periods of the calving season based on birth date. Heifers born in the first 21-d had reduced birth BW (36 ± 0.70 kg) compared with the other groups. Heifers born in the third 21-d had the greatest birth BW (38 ± 0.70 kg). One possible cause for increased birth BW in calves born later in the calving season is improved forage nutrient quality. Records were reported from spring calving cows with the average Julian birth date of calves from the first 21-d being 72 (March 13). The third group categorized was born on Julian date 113 (April 23). Previous research conducted at the Gudmundsen Sandhills Laboratory suggests a 2% improvement in range crude protein and nutrient value from February, coinciding with late gestation for calves born in the first 21-d period, to March/April, corresponding with late gestation for calves born in the third 21-d period of the calving season (Geisert, 2007). As previously mentioned, nutrient demand for fetal growth is greatest during late gestation due to the increased growth rate of the fetus prior to birth. Increased forage nutrient quality prior to birth for calves born later in the calving season could cause improved nutritional status of the cow, and thus increase nutrient supply to the growing fetus to increase birth BW.
Weaning BW, prebreeding BW, proportion of heifers cycling at the beginning of the breeding season, and percentage pregnant were greatest \((P \leq 0.03)\) for heifers born in the first 21-d of the calving season. Since more heifers born in the first 21-d of the calving season were cycling at the beginning of the breeding season, heifers from that group likely had more estrous cycles prior to breeding compared with heifers born later in the calving season. Byerley et al. (1983), observed increased pregnancy rates for heifers bred on third estrus compared with those bred on pubertal estrus. A greater \((P < 0.01)\) proportion of heifers born in the first 21-d also calved in the first 21-d as first-calf heifers and weaned heavier \((P = 0.10)\) calves (Funston et al., 2012). In addition, heifers calving earlier in their first calving season have greater lifetime productivity compared with those calving later (Lesmeister et al., 1973).

**Maternal nutrition and progeny health**

Several reports have linked maternal nutrition during gestation to calf health, including Corah et al. (1975), who reported increased morbidity and mortality rates in calves born to primiparous heifers receiving 65% of their dietary energy requirement over the last 90 d of gestation compared with calves from primiparous heifers receiving 100% of their energy requirement. One factor contributing to increased morbidity and mortality is decreased birth BW. Calves born to NR dams were 2 kg lighter at birth compared with calves from dams who received adequate nutrition (Corah et al., 1975).

Although protein supplementation did not increase progeny birth BW, Larson et al. (2009) reported reduced incidences of respiratory disease during the feedlot phase for steer progeny born to protein-supplemented dams. However, heifer cohorts did not have improved health owing to maternal nutrition (Funston et al., 2010b). These heifers
remained at the ranch postweaning and maintained a forage-based diet, which likely reduced stress compared with their steer cohorts who were transported to the feedlot two weeks postweaning and adapted to a concentrate-based diet.

A major factor influencing calf health is the absorption of immunoglobulin (Ig) from colostrum through the intestinal epithelium to the lymphatic system and into peripheral circulation, which improves neonate immune status while the immune system develops (Amer et al., 2008). Immunoglobulin absorption is greatest during the first 24–72 h after birth prior to the maturation of the intestinal epithelium cells (Parker and Nicol, 1990). Stalker et al. (2007) reported no differences in IgG concentrations in the colostrum of cows provided a protein supplement during late gestation compared with non-supplemented cows. However, Blecha et al. (1981) reported decreases in protein intake during late gestation linearly impaired calf serum Ig concentration despite having no effect on Ig concentrations in the colostrum. Furthermore, moderate NR from d 30–125 of gestation resulted in altered intestinal proliferation and vascularity of the fetus (Meyer et al., 2010), which could impact neonate Ig absorption and affect passive immunity transfer. Several researchers (Selman et al., 1975; Fallon, 1978) also indicated allowing calves to remain with their dams after parturition increased serum Ig levels when compared with calves immediately separated from their dams and hand-fed, which suggests lowering neonate stress levels may also impact Ig absorption. Maternal mineral intake during late gestation can also impact progeny Ig-absorption efficiency, with iodine being the most disruptive mineral affecting Ig absorption in newborn lambs (Boland et al., 2005).
**IMPACT OF PREWEANING NUTRITION ON HEIFER PRODUCTIVITY**

In addition to helping provide passive immunity to the calf, colostrum is a rich source of nutrients. DeNise et al. (1989) reported a correlation between calf plasma Ig concentration and future milk production if the calf was allowed to suckle their dam in the first 24 h postpartum. Milk production during the first and second lactation was reduced 10–15% for cows receiving 2 L of colostrum compared with those receiving 4 L of colostrum after birth (Faber et al., 2005). Bach (2011) suggested long-term effects of colostrum feeding at birth are most likely related to hormonal factors found in colostrum that aid in gastrointestinal-tract maturation, production of digestive enzymes, and nutrient absorption. Milk also contains hormones and growth factors, though at reduced levels compared to colostrum. Mammary gland development occurs during several different phases including fetal life, puberty, pregnancy and lactation (Sejrsen and Purup, 1997). Overnutrition has the greatest impact on mammary gland development during the peripubertal stage with heifers fed to gain above 600 g/d during the peripubertal period having reduced mammary growth (Bach, 2011). Furthermore, diet type impacts mammary growth with corn based diets having a greater reduction of mammary DNA compared with an alfalfa based diet (Bach, 2011).

The effects of preweaning milk product fed to dairy calves and its influence on milk production were summarized by Bach (2011) and are reported in Table 6. Allowing the calf to suckle 3 times per d improves milk yield by 4% compared with bottle-feeding milk replacer (Bar-Peled et al., 1997). Calves offered *ad libitum* whole milk for 30 min twice daily had greater first-lactation milk yield compared with those offered *ad libitum* milk replacer for 30 min twice daily (Moallem et al, 2010). Furthermore, bottle-feeding large quantities of whole milk twice daily also increased milk yield compared with
rearing calves on milk replacer. These studies suggest offering whole milk to calves during the first 1.5–2 months increases subsequent milk production compared with offering milk replacer. In beef cattle, cows weaned as heifers at 240 d tended to produce approximately 10% more milk compared with those weaned at 140 d, which suggests whole milk consumption past the 2 months reported in dairy cattle improves future milk yield (Holloway et al., 1973). Improved milk production in heifers raised on whole milk may result from the influence of leptin levels contained in whole milk, increasing food intake later in life (Bach, 2011).

Table 6. Influence of postpartum nutrition on future milk yielda.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Change in milk yield, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Suckling the dam 3x versus MR1x (360 g/d)</td>
<td>4.0c</td>
<td>Bar-Peled et al., 1997</td>
</tr>
<tr>
<td>WM 2x (ad libitum) versus MR 1x (450 g/d)</td>
<td>4.5d</td>
<td>Shamay et al., 2005</td>
</tr>
<tr>
<td>WM 2x (1.2 kg/d) versus MR 2x (1.1 kg/d)</td>
<td>13.0d</td>
<td>Moallem et al., 2001</td>
</tr>
<tr>
<td>MR 2x (1030) versus MR 2x (600 g/d)</td>
<td>4.1c,e</td>
<td>Rincker et al., 2011</td>
</tr>
<tr>
<td>MR 2x (1200) versus MR 2x (600 g/d)</td>
<td>-1.3</td>
<td>Morrison et al., 200</td>
</tr>
<tr>
<td>MR 2x (990) versus MR 2x (475 g/d)</td>
<td>5.2</td>
<td>Raeth-Knight et al., 2009</td>
</tr>
<tr>
<td>MR 2x (410) versus MR 2x (900 g/d)</td>
<td>5.9</td>
<td>Terré et al., 2009</td>
</tr>
<tr>
<td>Suckling the dam for 240 days versus suckling the dam for 140 days</td>
<td>10.5f</td>
<td>Holloway and Totusek, 1973</td>
</tr>
</tbody>
</table>

aAdapted from Bach 2011(120).
bx= number of times calves were fed each day; MR= milk replacer; WM= whole milk; values within parenthesis represent total grams of liquid food provided daily.
cDifference in milk yield was significant (P < 0.10).
dDifference in milk yield was found only when additional protein was offered after weaning (P < 0.05).
eEffect of postnatal nutrition correction for the effect of parent average.
fAverage difference in milk production over three years between treatments.
Maternal nutrient level provided during late gestation, as well as early lactation, influences maternal milk production (Wiltbank et al., 1962; Totusek et al., 1973; Lalman et al., 2000). Corah et al. (1975) reported reduced daily milk production for NR first-calf beef heifers compared with those fed at maintenance levels during late gestation. Heifer maintenance requirements, along with the high energy demand of the fetus in late gestation, likely alter adipose tissue development, reduce body reserves, and ultimately reduce milk production in first-calf heifers. Reductions in maternal milk production can influence weaning BW (Corah et al., 1975), and nutrient status in the first 2–3 months of age may impact the age and timing of puberty (Gasser et al., 2006). In addition, Gunn (1983) reported plane of nutrition in sheep during the first year of life plays an important role in influencing ovulation rate and litter size in adulthood.

**Impact of early weaning on heifer productivity**

In a production setting, most beef calves are weaned between 6 and 8 mo of age. A review of the literature (Thrift and Thrift, 2004) regarding early and late weaning reported early weaned calves had greater or equal BW compared with control-weaned calves at the time of the control weaning. However, in most cases, management of early weaned calves required a supplement of either concentrate or hay to achieve these BW (Thrift and Thrift, 2004). Similarly, Roth et al. (2009) reported reduced age at weaning for dairy calves weaned based on concentrate-consumption levels compared with conventional weaning at 12 wk of age; concentrate-dependent calves had longer rumen papillae length, which suggests a faster physiological development. Increasing calf concentrate intake is correlated with future milk yield and survivability to the second
lactation: first-lactation milk yields and cow longevity increase as age to consume 0.91 kg/d of starter decreases (Bach, 2011).

**POSTWEANING MANAGEMENT**

*Traditional heifer development*

Developing heifers in a traditional setting requires more resources and targets developing heifers to a greater BW prior to the breeding season. Although reports of heifer development research can be found from the early 1900’s (Short and Bellows 1971; Ferrell, 1982) the majority of heifer development literature dictating today’s production systems was published from 1950-1990. These studies reported the effect of over and undernutrition on pubertal attainment, reproductive performance and ultimately cow productivity (Joubert, 1954, Hansson, 1955; Lesmeister et al., 1973; Byerley et al., 1987; Patterson et al., 1992).

During this time heifer development and puberty attainment was linked to preweaning gain and weaning BW, with a large number of studies dedicated to determining if puberty attainment was controlled by age or BW. Heifers developed to 65% mature BW were suggested to have maximal reproductive performance (Patterson et al., 1992). Furthermore, during this time heifer development and production demands shifted, requiring heifers to produce their first calf at 2 yr rather than 3 yr old. In part this alteration in production practices was caused by increasing feed prices, but also by research reporting cows that calve at 2 yr old produce more calves over their lifetime compared with cows that produce their first calf at 3 yr old (Donaldson et al. 1968). These data have further been enhanced by current studies reporting increased productivity of heifers and cow that calve earlier in the calving season over their lifetime.
compared with cows calving later in the calving season (Funston et al., 2012; Kill et al., 2012).

Joubert (1955) reported heifers fed a high plane of nutrition prior to breeding were bred at 20.1 mo or 333 kg, while those on the low plane of nutrition were bred at 29.1 mo or 328 kg. These data as well as Joubert (1954) reporting nutrient restriction from 2 mo old to breeding increased the age at puberty 241 d compared with those heifers fed an adequate diet helped confirm the hypothesis that heifers needed to attain a proper proportion of mature BW or BW target in order to optimize reproductive performance. This led to increases in harvested forages and concentrate supplementation utilized in heifer development, resulting in heifers being developed to approximately 65% mature BW, which has been the industry standard for the past 20 yr (Patterson et al., 1992). However over the past decade, research has been conducted that challenges the requirement of developing heifers to 65% mature BW. These systems have either restricted nutrient intake early in the development system or developed heifers on dormant forages. These systems have helped alleviate some of the costs of heifer development due to increasing feed cost, but have maintained reproductive performance.

**Reduced input development systems**

In development systems in which heifers are limited in nutrient intake or managed for late post development BW gains, BW is reduced at the beginning of the breeding season (Funston et al., 2011). In reviewing the literature, pregnancy rates, calf birth date, and second–breeding season pregnancy rates for heifers developed on lower input systems (Table 7) are not different, although some studies reported increased age at puberty. Genetic selection may have decreased the heifer age at puberty, thus allowing
heifers developed in systems with reduced nutrient intakes to still attain puberty prior to the end of the breeding season (Funston et al., 2011).

Roberts et al. (2009b) reported restricted heifers consumed approximately 27% less feed and as a result had reduced ADG compared with control heifers during the individual feeding period. Average daily gain was greater for restricted heifers after the feeding period compared with control heifers, which suggests a compensatory gain effect for restricted heifers. Cow BW and BCS remained lower ($P < 0.01$) for individuals developed as restricted compared with control at the beginning of the breeding season at two to five years old (Roberts et al., 2009b). During the first 3 years of production, cow retention rates were greater for those developed on control compared with restricted diets; however, retention rates at 5 yr old and above are similar regardless of dam or individual treatment (Roberts et al., 2011).

Heifers nutrient restricted for 140 d after weaning had reduced proportions attaining puberty by 14 mo, but had similar overall pregnancy rates compared with their control-fed contemporaries (Roberts et al., 2009b). Lynch et al. (1997) also reported similar pregnancy rates for heifers developed to gain 0.11 kg/d for the first 112 d of development followed by 0.91 kg/d for 47 d compared with heifers developed to gain 0.45 kg/d throughout the development period. Funston and Deutscher (2004) reported heifers developed to 53% mature BW prior to the breeding season had reduced BW through weaning their second calf compared with heifers developed to 58% mature BW. In addition, calving interval, calving date through the third calving season, and pregnancy rates through the third breeding season were not different for heifers developed to 53% compared with 58% mature BW (Funston and Deutscher, 2004).
Table 7. Influence of postweaning nutrition on heifer reproductive performance.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age at puberty&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Heifer pregnancy rate&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Mean calving date&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Second-year pregnancy rate&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>Even gain vs. Late gain</td>
<td>INCR&lt;sup&gt;b&lt;/sup&gt;</td>
<td>NS</td>
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<td>Lynch et al., 1997</td>
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<td>Low gain vs. High gain</td>
<td>DECR&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<td>Funston and Deutscher, 2004</td>
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<td>Low supplement vs. High supplement</td>
<td>DECR&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<td>Winter grazing plus supplement vs. Dry lot</td>
<td>DECR&lt;sup&gt;c,d&lt;/sup&gt;</td>
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<td>Funston et al., 2011b</td>
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<td>Restricted vs. Control</td>
<td>INCR&lt;sup&gt;f&lt;/sup&gt;</td>
<td>NS</td>
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<td>Roberts et al., 2009b</td>
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<td>Low-high vs. High</td>
<td>—</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>Freetly et al., 2001</td>
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<sup>a</sup>Effect of reduced or late nutrient intake or growth compared with control; INCR = increased compared with control; DECR = decreased compared with control; NS = not significant.

<sup>b</sup>In year 2 only (P < 0.01).

<sup>c</sup>Reported as cyclic prior to breeding season.

<sup>d</sup>Means within study differ (P < 0.05).

<sup>e</sup>Reported first-service calving rate.

<sup>f</sup>Means within study differ (P < 0.10).

Winter grazing development systems

Grazing developing heifers on dormant WR or CR has been reported as an alternative to confinement feeding replacement heifers (Martin et al., 2008; Funston and Larson, 2011; Larson et al., 2011). However these dormant forages do not meet the nutrient requirements of the growing animal and protein supplementation is required (Fernandez-Rivera et al., 1989). Corn residue typically has greater nutrient value compared with dormant WR, thus utilization of CR in heifer development systems would improve heifer performance (Clanton, 1989). However, cattle grazing CR require an approximate 3 wk adaptation period to grazing CR (Fernandez and Klopfenstein, 1989).
Grazing habits can be developed through social interaction with young or naïve animals learning what to eat from their contemporary groups (Thorhallsdottir et al., 1990; Provenza et al., 1993).

Research suggests varying nutrient quality and digestibility of the differing components of corn residue. Corn cobs and the bottom two-thirds of the stalk have the lowest IVDMD (33-35 ± 1.74%) whereas the husk has the greatest IVDMD (59 ± 0.68%; McGee et al., 2012). At the beginning of the grazing season all components of CR are available for grazing but due to trampling and other environmental factors, not all the residue will be utilized effectively (reviewed by Klopfenstein et al., 1987). Over the course of the CR grazing period, diet quality is reduced for the first 4-5 wk before diet quality remains constant (Fernandez-Rivera et al., 1989). Furthermore, BW gain during CR grazing is associated with the CP content of leaf plus husk (Fernandez-Rivera and Klopfenstein, 1989). Due to the different nutrient values of CR components it would be crucial for animals grazing CR to recognize the most beneficial fractions of the CR. Fernandez-Rivera et al. (1989) also suggested cattle required an adaptation period for grazing CR of approximately 3 wk due to reduced starch content of the diets of naïve calves grazing CR which is suggested to be caused by an inability to find and consume grain in CR.

Martin et al. (2008) reported heifer grazing WR developed to 50% mature BW had reduced BW through the second pregnancy diagnosis compared with heifers developed to 56.5% mature BW. Similarly, Funston and Larson (2011) reported heifers developed on CR 51 kg (± 11 kg) lighter after the winter development period and remained 28 kg (± 4 kg) lighter at pregnancy diagnosis compared with heifers developed
in the drylot. However, prior to calving BW was similar for DL- and CR-developed heifers. In both studies, heifer reproductive performance was similar among treatments; however, Martin et al. (2008) did report a decrease in weaning BW for calves born to heifers developed to 50% mature BW compared with those developed to 56.5% mature BW. Although heifers in both studies were developed below the industry standard of 65% mature BW dystocia scores were ≤ 1.45. Dystocia scores were based on a scale of 1 to 5 with 1 = no assistance and 5= cesarean section. These data indicate reducing heifer BW prior to breeding does not have negative impacts on calving ease as scores for all groups were low.

Larson et al. (2011) developed heifer at two locations on either WR or CR. Heifers at each location were offered 0.45 kg/d 28% CP (DM Basis) distillers based supplement throughout the development period. Corn residue developed heifers had reduced BW after the approximate 93 d CR grazing period compared with heifers grazing WR. Average daily gain tended ($P = 0.06$) to be reduced during the winter grazing period compared with WR-developed heifers. Interestingly, CR-developed heifers tended ($P = 0.10$) to have increased ADG after returning to WR grazing and through pregnancy diagnosis compared with WR-developed heifers, suggesting a compensatory gain effect for heifers developed on CR (Larson et al., 2011). A second set of heifers was developed at the Agricultural Research and Development Center, Ithaca, NE. There was no difference in heifer performance based on winter development treatment in these heifers. It is suggested by the authors that the lack of statistical differences in heifers developed at the second location can be attributed to increased snow cover requiring supplemental feeding of hay during the CR grazing period (Larson et al., 2011). Reduced ADG of CR
heifers during this time, likely results from the reported grazing adaptation period required for cattle grazing CR (Fernandez-Rivera and Klopfenstein, 1989). Interestingly, ADG for heifers developed on CR is 2-times greater ($P = 0.03$) when placed on CR as pregnant heifers compared with DL-developed heifers (Summers et al., unpublished data). Corn residue developed heifers tend ($P = 0.07$) to have increased ADG compared with WR-developed heifers when placed on CR as pregnant heifers (Summers et al., unpublished data). The proportion of heifers attaining puberty prior to the breeding season and pregnancy rates were similar for heifers developed on WR and CR (Larson et al., 2011).

Although previous research indicated maximal reproductive rates when heifers were developed to approximately 65% mature BW prior to breeding (Patterson et al., 1992), more recent data suggest developing heifers to 50–57% mature BW at breeding will result in similar pregnancy rates to those of heifers developed to a greater percent of mature BW (Martin et al., 2008; Funston and Larson, 2011; Funston et al., 2011). Furthermore, these data suggest limiting nutrient intake after weaning could impact cow size and reduce maintenance requirements for the animal. Reports in literature suggesting longevity of heifers developed on limited inputs are limited. Funston and Deutcher (2004) suggested longevity will not be impacted, through the first two breeding seasons. Recently, Roberts et al. (2011) reported heifers fed a control diet for 140 d after weaning had greater retention rates at 2nd, 3rd, and 4th breeding, but retention rate at 5th breeding was not different between controls and heifers fed a nutrient restricted diet 140 d postweaning, however the dataset in this study is not completed.
Protein supplementation during heifer development

The previous studies regarding heifer development on winter forage, offered 0.45 kg/d protein supplement. As previously mentioned, dormant forages, although commonly abundant, have reduced nutrient quality, thus meeting animal protein requirements usually requires supplemental protein, especially in the form of rumen undegradable protein (RUP; Fernandez-Rivera and Klopfenstein, 1989). Protein provided in the diet of ruminants can either be degraded in the rumen by the microbial population (degradable intake protein; DUP) or by-passes degradation in the rumen and is digested and absorbed in the intestine (RUP). Although RUP bypasses degradation in the rumen, utilization of this fraction of protein in the animal is incomplete. MacDonald et al. (2007) reported RUP digestible ranged between 31.3 to 94.9% in various feedstuffs tested. Cattle performance while grazing dormant forages and being supplemented with either a DUP or RUP based supplement are mixed and seem to be affected by supplement type, length of supplementation period and physiological status of the animal (Lalman et al., 1993; Hollingsworth-Jenkins et al., 1996; Sletmoen-Olsen et al., 2000; Kane et al., 2004; Martin et al., 2007b).

Lalman et al. (1993) reported heifers supplement 421 g of RUP/d had increased age at puberty compared with control fed females. Similarly, Kane et al. (2004) reported decreased concentrations of FSH in the anterior pituitary of heifers consuming 321 g/d RUP compared with heifers consuming 115 g/d when slaughtered approximately 30 d after initiation of supplementation. Furthermore, IGFBP-2 and-4 concentrations were increased in follicular fluid of heifers supplemented high levels of RUP. Insulin-like growth factor and its binding proteins play an integral role in energy metabolism and
have been suggested to be one of many metabolic regulators in the reproductive axis 
(Zulu et al., 2002; Kane et al., 2004). Although these studies did not report any 
differences in reproductive performance, they suggest possible alterations in the 
reproductive axis and earlier studies in which RUP was in excess reported RUP may be 
detrimental to reproductive performance in cattle (Elrod et al., 1993; Appeddu et al., 
1997).

Conversely, Martin et al. (2007b) reported no difference in age at puberty for 
heifers feed a distiller based supplement compared with those fed a corn gluten based 
supplement. These supplements were designed to be isocaloric and isonitrogenous, but 
differed in RUP, with distillers supplemented heifers receiving approximately 267 g/d 
RUP and corn gluten supplemented heifers receiving approximately 90 g/d RUP. 
Furthermore, distillers supplemented heifers had improved AI conception and AI 
pregnancy rates compared with corn germ supplemented heifers, although estrus response 
was similar. These data differ from those reported by Lalman et al. (1993) who found 
increased age at puberty for RUP supplemented heifers. In their study, RUP 
supplemented heifers received approximately 421 g/d RUP supplement (Lalman et al., 
1993). Furthermore, this supplement was comprised of bloodmeal as the main protein 
source. In the study conducted by Martin et al. (2007b) heifers supplemented distillers 
consumed approximately 267 g/d RUP. This supplement was fed at 0.59% BW and thus 
take increased throughout the trail. However, maximum RUP intake levels were 318 
g/d. The average RUP (g/d) offered by Martin et al. (2007b) was approximately 63% and 
75% at maximum intake compared with the RUP supplement level offered to heifers by 
Lalman et al (1993). Thus differences in RUP intake in the two studies could be one
factor altering the results. Also, the source of RUP differed in these studies with the main protein source being animal compared with plant derived for Lalman et al (1993) and Martin et al. (2007b), respectively.

**Compensatory gain**

The phenomenon of compensatory growth was coined by Bohman (1955) and described as a period of accelerated and/or more efficient growth that usually follows a period of growth restriction (Drouillard and Kuhl, 1999). Typically compensatory growth results after a period of reduced nutrient intake; however, environmental factors such as extreme temperatures, disease, plant toxins, or parasite infection can also alter cattle growth rate and when alleviated can result in compensatory growth (Drouillard and Kuhl, 1999). Although the mechanisms by which compensatory gain occurs are not well understood, Carstens et al. (1991) and Sainz et al. (1995) hypothesized the increased growth rate was attributed to reduced maintenance requirements for smaller animals and increased feed intake and gastrointestinal tract fill, coupled with increased net energy content of gain, improving/increasing calf BW gain (Sainz and Bentley, 1997). Funston and Larson (2011) reported a compensatory gain effect for heifers developed on CR compared with those developed in the DL. During CR grazing, CR heifers had reduced ADG compared with DL-developed heifers. However, after AI, CR-developed heifers had greater ADG compared with DL-developed heifers (0.67 vs. 0.47 ± 0.05 kg/d). Grings et al. (2007) reported heifers developed to gain rapid weight at the end of development had reduced BW compared with constant gain heifers at the end of the low quality forage feeding period (approximately 90 d prior to the breeding season). However during the rapid growth phase, delayed gain heifers gain 0.44 kg/d (± 0.03 kg/d) faster
than control gain heifers, allowing heifer BW at prebreeding to be similar among constant and late- rapid growth heifers. Similarly, Freety et al. (2001) reported heifers fed a high ME diet (263 kcal ME/(BW_{kg}^{0.75}) from weaning to breeding had greater hip height and BW compared with heifers fed a low ME diet (157 kcal ME/(BW_{kg}^{0.75}) for the first 84 d post weaning followed by a higher ME diet (277 kcal ME/(BW_{kg}^{0.75}) from d 84 to the breeding season. Freety et al. (2001) also reported an increase in the ratio of BW gain:ME intake (kg/Mcal) during d 85 to 168 of development for heifers feed the low-high ME diet compared with those fed the high ME diet throughout development.

In each of these studies, heifers on reduced intake or energy diets, or grazing winter forage had reduced BW during development, but when nutrition/nutrient availability was increased BW gains improved. Furthermore, each study reported similar pregnancy rates among treatment groups (Freetly et al., 2001; Grings et al., 2007; Funston and Larson, 2011). Cows with reduced BW have reduced maintenance requirements, and Jenkins et al. (1986) suggested these animals have reduced liver mass. It has been reported that during reduced growth phases, body composition is altered (Hornick et al., 2000). Fat deposition in more effected during nutrient restriction compared with protein deposition, and thus the body becomes leaner. During this time (feed restriction) metabolism is reduced due to decreases in volume and metabolic activity of viscera (Hornick et al., 2000). In cases of prolonged nutrient restriction, plasma concentrations of anabolic hormones decrease and catabolic hormones increase. Growth hormone concentration is increased during restriction feeding and IGF-1 levels have been reported to be reduced (Breier and Gluckman, 1991). Jennings et al. (2011) also reported an increase in GH concentration at mid restriction for low energy fed cattle,
but also reported increased plasma leptin concentrations in high energy fed cattle. Interestingly, there was no difference in plasma ghrelin concentration based on d of treatment. However, when comparing steers at similar body fat deposition (1.0 cm) steers fed the high energy diet had increased plasma ghrelin concentrations. The increase in plasma ghrelin concentration at similar body fatness is surprising owning to previous literature suggesting ghrelin levels increase during feed restriction causing increases in feed intake (Wertz-Lutz et al., 2006, 2009; Jennings et al., 2011).

After nutrient restriction is alleviated, an adaptation period is required prior to the expression of compensatory growth. In ruminants it is suggested this adaptation time is approximately 1 mo and compensatory growth rates are considered cubic in nature (Hornicks et al., 2000). This maximal growth rate reached approximately 1 mo after resumption of improved nutrition lasts for approximately 1 mo prior to gain becoming reduced.

**Feed efficiency**

It is well understood differences among animals to convert feed into BW is correlated to profit for beef production (MacNeil et al., 2011). Feed costs for maintenance represent 60-75% of the total feed costs for the animal (Ferrell and Jenkins, 1985; Arthur et al., 2001). Two methods extensively utilized to determine the animal efficiency are feed conversion ratios and residual feed intake (RFI). Feed conversion ratios, such as feed:gain or gain:feed (G:F) measure the amount of feed consumed (DMI) by the difference in weight gain for a certain time period, or vice versa. The challenge with using feed conversion ratios as a selection criterion is this calculation takes into consideration measures of body size, growth rate, the composition of gain, and appetite
(Arthur et al., 2001). Thus, selecting animals based on feed conversion ratio would be beneficial for growing animals allowing for increased gains, but would also result in larger mature animals in the breeding herd which would increase maintenance demands (Archer et al., 1999).

Koch et al. (1963) suggested efficiency of feed use is not a directly measurable trait, but must be calculated from component traits. This calculation led to the residual feed intake (RFI) calculation which takes into consideration the mid-test BW of the individual and their cohorts, and their ADG compared with their cohorts. Furthermore, slope coefficients for midtest BW and ADG must be calculated as outlined by Cammack et al. (2005). Residual feed intake is the difference in the predicted DMI and actual DMI, thus animals consuming less than expected would have a negative RFI value and would be considered more efficient. Residual feed intake is considered an improved method of estimating efficiency, compared with growth ratios, due to its independence from growth and body size (Crews, 2005; Crowley et al., 2010). Heritability of RFI is moderate with studies reporting estimates to be between 0.27 and 0.54 (MacNeil et al., 1991; Crews, 2005; Nkruman et al., 2007; Lancaster et al., 2009). Furthermore, feed intake was more strongly correlated with RFI ($r_g = 0.69$) compared with feed conversion ratio ($r_g = 0.31$) in young Angus bulls and heifers (Arthur et al, 2001).

Although some variation has been reported concerning RFI, Kelly et al. (2010a), indicated 35% of this variation can be attributed to feeding behavior, carcass composition measures, and circulating blood metabolites. Animals with lower RFI (more efficient) have reduced daily feeding activity compared with high RFI (less efficient animals; Nkrumah et al., 2006; Kelly et al. 2010a, 2010b) with a positive relationship between
number of feedings events and eating rate. One hypothesis for improved RFI in low RFI animals would be increased sedentary time would decrease energy requirements of the animal and could serve as an energy-sparing mechanism (Kelly et al., 2010b). There are also some reports in the literature concerning the association of carcass fatness and RFI. These studies indicate RFI may be related to fatness (Koots et al., 1994; Herd and Bishop, 2000) leading some to believe an adjustment in RFI calculation is needed to account for animal fatness.

One challenge with utilizing RFI as a measure of animal efficiency is the potential of an animal changing its feed efficiency due to alterations in diet, age, or potentially physiological status. Durunna et al. (2011) reported 58% of steers fed a growing diet had a 0.24 to 0.38 kg/d change in RFI when steers were adjusted to the finishing diet. Similarly, Kelly et al. (2010b) reported a 0.30 kg/d change in RFI in 54% of heifers when comparing RFI at the yearling and finishing phase. Durunna et al. (2012) fed heifers a diet comprised of Barley straw and steam rolled barely for approximately 112 d. Although a similar diet was feed throughout the feeding period, the feeding trail was divided into two phases and heifer RFI calculated for each of the two phases. Heifers were grouped into 3 RFI groups based on phase 1 RFI: high, moderate, and low. Nearly half (49%) of the heifers remained in the same RFI class from period 1 and 2. Approximately 27% of heifers changed their RFI by 1 standard deviation (0.37 to 0.44 kg/d), whereas, 41% changed by less than 0.5 standard deviations. Furthermore, 88% of heifers changing RFI class moved up or down 1 classification group (Durunna et al., 2012) suggesting although change in RFI does occur, the magnitude of the change is
small. In addition, Durunna et al. (2011) reported RFI was still a better estimate of feed efficiency compared with G:F or ADG.

Genetic variation for maintenance energy requirements are moderately to highly heritable (Barsabab et al., 2003). This would suggest utilization of RFI would allow one to select for more efficient animals. One possible explanation for this is the incorporation of live BW and ADG utilized in the RFI calculation. Archer et al. (1999) suggested that using BW and ADG will help capture some of the variation in feed used for growth and maintenance, thus selection of animals based on improved RFI would produce more efficient progeny in the feedlot as well as mature cows. This would suggest estimates of feed efficiency can be a vital tool for different sectors of the beef industry.

**Economics of heifer development**

Management factors such as weaning date, development system, and breeding strategies are just a few considerations that must be made in determining heifer development systems. Feed costs represent the greatest expense to cow-calf producers. Utilizing dormant forages throughout the winter reduces production costs by increasing grazing season length and decreasing the amount of harvested forage needed in beef cattle production systems (Adams et al., 1994). Story et al. (2000) weaned heifers at 150, 210, or 270 d of age. After weaning, heifers were placed in the DL and developed to approximately 65% mature BW. These authors reported increased total development costs for each of the weaning systems with calves weaned at 150 d having the greatest development costs and calves weaned latest have the lowest development costs (Story et al., 2000). Increases in heifer development costs can be directly related to increased
amounts of harvested forage fed, with early weaned calves total feed costs being approximately $82/heifer greater than late weaned heifers.

Studies reducing harvested forages fed during the DL period, while not reporting economic ramifications, likely reduced heifer development costs. One example of reducing harvested forage utilized during development reported developing heifers with similar pregnancy rates on 27% less forage (Roberts et al., 2009a). Maintaining pregnancy rates in beef cattle production systems is vital for profitability. Trenkle and Willham (1977) reported reproduction had an economic value five times greater than calf growth and Renquist et al. (2006) reported longevity influenced profitability. Similarly, Meek et al. (1999) utilizing figures from a commercial cow herd, reported increasing pregnancy rate 1% in yearling heifers results in a net present value (NPV) increase of $4.30. The increase in NPV relates to the increased number of cows remaining in the herd to produce calves in subsequent years. If weaning BW is increased 1% in these same animals as 2-yr old cows, the change in NPV is $2.50, thus we see an increase of nearly $2.00 if improvements in reproduction are made compared with increased weaning BW. Describing the probability a cow would have X number of calves, Feuz (2001) reported the biggest decrease from 1 to 2 calves (~13%). Certainly the correlation of reproduction and economics relates to the ability to distribute development costs over a greater number of calves sold from cows remaining in the herd longer.

The challenge in determining the economic value for a producer to develop heifers compared with purchasing heifers is one must consider the future production of the heifer, the annual costs for keeping her in the herd, as well as the potential profit of her calves and her cull price if ownership is retained (Feuz, 2001). If a producer simply
purchases heifers they must consider the market price for the heifer calves they produce at weaning. Producers using this practice should utilize breeding systems that incorporate heterosis. Maximizing weaning weights would be most beneficial for profitability as all calves (bulls and heifers) would be sold at this time point.

Feuz (1992) developed a formula to determine the net cost of developing one pregnant heifer.

\[
\text{Net cost for 1 pregnant heifer} = \frac{(\text{yr 1 costs} - \text{cull heifer value})}{\text{yearling conception rate}} - \text{percent death loss}
\]

This formula uses the total heifer development cost for the entire group of heifers minus the value of all open and culled heifers. The total adjusted value is divided by the number of heifers exposed, and gives the producer the total cost of a heifer developed within the system. This value is divided by the pregnancy rate minus the percent death loss, giving the cost to develop one pregnant heifer.

Calculating heifer development costs based on this formula are beneficial since it accounts for heifers removed from the herd due to failure to conceive. However, producers should take care to calculate production costs through several calving seasons to determine the impacts their development system may have on overall profitability.

Feuz (2001) reviewed four studies published between 1980 and 1987. In his analysis he suggested heifers developed to 55% mature BW prior to the breeding season had reduced net costs for 1 bred yearling ($776 vs. $785) compared with heifers developed to 65% mature BW. This analysis would suggest producers develop heifers to reduced proportions of mature BW to capitalize on reduced feed costs. However, when these
same heifers are analyzed based on net cost of 1 bred 2-yr old cow, heifers developed to 55% mature BW cost $50/cow more compared with those developed to 65% mature BW. Reduced pregnancy rates as 2-yr olds and decreased calf weaning BW for cows developed to 55% mature BW are the main factors attributing to increased costs for developing heifers to lighter BW (Feuz, 2001).

It should be restated Feuz (2001) conducted his analysis on data recorded 25-30 yr ago. Current literature reports developing heifers to reduced BW reduces net cost of producing 1 pregnant heifer (Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011). Funston and Larson (2011) reported a $45/pregnant heifer decrease for heifers developed on a combination of WR and CR compared with those developed in the DL. Interestingly, if heifers are developed on either WR or CR or remain on WR there is no difference in net costs ($/pregnant heifer) due similarities in performance and prices for CR and WR (Larson et al., 2011).

Similar to Feuz (2001), Martin et al. (2008) reported a $24/pregnant heifer decrease for heifers developed to 51% mature BW compared with 57% mature BW. However, unlike Feuz (2001), Martin et al. (2008) report reduced net costs per pregnant heifer as 2-yr old cows in heifers with reduced BW at the beginning of their first breeding season. Although, Funston and Deutscher (2004) did not report economic results for their data, they did report pregnancy rates out to 4 pregnancies. In their study, pregnancy rates were similar for heifers developed to 53% compared with 58% mature BW at the beginning of the first breeding season. Furthermore, calf weaning BW was similar each yr between treatment groups. In the review conducted by Feuz (2001) both reduced pregnancy rates and decreased weaning BW were the driving factors impacting
profitability of heifers developed to reduced proportions mature BW. These contemporary data suggest economic returns can be greater for heifers developed to reduced proportions mature BW due to improvements in reproductive efficiency of cattle.

**PUBERTY ATTAINMENT**

To calve by 2 years of age, heifers must become pregnant at or before 15 months. Prior to becoming pregnant, the reproductive tract must mature and the heifer must attain puberty. Puberty has been defined as the time when ovulation is accompanied by signs of estrus and luteal function (Moran et al., 1989). Maturation of the neuroendocrine system, which allows for the development and ovulation of a mature oocyte, induces puberty. During the transition to sexual maturity, the negative feedback of estradiol (E2) at the hypothalamus is decreased, which leads to increased LH-pulse frequency, resulting in ovarian follicle development (Figure 7; Kinder et al., 1995). This period of time in which the change in E2 feedback occurs, in regard to LH secretion, is often referred to as the peripubertal period and begins approximately 50 d prior to puberty attainment in heifers (reviewed in Perry, 2012).

As ovarian follicles develop, enough E2 is produced to induce a preovulatory surge of gonadotropins and behavioral estrus (Kinder et al., 1995). Although a relatively low proportion of heifers can attain puberty prior to weaning (< 8 months old), most heifers attain puberty at approximately 11 months of age (Senger, 2003). The rate of BW gain from birth to puberty is correlated to the age at which a heifer will reach puberty, with preweaning rates of gain having a greater impact on age at puberty compared with postweaning gains (Patterson et al., 1992). Furthermore, heifer weaning BW has been
correlated to puberty, with an increased probability of heifers reaching puberty prior to the breeding season as weaning BW increases (Patterson et al., 1992).

Age at puberty attainment is influenced by several factors including nutrition, breed and management. Ciccioli et al. (2005) reported heifers fed 0.9 kg/d 42% CP (DM Basis) supplement for 30 and then a high-starch diet in the feedlot 60 d had reduced age at puberty compared with those self fed a low-starch diet 30 d while grazing grass pasture or control fed heifers. Wiltbank et al. (1969) fed straightbred or crossbred heifers either 1.5 kg/d concentrate or 0.2kg/d concentrate from weaning until puberty attainment. The authors reported straightbred heifers fed the reduced concentrate diet had a 191 d delay in puberty compared with the straightbred heifers fed the higher concentrate diet. However, the increased age at puberty for crossbred heifers fed the low concentrate diet was only 43 d compared with crossbred heifers fed the high concentrate diet. Similarly, Stewart et al. (1980b) reported decreased age at puberty for purebred compared with crossbred heifers and bulls and decreased age at puberty for pen confined fed cattle compared with pasture developed animals receiving supplemental hay and concentrates. These studies indicate the impact heterosis can have on puberty attainment, especially in a nutrient restricted development system. In a recent study, Freetly et al. (2011) reported the relationship of proportion mature BW and puberty attainment was conserved across several cattle breeds. Bos taurus cattle attained puberty at 0.56 to 0.58 proportion mature BW, whereas Bos indicus cattle attained puberty at 0.60. Similarly this study reported a 3-10 wk decrease in age at puberty for calves sired from Bos taurus compared with Bos indicus sires.
Decreasing age at weaning can also impact age at puberty. Gasser (2006) reported weaning heifers at 4 to 6.5 mo of age and feeding a high concentrate diet increased the proportion of heifers attaining puberty prior to 300 d compared with those fed a control diet. Unfortunately, this study did not have any heifers remaining with their lactating dam to determine the differences between early wean compared with normal wean. However, earlier data (Holloway and Totusek, 1973) reported heifers weaned at 140 d of age were lighter and had reduced gains at 240 d of age when cohorts were weaned. Although this study did not report pubertal status of animals on these treatments, it is likely heifers weaned at 140 d of age would have reduced proportions attaining puberty prior to the breeding season. This hypothesis would be supported by the literature showing an increased proportion of heifers attaining puberty as weaning BW increases (Patterson et
al., 1992). Although previous data support a positive relationship between preweaning ADG and pubertal status prior to the breeding season (Patterson et al., 1992), recent data suggests calf age has a greater impact on pubertal status (Funston et al., 2012). Heifers born in the first 21 d of the calving season were more likely to be cycling prior to their first breeding season compared with those born in the third 21 d of the calving season (Funston et al., 2012).

**ESTROUS CYCLE**

The estrous cycle marks the cyclical pattern of ovarian activity allowing females to go from a non-receptive to receptive reproductive state (Forde et al., 2011). These cycles last approximately 18-24 d in cattle and are comprised of two distinct phases: luteal and follicular. The follicular phase is the period following luteolysis of the CL until ovulation of the dominant follicle and is separated into the proestrus and estrus stages (Senger, 2003; Forde et al., 2011). The luteal phase of the estrous cycle is approximately 14-18 d long and follows ovulation and can be separated into the metestrus and diestrus stages (Senger, 2003). This period coincides with the presence of the corpus luteum (CL) and increased concentrations of P4.

Similar to events prior to pubertal attainment, the estrous cycle is regulated by hormonal stimulation. Hormones produced in the hypothalamus (gonadotropin-releasing hormone; GnRH), anterior pituitary (LH and FSH), ovaries (P4; E2, and inhibin) and the uterus (prostaglandin F2α; PGF) help regulate the estrous cycle via a positive and/or negative feedback system (Senger, 2003; Forde et al., 2011). Estrous cycle hormone concentration varies based on day of the estrous cycle and is indicated in Figure 8. Furthermore, the bovine estrous cycle typically contains 2 or 3 waves of follicular
growth. Following calving, beef cattle typically undergo anestrous or a quiescent period of time in which ovulation does not occur. This period can last for between 30-130 d (Forde et al., 2011) and can be impacted by physiological status and nutrient intake. Resumption of the estrous cycle early in the post partum period is crucial to maintain a 365 d calving interval in both dairy and beef cows.

**Figure 8.** Schematic depiction of the pattern of secretion of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone (P4); and the pattern of growth of ovarian follicles during the estrous cycle in cattle. Each wave of follicular growth is preceded by a transient rise in FSH concentrations. Healthy growing follicles are shaded in yellow, atretic follicles are shaded red. A surge in LH and FSH concentrations occurs at the onset of estrus and induces ovulation. The pattern of secretion of LH pulses during an 8-h window early in the luteal phase (greater frequency, lesser amplitude), the mid-luteal phase (lesser frequency, lesser amplitude) and the follicular phase (high frequency, building to the surge) is indicated in the inserts in the top panel (adapted from Forde et al., 2011).
**Follicular phase**

Although follicle growth from 0.3 to 5 mm in diameter takes more than 30 d, subsequent development can be rapid (Lussier et al., 1987). During the follicular phase, a cohort of follicles, typically 5-20, are recruited for growth and maturation. These follicles grow in wave-like patterns and are preceded by increased concentrations of serum FSH (Adams et al., 1992, 1994b; Evans 2003). As previously mentioned the number of follicles growing within a wave, although different among individuals, is highly repeatable in an individual (Jimenez-Krassel et al., 2009; Mossa et al., 2010). During this period, follicle growth is driven by FSH and receptors for the hormone are localized in the granulosa cells of the growing follicles as early as d 3 of the follicular wave (reviewed in Forde et al., 2010). Selection of a dominant follicle within the wave typically occurs between 36 and 48 h after the initiation of the follicular wave (reviewed by Bao and Garverick, 1998). Furthermore, dominance occurs with divergence in size of one follicle over the other follicles in the cohort and from that time forward there is only one healthy (dominant) follicle in each animal (reviewed by Bao and Garverick, 1998). When the selected dominant follicle reaches a mean diameter of approximately 4 mm, FSH concentrations are at their maximum level (Ginther et al., 1996). After emergence of the dominant follicle, other follicles in the cohort regress and undergo atresia. Furthermore, E2 and inhibin concentrations in the dominant follicle follicular fluid are able to have a negative feedback effect on FSH release reducing it to basal concentrations (Sunderland et al., 1994; Ginther et al., 2000).

As FSH concentrations decrease, the dominant follicle becomes more responsive to LH with receptors present in the granulosa and theca cells (Camp et al., 1991; Xu et
al., 1995). Throughout the follicular phase, P4 concentrations are reduced to basal level. Furthermore, E2 stimulates a GnRH surge from the hypothalamus resulting in sufficient concentrations of LH to be released from the anterior pituitary (reviewed in Forde et al., 2011). This wave of LH stimulates the final growth and maturation of the dominant follicle. With P4 concentrations at basal levels for 2-3 d and LH pulses occurring every 40-70 min, ovulation of the dominate follicle occurs (Roche, 1996). The increased concentrations of E2 also play an important role in the animal expressing estrus behavior required for successful mating (Ireland, 1987; Frandson et al., 2003).

Estrus behavior varies among animals and can be influenced by environmental factors such as footing surface type, size of the sexual active group, presence of a bull, and physiological status (Diskin, 2008). In dairy cows standing estrus duration averaged 8.1 h with 9.1 standing events/ mounts occurring during that time (Diskin, 2008). Interestingly, milk production level impacts standing estrus length, with cows yielding 25 kg milk/d standing estrus period 14.7 h. Alternatively, cows yielding 55 kg milk/d had a standing estrus period of approximately 2.8 h (Lopez et al., 2004). Beef cattle standing estrus behavior was reported to be less than 8.5 h (Diskin, 2008). However these animals were kept indoors and this environmental factor could have impacted length of standing estrus compared with cows remaining outdoors.

**Luteal phase**

Formation of the CL occurs within the first 3-4 d after ovulation of the dominate follicle from the location of the collapsed ovulated follicle. The primary role of the CL in the bovine is production of P4 to allow for establishment and maintenance of pregnancy. Although P4 concentrations are elevated during the luteal phase, FSH is secreted during
this time and follicular waves present. Although a dominant follicle from each wave will be selected, due to the negative feedback of P4 on the hypothalamus, GnRH levels typically will not stimulate a large enough release of LH to allow for ovulation of these follicles (Rahe et al., 1980). The dominant follicle in these estrous waves will undergo atresia similar to follicles not selected for dominance in ovulatory follicle waves. Formation of the functional CL coincides with the diestrus stage of the estrous cycle. This stage is the longest of the estrous cycle lasting 10-14 d (Senger, 2003).

High concentrations of P4 secreted from the CL during this stage prompt the uterus to prepare for pregnancy and eventual implantation to the endometrium (Senger, 2003). As previously mentioned in this review, low P4 serum concentrations have been related to increased embryonic mortality (Inskeep, 2004). If pregnancy does not occur, luteolysis, or degradation of the CL will occur, allowing the resumption of the estrous cycle in the animal. Luteolysis is controlled by the production of oxytocin and P4 produced from the CL and PGF produced by the uterine endometrium (Senger, 2003). As the luteal phase progresses, prolonged high concentration of P4 loses the ability to block E2 and oxytocin receptors located in the uterine endometrium. Increased levels of oxytocin secretion stimulate the release of PGF from the uterus. Prostaglandin F2α is transported to the ovary via a vascular countercurrent exchange mechanism, allowing for adequate quantities of PGF to reach the ovary and initiate luteolysis (Senger, 2003).

If the female becomes pregnant, interferon τ (INF-τ) is secreted from the trophoblast cells of the blastocyst. Interferon τ is important for regulation of early pregnancy due to its secretion prior to implantation. Interferon τ binds to the endometrium and inhibits oxytocin receptor synthesis by endometrial cells (Senger,
Due to decreased binding of oxytocin, PGF secretion will not be increased and inadequate levels of PGF will be synthesized for luteolysis of the CL. The CL is of vital importance in cattle to help maintain pregnancy and is the main source of P4 secretion until approximately 6-8 mo gestation, when the placenta takes over as the main P4 secretion source (Senger, 2003).

*Estrus synchronization*

Estrus synchronization has been reported to increase the proportion of cows conceiving earlier in the breeding season resulting in a more uniform calf crop (Dziuk and Bellows, 1983). Heifers calving earlier in the calving in season have been reported to wean heavier calves (Schafer et al., 1990) and have greater productivity throughout their lifetime (Lesmeister et al., 1973; Kill et al., 2012). Thus utilization of estrus synchronization could be beneficial for producer implementation. Typically estrus synchronization is utilized prior to artificial insemination to reduce labor inputs. However, recent reports indicate only 7.2% of all beef cattle operations in the United States utilize artificial insemination, a proportion similar to that reported nearly 20 yr earlier (NAHMS 1997, 2009). Thus it is likely to suggest the utilization of estrus synchronization protocols is limited in the United States.

Historical data indicates P4 was utilized as a treatment to synchronize estrous cycles of dairy cows and beef heifers in the mid 1950’s (reviewed by Lauderdale, 2009). These studies injected varying amounts of P4 suspended in corn oil or starch. Trimberger and Hansel (1955) reported cow receiving P4 injection displayed estrus 4.6 d later although pregnancy rates were low. Nellor and Cole (1956) reported increased length of time between P4 treatment and estrus as P4 treatment dose increased. These studies and
others stimulated research to develop commercially active compounds that would aid in control of the estrous cycle (Lauderdale, 2009).

In the 1960’s orally active synthetic progestins (medroxyprogesterone acetate, MAP; chlormadinine acetate, CAP; dihydroxyprogesterone acetophenonide, DHPA, melengestrol acetate, MGA) were reported to be a viable option for estrus synchronization in heifers (Hansel et al., 1961; Zimbelman, 1963; Wiltbank et al., 1967; Hansel et al., 1966; Zimbelman and Smith 1966a,b). These studies reported decrease pregnancy rates in animals if bred the estrus following the termination of supplement feeding; however, Hansel et al., (1966) reported heifers supplemented MAP or CAP had increased pregnancy rates for AI at synchronized estrus and subsequent estrus compared with control animals.

Today, MGA is utilized in heifer development and feedlot operations for estrus synchronization, or estrous inhibition. Early research conducted feeding MGA reported an effective dose of 0.5 mg/hd fed 14 to 18 d resulted in increased BW gain and efficiency in pubertal heifers due to depression of estrus (Bloss et al., 1966). In estrus synchronization protocols, MGA is fed for 14 d then 19 d after termination of MGA feeding a single injection of PGF is administered. Following PGF administration, estrus detection and AI are performed. Although heifers will display estrus shortly after the termination of MGA feeding, AI at this time will result in reduced fertility rates compared with after PGF administration. Supplementation of MGA and low progesterone type supplements results in the formation of large persistent follicles on the ovary due to continued low dose pulses of FSH and LH during supplementation. Fertility in these
follicles is compromised due to altered hormone production and likely due to increased age of the follicle (Inskeep, 2004).

Use of MGA is prohibited in mature beef cattle and most estrus synchronization is conducted using a protocol involving 2 injection of PGF given approximately 10-14 d apart, or utilizing an exogenous progestin with hormone treatment. Benefits of utilizing the 2 injections of PGF include reduced labor and ease of administration of the protocol. It is well established PGF is luteolytic (Lauderdale, 1972). Thus administration of the first injection should result in luteolysis of the CL in 70% of cattle if animals in a herd are evenly distributed across days of their estrous cycle (Odde, 1990). However, it has been reported PGF and its analogues are ineffective in causing luteolysis in early stages of the estrous cycle (reviewed in Odde, 1990). The second administration of PGF in the 10-14 d protocol should result in luteolysis of the CL in these as well as the other animals in the herd. Results utilizing this method of synchronization have been mixed, with early results indicating no improvement in pregnancy rates compared with control animals (reviewed in Odde, 1990).

Further efforts have been made to develop synchronization protocols that will allow for improved synchrony of estrus and increased pregnancy rates. Utilization of exogenous progestins in concert with GnRH at the beginning of the breeding season has proven to be successful at improving estrus synchronization. One reason administration of a progestin along with GnRH could be progestins can help aid in initiating estrus and ovulation in prepubertal heifers and anestrous cows (Anderson et al., 1996; Imewalle et al., 1998). A well known exogenous progestin is the controlled internal drug release (CIDR). This device contains 1.38 g of progesterone released into the animal after the
CIDR is inserted into the vagina. Lucy et al. (2001) conducted a study across several states and reported a greater proportion of beef cows treated with CIDR + PGF showed estrus activity within the first 3 d of the experiment compared with cows treated with PGF or controls. Increased cyclicity in CIDR + PGF treated cows led to an increased pregnancy rate during the 3 d period and tend to increase pregnancy rates over the 31 d breeding season compared with control and PGF treated cows. Similarly there was an increased proportion of heifers displaying estrus and pregnancy rates if treated with the CIDR + PGF protocol. However, this study also reported previous luteal activity impacted synchronization and pregnancy rates with prepubertal and anestrous cows having reduced pregnancy rates (Lucy et al., 2001).

**FOLLICLE MORPHOLOGY**

Follicular growth and development is driven by hormonal stimulation. During gestation primordial follicles form and arrest until stimulated by gonadotropins to begin the maturation process. Follicles develop from immature primordial follicles to mature antral follicles through a series of stages or developmental steps, with follicle growth and morphological changes taking place. Primordial follicles consist of an immature oocyte surround by a single layer of squamous (flattened) cells. Epithelial cells surrounding the oocyte undergo a structural change from squamous to cuboidal (cube-like) as the follicle develops into a primary follicle (Senger, 2003). As maturation continues, the follicle becomes a secondary follicle which is characterized as having two or more layers of follicle cells. A tertiary or antral follicle is characterized by the fluid filled cavity known as an antrum (Senger, 2003). Antral follicles differ in size, but these follicles, as mentioned earlier, are an important predictor of ovarian reserve (Cushman et al., 2009;
Ireland et al., 2012). Furthermore, one antral follicle from each estrous wave will be selected as the dominant follicle as previously discussed. Antral follicles contain three distinct follicle cell layers; theca interna, theca externa and granulosa cells, as well as the cumulus oocyte complex (COC). Upon ovulation the COC is expelled from the follicle and the CL formed with the luteinized granulosa and theca cells.

**Steroidogenesis**

The theca and granulosa cells of the follicle play an important role in steroidogenesis. The first evidence of both theca and granulosa cells interacting to produce E2 was reported by Flack (1959) indicating estradiol production in rat follicles required both cell types. Steroidogenesis is the process involving the conversion of cholesterol to androgens and estradiol in the ovary, and mineralocorticoids or glucocorticoids in the kidney. Although steroid hormones can be produced in the kidney, the majority of steroid hormone production occurs in the reproductive organs of the animal, thus we will focus on steroidogenesis in the ovary in this review. Steroidogenesis in humans and ruminants occurs through the $\Delta^5$ pathway whereas steroidogenesis in rodents and pigs can occur through either the $\Delta^4$ or $\Delta^5$ pathways (Figure 9; Conley and Bird, 1997).
Figure 9. The $\Delta^4$ and $\Delta^5$ pathways of gonadal steroid synthesis. Pathway utilized is species specific and dependant on enzymatic activity. Beef cattle utilized the $\Delta^5$ with cholesterol synthesized to pregnenolone (P5), 17OH-pregnenolone (17OH-P5), dehydroepiandrosterone (DHEA), androstenedione (A4) in the theca cells of the follicle and estrone (E1) and estradiol (E2) in the granulosa cells. Stage of the estrous cycle alters enzymatic activity with increased P450c17 enzymatic activity during the follicular phase driving estradiol production and increased $3\beta$-HSD enzymatic active during the luteal phase supporting increased progesterone (P4) formation (Adapted from Conley and Bird, 1997).

In order for cholesterol to be synthesized into E2 it must be transported from the cytosol into the mitochondria and this is referred to as the rate limiting step of steroidogenesis. Steroidogenic acute regulatory protein (StAR) is responsible for the transport of cholesterol into the mitochondria (Stocco and Clark, 1996). The binding of StAR to the outer membrane of the mitochondria alters the conformation of the membrane and creates a cholesterol binding pocket (Miller, 2007). Following entry into the mitochondria, cytochrome P450 side-chain cleavage (P450$_{scc}$ or CYP11A1) enzyme cleaves the side chain of cholesterol forming pregnenolone (P5). In the $\Delta^4$ pathway, P5 is converted to P4 via $3\beta$ –hydroxysteroid dehydrogenase ($3\beta$-HSD) enzyme activity. Through this steroidogenic pathway ($\Delta^4$) E2 is produced via a series of enzymatic
reactions from P4 (Figure 9) which will not be discussed in full here. The Δ⁵ pathway will be discussed in full due to it being the major steroidogenic pathway of the beef cow.

After conversion of cholesterol to P5, cytochrome P450 17α-hydroxylase (P450c17 or CYP17A1) enzyme oxidizes P5 to 17α-hydroxypregnenolone. Next, dehydroepiandrosterone (DHEA) is produced by the cleavage of the side chain from 17OH-P5. Prior to the conversion of DHEA to androstenedione (A4), DHEA is transported from the theca to the granulosa cells where the third Carbon is dehydrogenated by 3β-HSD to form A4. In the granulosa cells, cytochrome P450-aromatase (P450arom) enzyme hydroxylates A4 in two places forming estrone (E1). A final hydroxylation from E1 forms E2 via 17β-hydroxysteroid dehydrogenase (17β-HSD). It should be noted A4 can also react with 17β-HSD forming testosterone (T), which then is hydroxylated via P450arom to form E2. As described above, each conversion of substrate in the steroidogenic pathway is enzyme specific. Although certain enzymes can be utilized for more than one reaction in the conversion of cholesterol to E2 (i.e. 3β-HSD), production of substrates is site specific, with enzyme expression for each step of steroidogenesis being regionalized to the theca or granulosa cells of the follicle (Conley and Bird, 1997).

Early studies concerning steroidogenesis produced the two cell two gonadotropin model in which it was believed LH receptors on the theca cells increased androgen production whereas FSH receptors on the granulosa cells catalyzed the production of E2 (reviewed in Fortune and Quirk, 1988). While this model is a useful general model, Conley and Bird (1997) suggest the use of this model in species where the Δ⁵ pathway dominates may not be as applicable due to a Δ⁴ block. In the Δ⁵ pathway, CYP17A1 and
3β-HSD enzymatic activity is regulated in the theca and granulosa cells, respectively. This separation of the enzymes, which drives the two pathways may be important in determining the end product of steroidogenesis. Remember in the Δ⁴ pathway both enzymes are present in the theca cells and high concentrations of 3β-HSD will lead to the production of A4 and P4 in the theca cells (Figure 9). Whereas in species which undergo steroidogenesis via the Δ⁵ pathway, production of A4 in the theca cells is blocked due to the relative Δ⁴ block (Conley and Bird, 1997). Due to the dynamics of hormone production during the estrous cycle, it has been hypothesized expression of CYP17A1 is relatively high during the follicular phase compared with 3β-HSD to allow for maximal production of E2. Conversely, during the luteal phase production and expression of CYP17A1 is reduced compared with the level of 3β-HSD enzyme activity allowing for increase production of P4 in the CL (Conley and Bird, 1997).

**Theca cells**

Theca cells are important for the production of androgens during steroidogenesis (Conley and Bird, 1997). Although formation of the theca cell layer does not occur until the follicle contains two or more layers of granulosa cells (Young and McNeilly, 2010) we will discuss the theca cell first due to the initial intermediates of the steroidogenic pathway being produced in this cell type. Theca cell formation coincides with the follicle becoming responsive to LH and activation of steroidogenic enzymes (Magoffin and Weitsman, 1994). Theca cells are not present in the primordial follicle, but can be seen as early as the primary follicle. These cells are thought to be derived from the ovarian stroma although the factors that stimulate the formation of this cell layer are not well understood (reviewed in Young and McNeilly, 2010). Follicles < 3mm contain flattened
theca cells containing capillaries and bundles of collagen (O’Shea et al., 1978). As the follicle grows theca cells undergo hypertrophy and increase in amount of cellular organelles as well as lipid droplets. Theca cells are highly differentiated and contain large quantities of mitochondria and endoplasmic reticulum (reviewed in Magoffin, 2005). This is critical since the first step of steroidogenesis is the transportation of cholesterol into the mitochondria for cleavage of the side chain via CYP11A1. The remaining steps resulting in production of DHEA in the theca are completed in the endoplasmic reticulum (Young and McNeilly, 2010) prior to androgens being transported to the cytoplasm of the granulosa cells for conversion to E2.

The importance of theca cell function has been illustrated through several different experiments. McNeilly et al. (2000) reported although androgen and eventually E2 production can occur in an ovary devoid of oocytes, silencing of CYP17A1 results in declined production of A4, 17OH-P4, and P4 levels in the rat ovary (Lie et al. 2009). Remember, the rat and other rodents utilized the Δ⁴ pathway of steroidogenesis. Silencing of CYP17A1 would result in a direct reduction in the ability to produce A4, but should not impact P4 production since P4 is the substrate CYP17A1 hydroxylates to form 17OH-P4 (Figure 9). Impairment of CYP17A1 function must also impact steroidogenic mediators upstream in the steroidogenic pathway or causes a shift from the Δ⁴ to Δ⁵ pathway resulting in increased production of 17OH-P5 from P5. However, silencing of CYP17A1 would also inhibit the formation of DHEA from 17OH-P5 in the Δ⁵ pathway, which could also play a role in the reduction of A4 produced.

Cultured theca cells exposed to low LH concentrations stimulated androgen production, whereas high LH concentrations resulted in the inhibition of A4 and
stimulated secretion of P4 (Campbell et al., 1998; Ryan et al., 2009). These data support the previously described hypothesis by Conley and Bird (1997) in which low LH concentrations, as would be seen during the early follicular phase of the estrous cycle, would result in increased regulation of enzymes promoting E2 production. However, as follicles mature and near ovulation, large quantities of LH are present and result in a reduction of enzymatic activity promoting E2 production and increase enzymatic activity promoting P4 production. Theca cells initiate steroidogenesis just prior to the formation of the antrum in the follicle. Logan et al. (2002) reported mRNA expression for StAR, CYP11A1, CYP17A1, and 3β-HSD, and LHr present in preantral follicles. Furthermore, expression of these steroidogenic enzymes appears to increase as antral follicle growth continues (Xu et al., 1995; Boa et al., 1997).

**Granulosa cells**

Continuation of the steroidogenic pathway occurs in the granulosa cells with enzymatic activity for 3β-HSD, P450arom, and 17β-HSD present allowing for the conversion of DHEA to E2 (Conley and Bird, 1997). Similar to theca cell steroidogenic mRNA expression, abundance of steroidogenic enzymes in the granulosa cells (P450arom and 17β-HSD) increases as follicles progress from small to large antral stages (Bao et al., 1997). Production of E2 in the granulosa cells is important for the feedback mechanisms involving the hypothalamic-pituitary-gonadal axis, and thus reproductive function. In fact, mouse P450arom knock-out models do not ovulate and have reduced ovarian reserve, which are hypothesized to be due to the inability of these mice to produce E2 (Fisher et al., 1998; Britt et al., 2004). Granulosa cells are present in primordial follicles and comprise the squamous layer of cells surrounding the oocyte. The
transition of a primordial to primary, or small growing follicle, is characterized by the
morphological change of granulosa cells from squamous to cuboidal. Follicle stimulating
hormone receptors are present on the granulosa cells and FSH helps promote cell
proliferation, E2 production and LHr expression (Richards, 1994). Furthermore, FSH
prevents granulosa cell apoptosis and follicular atresia (Chun et al., 1996). The presence
of granulosa cells is also important for proper theca cell steroidogenic function. Fortune
(1986) reported increased androgen secretion from the theca if theca cells were combined
with granulosa cells in culture and treated with LH compared with cultures containing
only theca cells. Along with the production of E2, the granulosa cell is also essential for
the production of inhibin, follicular fluid, and growth factors (Senger, 2003).

**Growth factors**

There are several growth factors, or growth factor families secreted by the follicle. These factors include insulin-like growth factors (IGF), transforming growth factor-β (TGF-β), AMH, growth differentiation factor 9 (GDF9), and the vascular endothelial
growth factor family (VEGF), to name a few. Insulin-like growth factor-I can be
produced in granulosa cells and stimulates the expression of CYP17A1 in rat theca cells
(Magoffin et al., 1993). Similarly, IGF-1 has been reported to increase theca cell
proliferation in vitro (reviewed in Young and McNeilly, 2010). Furthermore, it appears
several growth factors including activin, inhibin, and IGFs play an important role in the
differential regulation of CYP17A1 and 3β-HSD expression through a variety of
mechanisms (reviewed by Conley and Bird, 1997).

Anti-Müllerian hormone is a member of the TGF-β superfamily (La Marca and
Volpe, 2006). Initial understanding of AMH was through its role in regression of the
Müllerian ducts in the fetal male. However, AMH is also expressed in granulosa cells of healthy follicles from the secondary to early antral stage of development (Duringer et al., 2002; La Marca and Volpe, 2006). Also, AMH concentration has been positively associated with the number of healthy oocytes in mouse ovaries (Kevenaar et al. 2006) as well as a reported 2-6 fold increase in AMH concentration as AFC increased from low to intermediate or high in young adult beef heifers (Ireland et al., 2008). One possible explanation for increased AMH concentrations in low AFC heifers is the negative correlation between AMH and the total number of primordial follicles recruited into a follicular wave (di Clemente et al., 1994). Furthermore, AMH decreases the responsiveness of FSH, which would also reduce the number of follicles recruited. Expression is increased in growing preantral and antral follicles but not present in primordial follicles. Expression of AMH is influenced by health of the follicle with most expression occurring in the outer layers of granulosa cells and cumulus cells. Interestingly, Rico et al. (2011) reported a decrease in AMH expression in atretic follicles, but an increase in AMH expression in cumulus cells of the same follicles.

Growth differentiation factor 9 is produced primarily in the oocyte, but has also been reported to be produced in the granulosa and theca cells of certain species (reviewed in Young and McNeilly, 2010). This growth factor is important for folliculogenesis with mutations in the GDF9 gene resulting in arrestment of the follicle at the primary follicle stage in sheep, mice, and humans (reviewed in Young and McNeilly, 2010). Furthermore, GDF9 plays a role in cumulus expansion and GDF9 null mice are unable to form a theca cell layer in early follicles (Elvin et al., 1999). As mentioned earlier, FSH plays a critical role in the recruitment of follicles into the follicle wave. Hayashi et al. (1999) found
treatment of FSH and GDF9 resulted in an enhanced effect of FSH with regard to initiating primordial follicle growth. Interestingly, results for treatment with GDF9 vary, thus it is believed there is a species specific effect of GDF9 of follicular cells (Spicer et al., 2008).

The vascular endothelial growth factor family or VEGF is comprised of 5 members VEGFA, VEGFB, VEGFC, VEGFD, and also includes placental growth factor. These factors are a family of growth factors and are polypeptides. Vascular endothelial growth factor A is a protein comprised of 8 exons and 7 introns and is spliced into pro-angiogenic or anti-angiogenic isoforms, with the most common of the angiogenic VEGFA isoforms being VEGFA_164 (Shima et al., 1996) while VEGFA_165B (VEGFA_164B in cattle) is the most common anti-angiogenic isoform (Harper and Bates, 2008). VEGFA plays an important role in stimulating vascularization and is up regulated by hypoxia (Dor et al., 2001). Furthermore, VEGFA plays an important role in follicle development and ovulation, VEGFA expression was increased as follicles grow and reached its maximum expression just prior to ovulation (Wulff et al., 2001; Einspainer et al., 2002). Expression of VEGFA has also been found in the CL with increased levels of VEGFA expression during the early luteal phase promoting vascularization of luteal tissue (Reynolds and Redmer, 1998).

Other experiments suggest VEGFA impacts follicle activation with in vitro administration of VEGFA significantly increasing the quantity of preantral follicles in the rat ovary (Danforth et al., 2003). Furthermore, treating ovaries with VEGFA reduced numbers of primordial follicles and increased numbers of developing follicles (Artac et al., 2009). Hormones involved with ovulation have also been reported to increase VEGFA
expression. Luteinizing hormone and FSH induced VEGFA transcription in the preovulatory follicle (Reynolds and Redmer, 1998). However, only low levels of FSH (1 ng/mL) increased expression of VEGFA_164 compared with higher doses (5 or 10 ng/mL).

Bovine granulosa cells also had increased expression of VEGFA_164 before and after the preovulatory rise in LH. VEGFA has been reported to not only improve vascularization of follicular granulosa cells, but also improves survival via reducing granulosa cell apoptosis (Kosaka et al., 2007). Furthermore, the expression of VEGFA has been reported in pregranulosa cells and granulosa cells of all stages, as well as, in theca cells of advanced stage follicles (McFee et al., 2009). The effects of VEGFA on granulosa cells suggest VEGFA effects follicle development through means other than vascularization due to the fact granulosa cells are not vascularized (Roberts et al., 2007).

**CUMULUS OOXYTE COMPLEX**

The cumulus oocyte complex (COC) consists of the oocyte and a group of closely associated cells directly adjacent to the oocyte (Tanghe et al., 2002). These cells along with the oocyte are largely surrounded by the antral follicular fluid, and separated from the mural (mature) granulosa cells (Thompson, 2006). Cumulus cells communicate with each other and the oocyte via gap junctions. The gap junctions are located at the end of cellular projections arising from cellular projections from the corona radiate and terminate in the oocyte oolemma (reviewed in Tanghe et al., 2002).

Tanghe et al. (2002) described three important biological functions of the COC; 1) before ovulation cumulus cells support oocyte maturation, 2) during ovulation cumulus
cells aid in directing the oocyte into the oviduct, 3) after ovulation cumulus cells aid in mechanisms controlling the access of spermatozoa to the oocyte. Although cumulus cells are present to aid in maturation of the oocyte, their presence at fertilization is species specific. In humans, cumulus cell removal prior to insemination improves fertilization potential of oocytes predicted to have low fertility (Lavy et al., 1988). However in both cattle and pigs cumulus cell removal before in vitro fertilization resulted in decreased sperm penetration (reviewed in Tanghe et al., 2002). Furthermore, cumulus cells facilitate delivery of usable metabolites to the oocyte. Metabolites such as glucose and cystine can be converted to pyruvate and cysteine, respectively, by cumulus cells and improve oocyte quality and fertilization (reviewed in Tanghe et al., 2002).

Oocytes arrest at prophase I of meiosis during gestation and nuclear development until the follicle reaches the antral stage of growth (Webb et al., 1999). Although meiosis is resumed, the competence of the oocyte is commiserate on several factors including oocyte diameter, activity of organelles and accumulation of RNA and proteins within the oocyte (reviewed in Webb et al., 1999). After activation of the arrested oocyte, several different factors will help determine the successfulness of pregnancy. Altered gene expression in several different classifications of genes including maternal effect genes, miRNA, and meiotic resumption factors may negatively impact embryogenesis.

**Maternal effect genes**

Maternal effect genes are required for the proper development and maturation of the oocyte and allow for fertilization to take place. Maternal factors such as mRNA, proteins, and biomolecules play a critical role in fertilization of the oocyte and formation of the zygote due to the transcriptional silence of the zygote for one or more cell cycles
prior to zygotic genome activation (Lindeman and Pelegri, 2009). Activation of the zygotic genome is species specific and can also be referred to as the embryonic block (Meirelles et al., 2004). The embryonic block coincides with the moment maternal genome activation is transferred to embryo genome activation (De Sousa et al., 1998). Cattle encounter the embryonic block at the fourth cell cycle, similar to humans (third to fourth cell cycle), but later than mice (second cell cycle; reviewed in Meirelles et al., 2004). Several maternal effect genes have been reported with alterations in their regulation having detrimental effects on embryonic survival.

Maternal effect genes have several different mechanisms by which they illicit their effects. Previously mentioned in this review the Dnmt family is important for methylation of DNA and ultimately embryonic survival, it is also considered a maternal effect gene. Furthermore, Dnmt-null mice report early embryonic mortality (Lei et al., 1996), and methyltransferase mutations can cause not only abnormal fetal growth, immunodeficiency, and brain abnormalities in humans (Clouaire and Stancheva, 2008). Another maternal effect gene, maternal antigen that embryos require (NLRP5) is transcribed specifically in the oocytes, accumulates during oogenesis, and plays a critical role in the progression of embryos beyond the 2-cell stage (Tong et al., 2000). Although Nlrp5-null mice have similar ovarian morphology, exhibit normal oocyte maturation, and estrous cycles compared with wild type mice, they are sterile. Interestingly, Nlrp5-null males are fertile supporting the hypothesis that NLRP5 is oocyte specific. Tong et al. (2000) reported although fertilization occurs in Nlrp5-null mice, embryos arrest at the 2-cell stage, which coincides with the activation of the embryonic genome. This would
suggest arrestment of bovine embryos would likely occur at the fourth cell cycle due to the later onset of embryonic genome activation if NLPR5 activity was reduced.

Similar to NLRP5, zygote arrest 1 (ZAR1) is hypothesized to be an ovary-specific maternal effect gene (Wu et al., 2003a). However, some studies report expression of ZAR1 in the testis as well as the ovary (Wu et al., 2003b). Zygote arrest1-null female mice are sterile, although no abnormalities in ovarian phenotype, ovulation, or fertilization are observed (Wu et al., 2003a). Interestingly, although expression of Zar1 has been reported in the male, Zar1-null males are fertile. Thus, perhaps the role of Zar1 in the male may not be as significant as in the female. Unlike Nlrp5-null mice, most embryos from Zar1-null mice fail to progress to the 2-cell stage suggesting arrestment shortly after fertilization (Wu et al., 2003a). These data suggest ZAR1 plays an important role in the transition from oocyte to zygote.

Developmental pluripotency associated protein 3 (DPPA3) was originally suggested to be necessary for early embryogenesis and is expressed in primordial germ cells and their descendents, which includes oocytes (Saltou et al., 2002; Liu et al., 2012). Furthermore, it was hypothesized DPPA3 was required to protect methylation patterns of the maternal genome (Nakamura et al., 2007). Bortvin et al. (2004) designed a Dppa3-null mouse and reported females expressed Oct4, a marker for oocytes, suggesting DPPA3 does not play a central role in germ cell programming. However, Dppa3-null females produced smaller litters suggesting a maternal factor role of Dppa3 (Bortvin et al., 2004). Small litter size of Dppa3-null mice was attributed to abnormalities in embryogenesis. Nearly all embryos reach the 2- to 4-cell stage; however, subsequent development was severely impaired.
**MicroRNAs**

Function and action of miRNA have been discussed previously in this review. Remember, miRNA are a class of regulatory, short non-coding RNAs consisting of approximately 22 nucleotides in their mature form (Iori et al., 2010). MicroRNAs are involved in gene expression in cumulus oocyte complex. Winter et al. (2009) explained the importance of the following miRNA and their function: DiGeorge syndrome critical region 8 (DGCR8), eukaryotic translation initiation factor 2C (EIF2C2), ribonuclease type III (RNASEN), exportin 5 (XPO5), and double-stranded RNA-specific endoribonuclease (DICER). MicroRNA synthesis involves the transcription of primary miRNA via RNA polymerase II or III from within polycistronic genes found within introns (Kim and Kim, 2010). Primary miRNA form a classic hair-pin structure and are cleaved in the nucleus to pre-miRNA by the RNASEN/DGCR8 complex. After formation of the pre-miRNA it is transported to the cytosol via XPO5. In the cytosol, DICER and other miRNA will cleave pre-miRNA to their mature length. This single strand of mature miRNA is combined with EIF2C2 and forms RNA-induced silencing complexes (Winter et al., 2009; Reviewed in Miles et al., 2012).

As would be expected, mice with the Dgcr8 deletion demonstrated reduced global production of miRNA in the oocyte (Suh et al., 2010). Transcripts of DICER are expressed in germline vesicle stage mouse oocytes and the expression of DICER declines during the 2-cell stage and then remains stable during morula and blastocyst formation (Cuia et al., 2007). Deletion of Dicer resulted in depletion of many miRNA in the mouse ovary and altered regulation gene expression in the oocyte (Murchison et al., 2007). Interestingly, Miles et al. (2012) reported gene expression for miRNA processing genes
was increased in the oocyte compared with COC or granulosa cells suggesting increased miRNA processing and function in the oocyte rather than other cell types of the follicle.

**Meiotic resumption**

Recall, during primordial follicle development, oocytes arrest at the diplotene stage of the first meiotic division until hormonal regulation stimulates the primordial to primary follicle transition (Webb et al., 1999; Nilsson and Skinner, 2009). The oocyte has a relatively short life span and timing of maturation must be tightly regulated to ensure a competent oocyte is ovulated. It has been established that meiotic resumption occurs after the LH surge. Although the oocyte is meiotically competent at the time of antrum formation (Erickson and Sorensen, 1974; Mehlmann et al., 2004) meiosis at this point is still arrested due to cAMP levels in the oocyte (Conti et al., 2002).

Immature follicles continually have tonic levels of cAMP transferred to the oocyte to maintain cell arrest (reviewed in Tanghe et al., 2002). Although cAMP could be transferred to the oocyte through the gap junctions between the cumulus and oocyte, some hypothesize the oocyte itself produces the cAMP to maintain meiotic arrest (Mehlmann, 2005). Although the complete meiotic pathway which cAMP levels arrest are not completely understood, it has been established that within the oocyte, cAMP activates protein kinase A (PKA). Downstream regulation of the meiotic promoting factor (MFP), a protein complex formed by the CDK/cyclin B (CYB) is impacted by PKA stimulated phosphorylation. Protein kinase A stimulates the action of WEE1 and MYT1. Stimulation of WEE1/MYT1 results in the phosphorylation of the MFP protein complex and maintains meiotic arrest (Mehlmann, 2005). After ovulation, the follicle is removed from the high cAMP environment and thus PKA activity is reduced and allows
for CDC25b to dephosphorylate the MFP complex allowing for meiotic resumption to occur.

**POLYCYSTIC OVARY SYNDROME**

Polycystic ovary syndrome (PCOS) is a reproductive disorder affecting nearly 6.6% women of reproductive age in the United States (Dumesic et al., 2007). The original description of the disease was first reported in 1935 (reviewed in Ehrmann, 2005). Diagnosis of PCOS is challenging due to the numerous conditions reported with the disease. A woman can be diagnosed with PCOS if at least 2 of the following symptoms are present: oligoovulation or anovulation (typically manifested as oligomenorrhea or amenorrhea), elevated levels of circulating androgens (hyperandrogenemia) or clinical manifestations of androgen excess (hyperandrogenism), and polycystic ovaries as defined by ultrasonography (reviewed in Ehrmann, 2005). Polycystic ovaries are characterized by the accumulation of small follicles (4-7mm diameter) containing hypertrophied theca cell layers (Legro et al., 1998). Furthermore, women with PCOS are more likely to develop metabolic and cardiovascular disease. In fact, 30% of women with PCOS are considered obese (Ehrmann, 2005) and increased visceral adiposity is associated with insulin resistance, glucose intolerance, and dyslipidemia (NCEP, 2002). Correlations between PCOS and metabolic syndromes are not surprising owning to the fact both share insulin resistance as a central pathogenic feature (Ehrmann, 2005). Furthermore, recent data suggest the PCOS phenotype can be programmed during fetal life (Franks et al. 2006; Dumesic et al., 2007)

In many cases of PCOS, hyperandrogenemia can be attributed to increased secretion of LH (Franks, 1995) and androgen excess in women can be attributed to
increased testosterone production in the ovary. Barbieri (1992) reported testosterone production was 4 times greater in PCOS women compared with normal women, but A4 was only 2 times greater. These data would suggest impairment of P450arom activity and the production of E2 from testosterone due to low E2 levels associated with PCOS compared with testosterone concentrations. However, other reports suggest increased testosterone production is a result of increased 17β-HSD activity (Reviewed in Nelson et al., 2001). Remember, Humans typically utilize the Δ⁵ steroidalgenic pathway; however, they can also utilize the Δ⁴ pathway (Figure 9). This alteration in steroidalgenic pathway can lead to the utilization of 3β-HSD to covert P5 to P4 in the theca cells and ultimately to the production of A4 in the theca, rather than DHEA. Increased 17β-HSD would suggest testosterone production from A4 in theca cells. However, Nelson et al. (2001) reported no differences in 17β-HSD activity for PCOS compared with normal theca cells. Furthermore, Nelson et al. (1999) indicated that there was no conversion of testosterone from DHEA in a 48 h de novo incubation suggesting the absence of 17β-HSD activity in normal theca cells. However, reports by Gilling-Smith et al. (1994) and Nelson et al. (1999) indicate P4, 17OH-P4, and DHEA secretions were increased in PCOS theca compared with normal theca cells.

Recall, StAR is important for the movement of cholesterol into the theca cell mitochondria to allow the initiation of steroidalogenesis. Interestingly, there is no difference in StAR promoter activity in normal or PCOS theca cells (Wickenheisser et al., 2001). These findings suggest similar levels of cholesterol are being transported to the theca cells for steroidalogenesis and an inability or alteration in steroidalgenic machinery efficiency is responsible for the altered testosterone and E2 production in PCOS patients.
Several studies have reported differences in other steroidogenic enzymes found in theca cells of PCOS patients. In the same study (Wickenheisser et al., 2001), the authors reported basal and forskolin-stimulated $CYP17A1$ activity was 4-fold greater in theca cells of PCOS patients compared with theca cells from normal ovaries. These data agree with, Nelson et al. (1999) reporting a 22-fold increase in 17OH-P4 production under basal conditions and a 20-fold and 4-fold increase in P4 and testosterone concentrations, respectively, for PCOS compared with normal theca cells. Furthermore, $CYP11A1$ mRNA abundance was increased 5-fold over the basal levels in PCOS compared with normal theca cells.

Along with increased steroidogenic enzymes, PCOS theca cells also contain increased expression of other enzymes. The transcription factor GATA-6 stimulates the promoter activities of the $CYP11A1$ and $CYP17A1$ in PCOS theca cells and its protein expression was approximately 3-fold greater in PCOS ovaries (Wood et al., 2003). Aldehyde dehydrogenase 6 ($ALDH6$) converts retinaldehyde to all-trans retinoic acid (atRA). Increased expression of $ALDH6$ in theca cells of PCOS ovaries is likely due to increased retinol dehydrogenase 2 (RoDH2) expression, which acts on several androgen precursors to allow for atRA synthesis (Wood et al., 2003). The authors reported atRA regulates the production of DHEA as well as $CYP17A1$ and $StAR$ gene expression in normal theca cells. Coupled with the knowledge that expression of these steroidogenic pathway intermediate and enzymes have altered expression in PCOS theca cells, it is likely that atRA plays a role in the PCOS phenotype (Wood et al., 2003).
Ruminants as a human model

For decades, the scientific community has utilized rodents as a model for human disease and to understand human physiologic function. In fact, a recent review (Adams et al., 2012) reported 98% of grants from the National Institutes of Health and the Department of Health and Human Services utilized rodents as the model. Thus the authors suggested only 2% of grants funded by these agencies involved using larger domestic species (Adams et al., 2012). However, one must also take into consideration the use of other species such as Drosophila, C. elegans, zebra fish, or Xenopus in research. Furthermore, the use of the term “larger” is fairly broad when used by the authors, since this group of animals included cows, pigs, sheep, goats, horses, chickens, turkeys, and ducks. It is likely that the rodent is utilized in current research due to the ease of handling, relatively fast generation interval, and reduced maintenance costs, especially when compared with ruminants, horses, or pigs. However, recent reviews have described the benefits of utilizing large animal models due to similar fetal:dam weight ratio as reported in humans and the increased gestation lengths compared with rodents allowing for greater understanding on therapeutic approaches to improving pregnancy success (Morel et al., 2012).

Although the rodent model is very beneficial for use in biomedical research, differences in ovarian dynamics when compared with humans are great (Adams et al., 2012). Rodents are a polytocous species, have a short estrus cycle and the luteal phase is only expressed if mating occurs. Furthermore, primordial follicle development occurs after gestation, when the neonate is removed from the high P4 environment of the dam. Conversely, large animal species, like humans, are monovular and polycyclic (Adams et
al., 2012). Also similarities in ovarian size and morphology exist between humans and cattle. Ovarian follicle size is similar between species (15 to 20 mm in diameter) and similar pathologic conditions can occur in both species (Adams and Pierson, 1995). Primordial follicle assembly occurs during gestation in humans as well as cattle and sheep. In humans oocyte nest breakdown begins on approximately d 112 of gestation (Hirshfield, 1991; Sforza and Forabosco, 1998; Abir et al., 2005) whereas cattle undergo this transition slightly earlier (d 80). The period of follicle assembly occurs through gestation d 133 and 142 in humans and cattle, respectively. However, both species undergo this transition at a similar time point coinciding with mid-gestation.

Previous reports in humans have related maternal nutrition and long term fetal health. Early work involving fetal programming was conducted through epidemiological studies. These studies reported children born to undernourished mothers had increased hypertension, cardiovascular disease, insulin resistance, type II diabetes, and obesity (Barker et al., 1989; Barker et al., 2002; McMillen and Robinson, 2005). Utilizing sheep as a model for undernutrition, similar results were reported with lambs born to NR females having altered fetal pancreatic β-cell number, increased appetite, obesity and impaired cardiovascular function (reviewed in Ford and Long, 2012).

Unfortunately, to date there are no reports utilizing an animal model with naturally occurring symptoms of PCOS. However, scientists have produced PCOS models in both non-human primates and sheep through androgen treatment during gestation (reviewed in Dumesic et al., 2007; Steckler et al., 2009). Prenatal testosterone treatment altered LH secretion, reduced hypothalamic sensitivity to steroid negative feedback, and increased insulin secretion due to increased abdominal adiposity in rhesus
monkeys. These monkeys had ovarian morphology similar to PCOS patients with hypergoadotropism, neuroendocrine feedback defects and polycystic ovaries (reviewed in Dumesic et al., 2007). Similarly sheep exposed to testosterone during early to midgestation exhibited reproductive defects as well as increased adiposity during overfeeding compared with control sheep (Steckler et al., 2007). As previously stated these models of PCOS are developed via exogenous androgen treatment. Identification of a naturally occurring model of PCOS in domestic livestock could further our understanding of the disease and potential treatment options.

CONCLUSIONS

Several factors influence cow productivity and performance, including nutrient status, maternal age, maternal milking ability, weaning weight, and maternal nutrition during gestation. Maternal nutrient status can alter the epigenome via posttranslational modifications. The timing of maternal nutrient alteration also plays an important role in determining the effect nutrition will have on the developing placenta, fetus and progeny performance. Fetal programming can occur prior to implantation, and placental development can be compromised through reduced nutrient intake. Currently, the amount of literature reporting maternal nutrition effects on heifer progeny longevity is limited; however, in most of these studies, performance through the first production cycle has been documented. Providing adequate nutrition has resulted in improved progeny health and increased body weight. Furthermore, studies have reported reduced age at puberty, increased fertility, and earlier calving dates for heifers born to dams supplemented with protein while grazing winter range. Furthermore, the effects of maternal nutrition on steer progeny performance have been well documented. Improved maternal nutrient status
during late gestation can improve calf weaning BW, feedlot performance and carcass characteristics. Protein supplementation during late gestation also increases net profits per cow due to increased quality grade and HCW of steer progeny.

Early neonatal life is also crucial for future calf performance. Milk production and puberty attainment can be altered based on nutrient intake as well as time of weaning. Studies have also reported by limiting feed during the developmental period, heifer development costs can be reduced without compromising reproductive efficiency. Perhaps the improvement in BW gains prior to the breeding season in nutrient restriction developed heifers is one of the main factors allowing for similar reproductive efficiency due to compensatory gain mechanism and a flushing effect. Exposing heifers to the environment they will be managed as cows early in development may potentially improve productivity and longevity.

Longevity in beef cattle production is related to live calf production. Although reproductive characteristic are considered to have low heritability, certain phenotypic measurements (AFC, uterine horn diameter, RTS) can be utilized to predict heifer fertility. Heifers with increased AFC have altered gonadotropin and steroid hormone secretion compared with low AFC heifers. Furthermore, understanding of steroidogenic pathway intermediates can help determine the overall health of the ovary and developing follicles.

In humans, PCOS is a condition affect nearly 7% of reproductive age women in the United States. Hyperandrogenism, polycystic ovaries, and metabolic syndrome are three symptoms typically presented in cases of PCOS. Impaired enzymatic activity in the steroidogenic pathway results in hypersecretion of testosterone in PCOS and is typically
the result of altered theca cell function. Currently 2 animal models (non-human primate and sheep) exist to study PCOS. However, these models are produced via prenatal treatment with androgens. Development of a naturally occurring model for PCOS in domestic livestock could allow for greater understanding altered gene expression and androgen production in PCOS women.

OBJECTIVES

- Determine the effect of developing heifers on winter range and corn residues compared with winter range and drylot ADG and reproductive performance.
- Assess the economic ramifications of moving heifer development from the drylot to winter range.
- Determine the effect of development system on feed efficiency as a pregnant heifer and calving performance.
- Evaluate the effects of two differing rumen undegradable protein (RUP) supplements during late gestation on primiparous heifer calving and reproductive performance.
- Determine the effects of maternal RUP supplement on progeny growth and preweaning performance, feedlot performance and carcass characteristics.
- Identify the effect of ovarian and follicular environment on the oocyte molecular phenotype.
LITERATURE CITED


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CHAPTER II: Effect of beef heifer development system on ADG, reproduction, and feed efficiency during first pregnancy


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ABSTRACT

A 3-yr study was conducted to determine the effect of heifer development system on ADG, reproductive performance, and subsequent feed efficiency as a pregnant heifer. Crossbred Angus heifers (n= 299) were assigned by initial BW to be developed grazing corn residue (CR) or in a drylot (DL). Corn residue heifers grazed native pasture 33 d prior to grazing CR 73 d. Corn residue heifers were then placed on dormant forage pastures 67 d and then combined with DL heifers for approximately 39 d for estrus synchronization and AI. Heifers assigned to DL grazed dormant forage for 98 d and then placed in a DL for 114 d. Prebreeding BW was greater (P = 0.01) for DL heifers compared with CR heifers (349 vs. 314 ± 9 kg). At pregnancy diagnosis BW remained greater (P = 0.05) for DL compared with CR heifers (422 vs. 403 ± 9 kg). Drylot heifers had greater (P = 0.01) overall ADG during development compared with CR heifers. Corn residue-developed heifers tended (P = 0.11) to have increased AI conception rates compared with DL heifers (74 vs. 65 ± 8). However, there was no difference (P ≥ 0.32) in percent cycling (42 vs. 52 ± 15%) prior to the breeding season or final pregnancy rates (95 vs. 92 ± 2%) for CR and DL heifers, respectively. Gestation length, calving date, and calf birth BW were similar (P ≥ 0.37) between development systems. At the beginning of the second winter, a subset of AI pregnant heifers (n = 114) were stratified by BW and
development system, and placed in a Calan Broadbent individual feeding system for 82 d during late gestation. For heifers placed in the Calan system, initial BW was similar \((P = 0.77)\) between development systems; however, pre-calving BW tended \((P = 0.10)\) to be greater for DL heifers compared with CR. Dry matter intake and RFI were similar \((P \geq 0.36)\) between treatments. Drylot heifers tended to have greater \((P \leq 0.09)\) ADG (0.80 vs. 0.70 ± 0.14 kg/d) and G:F compared with CR. Overall, heifers developed on CR had reduced BW through early pregnancy, however reproductive performance was similar to DL-developed heifers.

**Key words:** beef cattle, feed efficiency, heifer development

**INTRODUCTION**

Previous literature suggests heifers must be developed to approximately 65% mature BW to maximize pregnancy rates (Patterson et al., 1992). These early data also indicate an inverse correlation between postweaning growth rate and age at puberty (Patterson et al., 1992; Funston et al., 2012).

The single greatest cost of heifer development is feed. Reducing harvested forage use could decrease heifer development costs. Decreasing development BW 5-10% below contemporaries resulted in a $19-45/heifer decrease in development cost (Feuz, 2001; Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011). Furthermore, reduced input production systems report similar reproductive performance when compared with contemporaries fed a higher plane of nutrition or to a greater proportion of mature BW (Freetly et al., 2001; Funston and Deutscher, 2004; Martin et
Grazing developing heifers on dormant winter range or corn residue has been reported as an alternative to confinement-feeding replacement heifers (Martin et al., 2008; Funston and Larson, 2011; Larson et al., 2011). However, these dormant forages do not meet the nutrient requirements of the growing animal and protein supplementation is required (Fernandez-Rivera et al., 1989).

In addition to reduced feed inputs, reports indicate decreased BW through pregnancy diagnosis in heifers developed to reduced proportions of mature BW (Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011) and reduced BW through 5 yr of age in heifers restricted feed 140 d post weaning (Roberts et al., 2009) compared with their nonrestricted contemporaries. Reduction in BW would suggest reduced maintenance requirements which would decrease production costs. The objective of this study was to determine the effect of heifer development system on ADG, reproductive performance, and subsequent feed efficiency as a pregnant heifer.

**METHODS AND MATERIALS**

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved all procedures and facilities used in this experiment.

*Developing heifer management*

Weaned, crossbred Angus heifers (n = 299) were received at the University of Nebraska West Central Research and Extension Center (WCREC), North Platte, NE. After a 14 d acclimation period heifers were blocked by BW and randomly assigned to be developed either grazing corn residue (CR) or in a drylot (DL). During winter grazing (native pasture and corn residue) heifers were offered 0.45 kg/d dried distillers grain based protein supplement (28% CP) containing 80 mg·animal\(^{-1}\)·d\(^{-1}\) monensin (Rumensin,
Elanco Animal Health, Indianapolis, IN) and vitamins and trace minerals to meet heifer requirements (Table 1). Corn residue heifers grazed native pasture 33 d prior to grazing CR for 73 d. Subsequently, CR heifers were placed on dormant pastures for 67 d prior to being placed in the DL for approximately 39 d for synchronization of estrus and AI (Figure 1).

Corn residue fields were located in Keith County, NE and CR developed heifers were transported about 100 km from WCREC to the CR fields. Heifers were transported on approximately December 2 and returned to WCREC approximately February 14 each yr. Heifers grazed CR for 77, 57, and 88 d in yr 1, 2, and 3 respectively. Corn residue grazing length was dictated by corn harvest date and weather conditions. The CR fields were approximately 40 ha, irrigated and planted in April. Corn harvest was completed in October with an average yield of 12,544 kg/ha. Heifers were placed on CR with a 2.5 heifer/ha stocking rate during the grazing period.

Drylot heifers grazed native forage pastures 98 d and then placed in the DL for 114 d (Figure 1). Drylot-developed heifers were offered a diet formulated to target 65% of mature BW (360 kg) at breeding. Individual feed intake for DL heifers was 7.6 kg/d (DM basis). Diet for DL heifers, on a 3 yr average, was composed of: 16% wet corn gluten, 10% cracked corn, 70% brome hay, and 4% supplement (Table 2). Diets were provided in the morning for DL heifers once daily. Thirty-nine d prior to AI, CR and DL heifers were combined, managed together and fed a common diet.

Estrus was synchronized utilizing the melengestrol acetate-prostaglandin (MGA-PGF) synchronization protocol with heifers fed MGA (Pfizer Animal Health, New York, NY) for 14 d and administered a single i.m. injection of PGF (Lutalyse, Pfizer Animal Health, New York, NY).
Health, New York, NY) 19 d after the end of MGA feeding. Estrus detection was performed for 5 d following PGF administration. Each year heifers were randomly AI to 1 of 4 bulls approximately 12 h after standing estrus. Approximately 10 d following the last d of AI, heifers were exposed to bulls at a bull to heifer ratio of 1:50 for 60 d. Artificial insemination and overall pregnancy rates were determined 45 d after AI and 45 d after bull removal, respectively, via transrectal ultrasonography.

**Blood collection and RIA**

Prior to the breeding season blood samples were collected 10 d apart via coccygeal venipuncture to determine plasma progesterone concentration. Blood was collected in vacuum tubes, cooled on ice, and centrifuged at 2,500 × g. Serum was isolated and stored at -20° C until analysis. Serum progesterone assays were carried out without extraction (Melvin et al., 1999) using a direct solid-phase RIA (Coat-A-Count, Diagnostics Products Corp., Los Angeles, CA). Intra- and interassay CV was 3.1% and 3.6%, respectively. Heifers with progesterone concentrations > 1.0 ng/mL were considered to have attained puberty (Henricks et al., 1971).

**Primiparous heifer management**

All heifers remained in a common group through the summer grazing native pasture. After final pregnancy diagnosis, a subset of heifers (yr 1 = 38; yr 2 = 40; yr 3 = 36) confirmed AI pregnant were placed in a Calan Broadbent individual feeding system during late gestation. Heifers were allowed approximately 25 d to adapt to the individual feeding system followed by an 84 d (yr 1 and 3) or 80 d (yr 2) feeding trial. Heifers were offered *ad libitum* grass hay and either no supplement; 0.82 kg/d distillers based supplement; or 0.82 kg/d dried corn gluten based supplement. Supplements were
formulated to be isocaloric and isonitrogenous, but differed in rumen undegradable protein. Feed offered was recorded daily and refusals removed and weighed weekly. Residual feed intake (RFI) was calculated as the actual DMI – predicted DMI, with DMI calculated based on NE values of the feed to account for different energy levels of the supplement compared with the control diet.

Heifers (n = 185) not placed on the individual feeding trial grazed CR during late gestation. Heifers were transported to corn fields and grazed approximately 79 d, based on CR availability over the 3 yr. While grazing, all heifers received the equivalent of 0.45 kg/d of 28% CP supplement three times weekly. Heifers were returned to WCREC approximately mid-February and placed in a common group with Calan heifers through calving. Heifers grazing CR during the second winter were sold as cow/calf pairs each yr in May and removed from the study.

After calving, Calan heifers remained at WCREC through breeding. Artificial insemination utilized a fixed-timed AI protocol and pairs were transported 43km to a commercial ranch in the Nebraska Sandhills for summer grazing. A single bull was placed with heifers approximately 10 d after AI for 60 d. Pairs were returned to WCREC prior to weaning for final pregnancy diagnosis.

**Economic analysis**

Cost of grazing for weaned heifers was estimated to be one half of mature cow cost based on heifer BW at weaning. Average cost for weaned CR heifers grazing during the winter and spring period was estimated to be $0.46/d, including supplement cost. Cost of grazing WR with supplement and DL feeding was calculated across the entire development period and valued at $0.75/d. Cost for grazing heifers in central Nebraska
during the summer on upland grass was estimated to be $0.55/d (Johnson et al., 2010). Additional development cost including feed delivery costs, nonfeeding costs including estrus synchronization, breeding costs, health/veterinarian costs, and trucking were assessed to be $0.36/d in both systems. A 7% interest rate was considered and interest charged on the opportunity cost of the heifer and ½ the value of the variable expenses and feed costs. Fixed cost accounted for insurance and depreciation of animals, facilities, and equipment and was considered $15 for both systems. Purchase and cull prices were determined using USDA market prices reported by USDA Agricultural Marketing Services (USDA-AMS, 2012). Net cost of one pregnant heifer was calculated using the formula developed by Feuz (1992). Total value of cull heifers was subtracted from the total cost of all developed heifers. Total costs were then divided by the number of heifers exposed at breeding, to determine the total cost of one heifer. Lastly, the total development cost was then divided by the final pregnancy rate minus percent death loss, to determine the total net cost per one pregnant heifer.

**Statistical analysis**

Data were analyzed using the MIXED and GLIMMIX procedures of SAS (SAS Inst., Inc., Cary, N.C.). Treatments were applied by grazing heifers on CR and WR or WR and then place in the DL, replicated for 3 yr, thus CR field (n=3) and DL pen (n=3) were considered the experimental unit with development system classified as a fixed effect. Artificial insemination technician and yr were included as random effects in the model for AI pregnancy and overall pregnancy data. A subset of animals from each development system were placed in 1 of 4 pens where individual feeding occurred. This development by pen classification was included as a random variable and considered the
experimental unit for individual feeding data with developmental treatment and barn diet as fixed effects and yr considered a random effect. Heifer development × second winter treatment interaction was not significant and removed from the model. For the individual feeding period, heifer development × Calan treatment interaction was not significant, thus all data are presented as the effect of heifer development system. A P-value ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

Heifer development BW gain and reproduction

Data for heifer development BW gain are reported in Figure 2. Body weight was similar for CR and DL heifers at the beginning of the experiment (220 vs. 220 ± 3 kg; \( P = 0.93 \)). However, prior to breeding, DL heifers were 35 kg (± 9 kg) heavier (\( P = 0.01 \)) than CR-developed heifers. Body weight remained greater for DL heifers at AI pregnancy diagnosis (370 vs. 348 ± 11 kg; \( P = 0.02 \)) and final pregnancy diagnosis (422 vs. 403 ± 9 kg; \( P = 0.05 \)) compared with CR heifers. These data agree with previous literature (Funston and Larson, 2011) reporting DL-developed heifers had greater (\( P < 0.05 \)) BW from the end of the development period through pregnancy diagnosis compared with heifers developed on CR and winter range. However, in that study DL heifers did not graze dormant forage pastures prior to entering the DL and were thus in the DL between 150- 204 d, whereas DL heifers in the current study grazed dormant forage pasture for 98 d and were then placed in the DL for 114 d.

Overall ADG was greater (\( P = 0.01 \); Table 3) for DL heifers compared with CR heifers. Drylot-developed heifers also had greater (\( P = 0.04 \)) ADG during the Spring, which coincides with the DL heifers entering the DL after grazing dormant pasture, while
the CR heifers moved from CR to native dormant pasture. Average daily gain was similar (0.81 vs. 0.58 ± 0.13 kg/d; \( P = 0.25 \); Table 3) between CR and DL heifers during estrus synchronization and remained similar through AI pregnancy diagnosis (\( P = 0.24 \)).

Funston and Larson (2011) reported a similar increase in DL heifer ADG during the DL period compared with heifers grazing CR and winter range (WR); however, after the breeding season they reported an increase in ADG for heifers developed on CR- and WR-compared with DL-developed heifers, suggesting a compensatory gain effect.

Although ADG was greater through development for DL compared with CR heifers, the proportion of heifers attaining puberty prior to the breeding season was not different (\( P = 0.46 \); Table 3). Martin et al. (2008) also reported no significant difference in puberty attainment for heifers fed to 51 vs. 57% mature BW. However, Funston and Larson (2011) reported decreased puberty attainment in heifers developed on WR- and CR-compared with DL-developed heifers (56 vs. 65% mature BW, respectively). In the current study, DL-developed heifers were developed to 63% mature BW (554 kg mature BW) compared with 57% (\( P = 0.01 \)) for CR-developed heifers (Table 3).

Artificial insemination pregnancy rate tended (\( P = 0.11 \)) to be greater for CR compared with DL-developed heifers (74 vs. 65 ± 8%). Previous literature reports post insemination diet may impact heifer AI pregnancy rates and embryonic survival (reviewed in Perry, 2012). Feeding heifers 85% maintenance requirements for protein and energy resulted in decreased embryonic development on d 3 and 8 of gestation compared with heifers fed 100% maintenance requirements (Hill et al., 1970). In a recent review, Perry (2012) reported heifers developed in a DL and placed on spring forage pastures just after insemination had reduced AI pregnancy rates compared with heifers
developed in a DL, placed on spring pastures after AI and supplemented 2.2 kg·animal$^{-1}$·d$^{-1}$ dried distillers grains with solubles for 42 d. In the current study heifers were removed from DL after the 5 d AI period and placed on summer pastures. Although not significant ($P = 0.24$), ADG was 0.13 kg/d greater for CR compared with DL-developed heifers from AI to AI pregnancy diagnosis and it is suggested post insemination ADG may alter the uterine environment by various mechanisms (reviewed in Perry, 2012). Furthermore, CR-developed heifers were placed in the DL for 39 d prior to the beginning of the breeding season to allow for estrus synchronization, improving nutrient intake for CR-developed heifers just prior to breeding, which could result in a flushing effect for CR-developed heifers (Utter, 1994; Lynch et al., 1997). Lynch et al. (1997) also reported a tendency for increased first service pregnancy rates in heifers developed to gain 0.11 kg/d for the first 112 d of development followed by 0.91 kg/d for 47 d compared with heifers developed to gain 0.45 kg/d throughout the development period in yr 2, but not yr 1. The differences reported for yr 1 and yr 2 were attributed to the increased improvement in ADG for late-gain heifers during the last 45 d of development in yr 2 compared with yr 1 suggesting a more severe dietary restriction in yr 2 and increased flushing effect (Lynch et al., 1997).

There was no difference ($P = 0.32$) in final pregnancy rate (95 vs. 92 ± 2%; Table 3) for CR and DL, respectively. Previous research indicates maximal reproductive rate when heifers were developed to approximately 65% mature BW prior to breeding (Patterson et al., 1992). However, more recent data suggests developing heifers to 50-57% mature BW at breeding will result in similar pregnancy rates as heifers developed to
A greater percent of mature BW (Martin et al., 2008; Funston and Larson, 2011; Funston et al., 2012). This agrees with data reported in the current study.

**Economic evaluation**

Development period feed costs were $50 greater/heifer for DL-developed heifers. Increased costs associated with DL-development can be attributed to the increased amount of harvested forages being utilized in that system and is similar to previously published data (Funston and Larson, 2011). In that study, DL-developed heifers were fed in the DL for approximately 184 d. In the current study, DL-developed heifers were on WR for 98 d followed by DL for 114 days, reducing DL feeding length 70 d. Even with reduced time in the DL total development costs were $52/heifer greater for DL-compared with CR-developed heifers in the current study. Net cost of one pregnant heifer was $47/heifer greater for DL-compared with CR-developed heifers. These data are similar to previously reported data suggesting a $19-45/heifer savings when BW is target to be 5-10% less prior to the first breeding season (Feuz, 2001; Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011).

**Primiparous heifer feed efficiency**

Heifer BW at the beginning of the second winter was 18 kg (± 4 kg) greater for heifers developed on CR compared with DL (Table 5). Furthermore, BW tended \((P = 0.09)\) to be greater prior to calving for DL-developed heifers. Previous reports differ on the length of time BW will differ in heifers developed on reduced planes of nutrition (Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011), but
Roberts et al. (2009) reported difference in cow BW up to 5 yr in age when heifers were fed a nutrient-restricted diet for 140 d after weaning.

Data for primiparous heifers placed in the Calan Broadbent individual feeding system during late gestation are reported in Table 5. Initial BW for the subset of heifers placed in the individual feeding system was similar \((P = 0.77)\) between DL and CR heifers. However, pre-calving BW tended to be greater \((P = 0.10)\) for DL-developed heifers at the end of the 80 to 84-d individual feeding period. Dry matter intake or DMI based on feed NE \((P \geq 0.93)\) was not different between treatments. Average daily gain \((P = 0.09)\) and G:F \((P = 0.08)\) tended to be greater) for DL- compared with CR-developed heifers. Residual feed intake based on NE values of the diet \((P = 0.36)\) was not different between treatments.

Similarities observed in pregnant heifer performance likely results from a subset of heifers being selected from the CR and DL groups. Although BW prior to the second winter differed for the groups \((P = 0.04)\), a subset of heifers with similar \((P = 0.77)\) initial BW was placed in the Calan feeding system due to objectives of concurrent studies (Summers et al., 2012). This resulted in selection of heifers from the CR-developed group having a mean BW approximately 13 kg heavier than the overall CR-developed group, whereas the difference in BW for DL-developed heifer selected for the individual feeding system was 3 kg less than the overall DL-developed group (Table 6). Although RFI is considered to be a better indicator of feed efficiency than feed conversion ratios, due to its independence from growth and body size (Crews et al., 2005) some reports suggest an adjustment for carcass fatness be considered (Koots et al., 1994; Herd and Bishop, 2000). Body weight is a predictor of BCS and although BCS was not recorded on these animals
it could be suggested DL-developed heifers had greater BCS at the beginning of the second winter feeding period. The NRC (2000) suggests an empty BW difference of 50-75 kg per BCS. Although differences in final BW was not the equivalent of 1 BCS greater in DL- compared with CR-developed heifers, the 18 kg (± 4 kg) difference in BW could result in a slight increase in adipose storage in DL heifers.

Limited data are available on the effect pregnancy may have on RFI (McDonald et al., 2010). Fetal growth is greatest during the last 2 mo of gestation (Robinson et al., 1997), thus fetal growth requirements could play a role in feed efficiency. All heifers utilized in the Calan system were confirmed AI pregnant, thus at similar stages of gestation. When accounting for calf birth BW in the model, RFI remained similar among groups (not reported). Although calf birth BW varied among individuals, group calf birth BW was similar. Finally, development system was considered the treatment in this experiment. Heifers were removed from their allotted treatments and comingled for approximately 8 mo prior to individual feeding period, this could possible confound the results for RFI. Durunna et al. (2012) reported 51% of heifers had a different RFI class during period 2 compared with 1, with 27% of heifers changing RFI by 1 SD (0.37 to 0.44 kg of DM·d⁻¹). However, it should be noted the two feeding periods utilized by Durunna et al. (2012) were approximately 55 d and 56 d for the two periods respectively, whereas the feeding period in this study was 80 to 84 d. Conversely, Herd et al. (2006) and Arthur et al. (1999) reported a correlation for RFI measured in heifers post-weaning and again as 4 yr old cows, however; these animals were not tested during gestation.

Gestation length, calf birth BW, and calving ease did not differ ($P \geq 0.19$) among treatments (Table 6). One concern with developing heifers to a reduced proportion
mature BW at the beginning of the first breeding is dystocia. Body weight gain prior to the first breeding season has been associated with hip height and pelvic area. Freetly et al. (2001) reported heifers offered 263 kcal ME(BW_{kg}^{0.75}) had greater hip heights prior to the breeding season compared with heifers either offered 238 kcal ME(BW_{kg}^{0.75}) or 157 kcal ME(BW_{kg}^{0.75}) daily for the first 83 d and then offered 277 kcal ME(BW_{kg}^{0.75}). Heifers fed to gain 0.23 kg/d over a 153-d feeding period had reduced BW, body condition, and pelvic area compared with those heifers fed to gain 0.45 or 0.68 kg/d (Short and Bellows, 1971). Conversely, Funston and Deutscher reported heifers developed to 53% mature BW prior to the first breeding season had similar pelvic area compared with heifers developed to 58% mature BW. Although pelvic area was not measured in this study, % mature BW at breeding was 6% (± 2%) lower for CR compared with DL heifers. Current research has reported heifers developed to reduced proportions of mature BW prior to the first breeding season have similar dystocia scores compared with heavier developed contemporaries (Funston and Deutscher, 2004; Martin et al., 2008; Funston and Larson, 2011).

Pre-breeding BW did not differ (P = 0.29) between treatments as first calf heifers and proportion pregnant at weaning was also similar (P = 0.65) for DL- compared with CR-developed heifers (Table 6). Funston and Deutscher (2004) reported similar cow BW and proportions of cows pregnant with their second calf at weaning for cows developed to 53 compared with 58% mature BW; however, BCS at weaning was greater for cows developed to 58% mature BW as heifers. Roberts et al. (2011) fed heifers a control or nutrient restricted (80% of control) diet for 140 d post weaning and then fed adequate (~1.81 kg alfalfa hay hd/d) or a restricted diet (~ 1.08 kg alfalfa hay hd/d) from
December to March. At the 2\textsuperscript{nd}, 3\textsuperscript{rd} and 4\textsuperscript{th} breeding seasons, few restricted heifers remained in the cow herd compared with control heifers.

**IMPLICATIONS**

Traditional DL development systems would place heifers in DL shortly after weaning. In this experiment, developing heifers on dormant pasture followed by DL increased heifer BW from the end of the development period through pregnancy diagnosis compared with CR-developed heifers. However, reproductive and calving performance was similar between treatments. Reproductive performance was maintained by developing heifers with reduced harvested forage.
LITERATURE CITED


FIGURE LEGENDS:

Figure 1. Illustration depicting time frame corn residue (CR) and drylot (DL) developed heifers grazed dormant winter pasture (WR), CR, or placed in the DL. Corn residue developed heifers were placed on dormant pasture 33 d, and then shipped to CR for 73 d. Following CR grazing heifers were returned to dormant pasture for 67 d prior to being placed in the DL for 39 d to allow for estrus synchronization. Drylot developed heifers grazed dormant pasture 98 d then placed in the DL for 114 d. After AI, all heifers grazed summer pasture for 111 d until final pregnancy diagnosis. Period associated with calculated ADG is reported on the right.

Figure 2. Heifer BW during development and summer grazing. Corn residue developed heifers (CR= black line) were placed on dormant pasture 33 d, and then shipped to CR for 73. Following CR grazing heifers were returned to dormant pasture for 67 d prior to being placed in the drylot (DL) for 39 d to allow for estrus synchronization. Drylot developed heifers (grey line) grazed dormant pasture 98 d then placed in the DL for 114 d. After AI, all heifers grazed summer pasture for 111 d until final pregnancy diagnosis.
Figure 1.
Figure 2.
Table 1. Composition and nutrient analysis of supplement offered to heifers grazing corn residue or winter range

<table>
<thead>
<tr>
<th>Item</th>
<th>DM %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Supplement(^2)</td>
<td>7</td>
</tr>
<tr>
<td>DDGS(^3)</td>
<td>62</td>
</tr>
<tr>
<td>Wheat middlings</td>
<td>11</td>
</tr>
<tr>
<td>Cottonseed meal</td>
<td>9</td>
</tr>
<tr>
<td>DCGF(^4)</td>
<td>5</td>
</tr>
<tr>
<td>Molasses</td>
<td>5</td>
</tr>
<tr>
<td>Calcium carbonate</td>
<td>3</td>
</tr>
<tr>
<td>Urea</td>
<td>2</td>
</tr>
<tr>
<td>Other</td>
<td>3</td>
</tr>
</tbody>
</table>

Nutrient composition

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein, %</td>
<td>8.5</td>
</tr>
<tr>
<td>RUP, % CP</td>
<td>32.3</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>2.5</td>
</tr>
<tr>
<td>NE(_g), Mcal/kg</td>
<td>0.53</td>
</tr>
</tbody>
</table>

\(^1\)Heifers were offered 0.45 kg/d while grazing corn residue or winter range during development.

\(^2\)Provided 80 mg animal\(^-1\)d\(^-1\) monensin (Rumensin, Elanco Animal Health, Indianapolis, IN).

\(^3\)Dried distillers grains plus solubles.

\(^4\)Dried corn gluten feed.
Table 2. Composition of diet provided to drylot heifers from February to May

<table>
<thead>
<tr>
<th>Item (DM basis)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Brome hay, %</td>
<td>68.9</td>
</tr>
<tr>
<td>WCGF(^1), %</td>
<td>16.8</td>
</tr>
<tr>
<td>Cracked corn, %</td>
<td>10.2</td>
</tr>
<tr>
<td>Supplement(^2), %</td>
<td>4.1</td>
</tr>
<tr>
<td>Intake, kg/d</td>
<td>7.8</td>
</tr>
</tbody>
</table>

Nutrient composition\(^3\)

<table>
<thead>
<tr>
<th></th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>12.2</td>
<td>12.1</td>
<td>12.4</td>
</tr>
<tr>
<td>RUP, %CP</td>
<td>22.0</td>
<td>22.9</td>
<td>22.9</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>2.8</td>
<td>2.8</td>
<td>2.9</td>
</tr>
<tr>
<td>NE(_g), Mcal/kg</td>
<td>0.82</td>
<td>0.83</td>
<td>0.86</td>
</tr>
</tbody>
</table>

\(^1\)Wet corn gluten feed.

\(^2\)Ground corn, calcium carbonate, trace mineral mix, and vitamin mix, formulated to provide 200 mg monensin (Rumensin, Elanco Animal Health, Indianapolis, IN).

\(^3\)Wet Chemistry, Ward Laboratories Inc., Kearney, NE; RUP based on NRC (2000).
Table 3. Effect of winter heifer development system on ADG, and reproductive performance

<table>
<thead>
<tr>
<th>Item</th>
<th>CR</th>
<th>DL</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADG</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall^3, kg/d</td>
<td>0.43</td>
<td>0.59</td>
<td>0.03</td>
<td>0.01</td>
</tr>
<tr>
<td>Winter^4, kg/d</td>
<td>0.04</td>
<td>0.06</td>
<td>0.12</td>
<td>0.64</td>
</tr>
<tr>
<td>Spring^5, kg/d</td>
<td>0.55</td>
<td>1.09</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>Estrus synchronization^6, kg/d</td>
<td>0.81</td>
<td>0.58</td>
<td>0.13</td>
<td>0.25</td>
</tr>
<tr>
<td>Breeding^7, kg/d</td>
<td>0.52</td>
<td>0.39</td>
<td>0.06</td>
<td>0.24</td>
</tr>
<tr>
<td>Summer^8, kg/d</td>
<td>0.77</td>
<td>0.75</td>
<td>0.03</td>
<td>0.61</td>
</tr>
<tr>
<td>Pubertal^9, %</td>
<td>42</td>
<td>52</td>
<td>15</td>
<td>0.46</td>
</tr>
<tr>
<td>AI pregnant, %</td>
<td>74</td>
<td>65</td>
<td>8</td>
<td>0.11</td>
</tr>
<tr>
<td>Pregnant, %</td>
<td>95</td>
<td>92</td>
<td>2</td>
<td>0.32</td>
</tr>
<tr>
<td>Mature BW, %</td>
<td>57</td>
<td>63</td>
<td>2</td>
<td>0.01</td>
</tr>
</tbody>
</table>

^1 CR= heifers grazed dormant pastures 33 d, corn residue 73 d, and were placed on dormant winter pastures 67 d prior to entering the drylot 39 d before AI.

^2 DL= heifers grazed dormant pastures 98 d prior to entering the drylot 114 d before AI.

^3 ADG from initiation to prebreeding.

^4 ADG while grazing dormant pasture and corn residue (CR) or dormant pasture (DL).

^5 ADG while grazing dormant pasture (CR) or while in drylot (DL).

^6 ADG while in the drylot.

^7 ADG from breeding to AI pregnancy detection.

^8 ADG from AI pregnancy to final pregnancy detection.

^9 Considered pubertal if blood serum progesterone concentrations were > 1 ng/mL.
### Table 4. Partial budget analysis of developing beef heifers on corn residue or drylot

<table>
<thead>
<tr>
<th>Description</th>
<th>CR ($/heifer)</th>
<th>DL ($/heifer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opportunity cost of the heifer&lt;sup&gt;1&lt;/sup&gt;, $/heifer</td>
<td>520</td>
<td>520</td>
</tr>
<tr>
<td>Feed costs, $/heifer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Development&lt;sup&gt;4&lt;/sup&gt;</td>
<td>80</td>
<td>130</td>
</tr>
<tr>
<td>Estrus synchronization&lt;sup&gt;5&lt;/sup&gt;</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>Summer&lt;sup&gt;6&lt;/sup&gt;</td>
<td>61</td>
<td>61</td>
</tr>
<tr>
<td>Other variable costs&lt;sup&gt;7&lt;/sup&gt;, $/heifer</td>
<td>76</td>
<td>76</td>
</tr>
<tr>
<td>Interest&lt;sup&gt;8&lt;/sup&gt;, $/heifer</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>Fixed expenses&lt;sup&gt;9&lt;/sup&gt;, $/heifer</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Total 1st yr costs, $/heifer</td>
<td>830</td>
<td>882</td>
</tr>
<tr>
<td>Less cull heifer value, $/heifer exposed</td>
<td>62</td>
<td>88</td>
</tr>
<tr>
<td>Net 1st year's costs, $/heifer</td>
<td>768</td>
<td>794</td>
</tr>
<tr>
<td>Net cost for 1 bred heifer, $</td>
<td>826</td>
<td>873</td>
</tr>
</tbody>
</table>

<sup>1</sup>CR= heifers grazed dormant pastures 33 d, corn residue 73 d, and were placed on dormant winter pastures 67 d prior to entering the drylot 39 d before AI.

<sup>2</sup>DL= heifers grazed dormant pastures 98 d prior to entering the drylot 114 d before AI.

<sup>3</sup>Caculated as heifer initial BW × $2.37/kg.

<sup>4</sup>Feed costs valued at $0.75/d and $0.46/d for DL and CR heifers, respectively during the first 173 d of the development system.

<sup>5</sup>Feed costs associated with estrus synchronization period valued at $0.85/d for both DL and CR heifers during 39 d synchronization period.

<sup>6</sup>Feed costs associated with 111 d summer grazing period valued at $0.55/d.

<sup>7</sup>Includes costs associated with feed delivery, health and veterinary cost, trucking, estrous synchronization, and breeding costs.

<sup>8</sup>Caculated as opportunity cost plus ½ feed and variable costs based on a 7% interest rate.

<sup>9</sup>Includes insurance and depreciation of livestock, buildings, and equipment.
Table 5. Effect of winter heifer development system on late gestation ADG and feed efficiency

<table>
<thead>
<tr>
<th>Item</th>
<th>CR</th>
<th>DL</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All pregnant heifers</td>
<td></td>
<td></td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Winter initial BW, kg</td>
<td>435</td>
<td>453</td>
<td>4</td>
<td>0.04</td>
</tr>
<tr>
<td>Pre-calving BW, kg</td>
<td>448</td>
<td>466</td>
<td>16</td>
<td>0.09</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.45</td>
<td>0.33</td>
<td>0.10</td>
<td>0.09</td>
</tr>
<tr>
<td>Calan heifers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>449</td>
<td>450</td>
<td>9</td>
<td>0.77</td>
</tr>
<tr>
<td>Pre-calving BW, kg</td>
<td>506</td>
<td>516</td>
<td>9</td>
<td>0.10</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>10.13</td>
<td>10.14</td>
<td>0.10</td>
<td>0.93</td>
</tr>
<tr>
<td>NE DMI, kg/d</td>
<td>4.96</td>
<td>4.97</td>
<td>0.07</td>
<td>0.89</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>0.70</td>
<td>0.80</td>
<td>0.14</td>
<td>0.09</td>
</tr>
<tr>
<td>RFI, NE</td>
<td>0.028</td>
<td>-0.050</td>
<td>0.137</td>
<td>0.36</td>
</tr>
<tr>
<td>G:F</td>
<td>0.068</td>
<td>0.078</td>
<td>0.013</td>
<td>0.08</td>
</tr>
</tbody>
</table>

1 CR= heifers grazed dormant pastures 33 d, corn residue 73 d, and were placed in dormant winter pastures 67 d prior to entering the drylot 39 d before AI.
2 DL= heifers grazed dormant pastures 98 d prior to entering the drylot 114 d before AI.
3 Includes all corn residue and drylot developed heifers.
4 Heifers selected from either corn residue or drylot development system and placed in the Calan Broadbent individual feeding system for a 25 d adaptation period and approximately 82 d feeding trial during late gestation.
Table. 6. Effect of heifer development system on calving and reproductive performance through the subsequent breeding season

<table>
<thead>
<tr>
<th>Item</th>
<th>CR(^1)</th>
<th>DL(^2)</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>3</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>283</td>
<td>288</td>
<td>2</td>
<td>0.29</td>
</tr>
<tr>
<td>Birth date, Julian</td>
<td>66</td>
<td>69</td>
<td>1.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Calf birth BW, kg</td>
<td>33</td>
<td>34</td>
<td>0.4</td>
<td>0.19</td>
</tr>
<tr>
<td>Dystocia score(^3)</td>
<td>1.3</td>
<td>1.4</td>
<td>0.1</td>
<td>0.62</td>
</tr>
<tr>
<td>Prebreeding BW, kg</td>
<td>451</td>
<td>457</td>
<td>13</td>
<td>0.29</td>
</tr>
<tr>
<td>Pregnancy check BW, kg</td>
<td>484</td>
<td>482</td>
<td>16</td>
<td>0.83</td>
</tr>
<tr>
<td>Pregnant, %</td>
<td>89</td>
<td>86</td>
<td>11</td>
<td>0.65</td>
</tr>
</tbody>
</table>

\(^1\)CR= heifers grazed dormant pastures 33 d, corn residue 73 d, and were placed on dormant winter pastures 67 d prior to entering the drylot 39 d before AI.

\(^2\)DL= heifers grazed dormant pastures 98 d prior to entering the drylot 114 d before AI.

\(^3\)Scoring system 1 to 5: 1 = no assistance; 2 = easy pull; 3 = mechanical pull; 4 = hard mechanical pull; and 5 = cesarean section.
CHAPTER III: Late gestation supplementation impacts primiparous beef heifers and progeny

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ABSTRACT

A 3-yr study utilizing primiparous crossbred beef heifers (n = 114) was conducted to determine the effects of protein supplement during late gestation on cow and progeny performance. Pregnant heifers were stratified by heifer development system, initial BW, and service sire and placed in an individual feeding system. Heifers were offered meadow hay (10 % CP) from early November to mid-February and provided no supplement (CON; n = 37), 0.82 kg/d of a dried distillers grain based supplement (HIGH; n = 39) or 0.82 kg/d of a dried corn gluten feed based supplement (LOW; n = 38). Supplements were designed to be isonitrogenous (29% CP) and isocaloric, but differ in RUP with HIGH (59 % RUP) having greater levels of RUP than LOW (34% RUP). After the individual feeding period, heifers were placed in a drylot for calving. All heifers were bred using a fixed-timed AI protocol and pairs moved to a commercial ranch in the Nebraska Sandhills for summer grazing. Approximately 10 d after AI, a bull was placed with the heifers for 60 d. Pregnancy rates were determined via rectal palpation at weaning and calves placed in a feedlot. Final BW was greater ($P < 0.01$) for HIGH heifers compared with CON heifers (519 vs. 501 ± 10 kg). Heifers offered the HIGH diet had greater ($P < 0.01$) ADG and G:F compared with CON heifers. Similarly, supplemented heifers had increased ($P < 0.01$) DMI based on diet NE compared with CON heifers.
However, CON heifers had improved ($P < 0.01$) RFI compared with supplemented heifers. Pre-breeding BW was greater ($P = 0.03$) for supplemented heifers compared with CON, however there was no difference ($P = 0.58$) in proportion of heifers pregnant with their second calf. Weaning calf BW tended to be greater ($P = 0.09$) for steers born to HIGH dams (270 kg) compared with steers born to CON heifers (247 kg). Feedlot entry BW was greater ($P < 0.03$) for HIGH compared with CON calves (303 vs. 266 ± 23 kg). However, there was no difference in final BW among treatments ($P ≥ 0.51$). Marbling score tended ($P = 0.06$) to be greater for steers from CON dams compared with LOW (688 vs. 591 ± 52). Heifers from LOW dams tended ($P = 0.09$) to have reduced yield grade compared with CON. Crude fat levels were greater ($P < 0.01$) for steers from CON and HIGH dams compared with LOW. Protein supplementation improved primiparous heifer performance but did not impact reproductive efficiency. Furthermore, maternal protein supplementation improved steer calf weaning BW and altered carcass characteristics.

**Key Words:** beef cattle, maternal nutrition, protein supplementation

**INTRODUCTION**

Previous research indicates late gestation protein supplementation influences multiparous cow progeny performance, carcass quality, and health (Stalker et al., 2006, 2007; Martin et al., 2008; Mulliniks et al., 2008; Larson et al., 2009). These results support the fetal programming hypothesis, which suggests maternal environment during gestation can influence progeny postnatal growth and health (Barker et al., 1993). Most
studies concerning primiparous heifer prepartum nutrition focus their objectives on the impacts of nutritional treatments on reproductive performance (Bellows et al., 1982; Wiley et al., 1991; Lammoglia et al., 1997; Bellows et al., 2001; Patterson et al., 2003; Martin et al., 2005; Engel et al., 2008; Sullivan et al., 2009). However, a limited number of studies report the impact primiparous heifer nutrition during late gestation may have on subsequent progeny performance through weaning (Corah et al., 1975; Martin et al., 2005; Engel et al., 2008). Corah et al. (1975) reported altered birth and weaning BW for calves born to heifers receiving 65% of their energy requirement during late gestation. Pregnant heifers have added nutrient requirements during late gestation compared with mature cows due to their own growth requirement, as well as the growing fetus and maintenance requirements (Caton et al., 2007). Not meeting energy requirements of the dam not only impacts her productivity, but the performance of subsequent offspring as well (Houghton et al., 1990; Dunn et al., 1992; Beaty et al., 1994; Wu et al., 2004; Hess et al., 2005; Underwood et al., 2010). The objective of the current study was to evaluate the effects of RUP supplementation levels on primiparous heifer production and subsequent progeny growth, feed efficiency, and carcass quality.

**METHODS AND MATERIALS**

*Primiparous heifer management*

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in this experiment.

Each October, 40 pregnant heifers were placed in a Calan Broadbent individual feeding system and acclimated to the individual feeding bunks for approximately 25 d
prior to the beginning of the feeding trial. Heifers were offered an initial diet of 19% wet corn gluten feed (WCGF) and 81% grass hay (DM basis) *ad libitum* during the acclimation period. Wet corn gluten feed was slowly removed from the ration during the acclimation period until heifers received only grass hay at the beginning of the feeding trial. Six heifers were removed (yr 1 = 2, yr 3 = 4) from the feeding system during the acclimation period due to temperament. Following the acclimation period, heifers (n=114) were offered meadow hay (10.2% CP, DM basis) from early November to mid-February (yr 1 and 3 = 84 d; yr 2 = 80 d) and provided no supplement (CON; n = 37); 0.82 kg/d (DM basis) of a dried distillers grains with solubles (DDGS) based supplement (HIGH; n = 39); or 0.82 kg/d (DM basis) of a dried corn gluten feed based supplement (LOW; n = 38). Supplements (Table 1) were designed to be isonitrogenous (29% CP, DM basis) and isocaloric, but differ in RUP with HIGH (59% RUP) having greater levels of RUP than LOW (34% RUP).

Hay and supplement were offered once daily at approximately 0800. Hay offered was recorded daily and estimated to allow for *ad libitum* intake with 0.10-0.23 kg refusals daily. Hay was measured and fed followed by 0.82 kg supplement offered on top of the hay for HIGH and LOW heifers, respectively. Feed refusals were measured and recorded weekly and heifer BW measured every 14 d in yr 1 and 21 d in yr 2 and 3. Nutrient balance for heifers based on late gestation nutrition was calculated using the 1996 NRC computer model (Table 2). Model considerations included the average d of gestation for the feeding period (220 d), age of heifer (22 mo), and average BW during the feeding period (CON = 476 kg; HIGH = 483 kg; LOW = 482 kg) with thermoneutral conditions.
Dietary nutrient intake was based on nutrient analysis (Ward Laboratories, Kearney, NE) and heifer DMI.

After the individual feeding period, heifers were placed in a dry lot for calving. Heifers were given a calving ease score (1= no assistance; 2= easy assist; 3= difficult assist; 4= caesarian section; 5=breech/abnormal presentation) and calves received a vigor score (1= nursed immediately; 2= nursed on own, took some time; 3= required some assistance to suckle; 4= died shortly after birth; 5= dead on arrival) at parturition. Heifers remained in a single group and were offered 7.9-9.5 kg/d (DM basis) grass hay and 2.8 kg/d (DM basis) corn and WCGF-based supplement until placed on summer pasture after the breeding season. Prior to breeding, cows were vaccinated against infectious bovine rhinotracheitis/bovine viral diarrhea type I and II/ parainfluenza-3 virus/Vibriosis/Leptospirosis (PregGaurd Gold FP10, Pfizer Animal Health, New York, NY). Estrus was synchronized using a fixed-timed AI protocol. Ten d prior to AI cows were administered a single injection GnRH (100 μg; i.m.; Fertagyl, Intervet, Millsboro, DE) and a controlled internal drug release vaginal insert (Eazi-Breed CIDR, Pfizer Animal Health, New York, NY). Sixty h prior to AI, CIDR were removed and cows given a single injection of prostaglandin (25 μg; i.m.; Lutylase, Pfizer Animal Health, New York, NY). Artificial insemination was performed by a single technician and approximately 1 wk later pairs were moved 43 km to a commercial ranch in the Nebraska Sandhills for summer grazing. A single bull was placed with cows approximately 10 d after AI for 60 d. Pairs were returned to WCREC in late October for final pregnancy diagnosis and weaning.
**Preweaning calf management**

At approximately 2 mo of age, all calves received an infectious bovine rhinotracheitis/parainfluenza-3 virus/bovine respiratory syncytial virus/bovine viral diarrhea type I and II vaccine (BoviShield 5, Pfizer Animal Health, New York, NY). At the time of vaccination calves were weighed, branded, and male calves castrated. Calves were shipped with cows to summer grazing pastures and returned to WCREC in late October for weaning. Upon arrival at WCREC calves were given an injection of BoviShield 5, (Pfizer Animal Health, New York, NY) prior to weaning. At weaning, calves were weighed, electronic identification tags applied (yr 2), vaccinated against bovine rotavirus-coronavirus/clostridium perfringens type C and D/Escherichia coli bacterin-toxoid (Guardian, Intervet, Millsboro, DE), and topical endectocide was applied (Ivermectin, Aspen Veterinary, Liberty, MO).

**Calf feedlot management**

After weaning calves were limit fed a starter diet for 5 d at 2.0% BW prior to determining initial feedlot BW. Implants were administered providing 20 mg of estradiol benzoate and 200 mg progesterone (Synovex S, Pfizer Animal Health, New York, NY) to steers and 20 mg of estradiol benzoate and 200 mg testosterone to heifers (Synovex H, Pfizer Animal Health, New York, NY). Calves were transitioned (21 d) to a common finishing diet of 48% dry rolled corn, 40% corn gluten feed, 7% prairie hay, and 5% supplement (DM basis; Table 3). Approximately 100 d prior to slaughter, calves were implanted with 28 mg estradiol benzoate and 200 mg trenbolone acetate (Synovex Plus, Pfizer Animal Health, New York, NY). Calves were slaughtered at a commercial abattoir when visually estimated to have 1.3-cm fat thickness over the 12th rib. Carcass data and
HCW was collected after a 24-h chill. Final BW was calculated using the HCW adjusted for a common dressing percentage (63%).

In yr 1, calf DMI was calculated using the DMI prediction equation established by Tedeschi et al. (2006) where $DMI = 4.18 + (1.98 \times ADG) + (0.0013 \times (MBW^{0.75}) + (0.019 \times EBF)$ with EBF representing estimated body fat percentage. Estimated body fat percentage was calculated using the equation developed by Guiroy et al. (2001) where $EBF = 17.76107 + (11.8908 \times 12\text{th rib fat depth}) + (0.0088 \times \text{HCW}) + (0.81855 \times [(\text{marbling score/100}) + 1] – (0.4356 \times \text{longissimus muscle area})$. In yr 2 calves were placed in a GrowSafe feeding system (GrowSafe Systems Ltd, Airdrie, AB, Canada) approximately 1 mo after weaning. Calf BW was measured on 2 consecutive d prior to GrowSafe entry and again 10 d after GrowSafe entry to account for the acclimation period to the feeding system. The average of the second 2 d BW was considered the initial feedlot entry BW in yr 2 and data concerning feedlot performance (BW change, DMI, ADG) was calculated from this average BW.

**Statistical analysis**

Heifers were offered hay and supplement on an individual basis (yr 1 = 38; yr 2 = 40 yr 3 = 36), therefore animal was considered the experimental unit and diet the treatment. Data were analyzed using PROC MIXED and PROC GLIMMIX of SAS with a $P \leq 0.05$ considered significant. The statistical model for heifers included treatment as the fixed effect with pen and year as random effects. Artificial insemination technician was included as a random effect in the model for AI pregnancy and overall pregnancy data. The initial statistical model for calves included dam treatment as the fixed effects with sex included as a covariate and sire and yr included as random effects. Calf sex was
a significant source of variation and thus was placed in the final model as a fixed effect. Due to the differences in calf performance based on sex, calf performance data will be presented for steers and heifers separately. Residual feed intake (RFI) was calculated utilizing PROC GLM of SAS. Coefficients for ADG and mid BW were calculated for steers and heifers separately. Year 2 RFI was calculated with the initial period being GrowSafe entry to re-implant and the re-implant period being calculated from re-implant to slaughter to determine differences in RFI during the feedlot period.

RESULTS AND DISCUSSION

Primiparous heifer production

Primiparous heifer performance data are reported in Table 4. Initial individual feeding period BW was similar for all three treatment groups \((P = 0.44)\). However, HIGH heifers had greater \((P < 0.01)\) final BW compared with CON heifers \((519 \text{ vs. } 501 \pm 10 \text{ kg})\), and final BW tended \((P = 0.07)\) to be heavier for LOW compared with CON heifers. Similarly, at prebreeding, HIGH and LOW heifers had increased \((P = 0.03)\) BW compared with CON heifers, although BW were similar \((P = 0.23)\) between all treatments at weaning. Previous data indicate supplementing multiparous cows with 0.45 kg/d dried distillers grains with solubles (DDGS) during late gestation improves pre-calving BW (Stalker et al., 2006). However, cows in that study were grazing dormant winter range during supplementation compared with heifers in the current study receiving grass hay. Previous data suggest dormant winter range in the Nebraska Sandhills contains approximately 5.4% CP (DM basis; Stalker et al., 2006) whereas hay offered heifers in the current study contained approximately 10.2 % CP (DM basis). These data would suggest non-supplemented cows in the study conducted by Stalker et al. (2006) were
likely more nutrient deficient than supplemented cows, whereas estimated dietary
nutrient intake in the current study indicate no deficiencies in energy or CP based on late
gestation treatment (Table 2).

Klopfenstein (1996) reported young, growing cows may require some RUP
supplementation to meet MP requirements when consuming low quality forages, but diets
fed in the current study met MP requirements of the animals in late gestation (Table 2),
however HIGH heifers did receive an additional 109 g/d of MP in the form of RUP
compared with CON heifers. Engel et al. (2008) offered heifers a grass hay and DDGS
diet (DDG; 17.1% CP; 39% RUP; 6.3% EE) or a grass hay and soybean hulls diet (SBH;
12.8% CP; 19.8% RUP; 3.4%EE) for 70 d during late gestation. Both diets provided
adequate MP until the last mo of gestation when SBH heifers were -48g/d deficient.
During early gestation, fetal growth is slow and nutrient demand for pregnancy low. In
ruminants 75% of fetal growth occurs during the last 2 mo of gestation (Robinson et al.,
1977). Increased fetal nutrient demand is responsible for the increased MP requirement in
the last mo of gestation for both DDG and SBH heifers (Engel et al., 2008). Final BW
was 7 kg (± 1.8 kg) greater for DDG compared with SBH heifers. Furthermore, Engel et
al. (2008) reported improved pregnancy rates for DDG heifers compared with SBH.
These data along with the data reported in the current study could suggest both increased
fat and RUP of DDG allows for improved cow performance, although the mechanistic
action is unclear.

Average daily gain was greater ($P < 0.01$) for HIGH compared with CON heifers
(0.89 vs. 0.61 ± 0.14 kg/d). Furthermore, DMI tended ($P = 0.09$) to be greater for HIGH
compared with CON heifers. Energy density of the diets also differed (Table 2). Control
heifers were offered a diet of grass hay only whereas HIGH and LOW heifers were offered a DDGS-based or corn gluten feed based supplement, respectively. Also, the HIGH and LOW supplements were designed to be isocaloric and isonitrogenous, but differ in RUP. Similarities ($P = 0.62$) in DMI based on NE for HIGH and LOW heifers suggest supplements were similar in energy density and protein content. The differences in diet nutrient density resulted in a greater ($P < 0.01$) nutrient intake for the HIGH and LOW heifers compared with the CON heifers. Although DMI tended to be greater for HIGH compared with CON heifers, G:F was still greater ($P < 0.01$) for HIGH compared with CON heifers. The increase in G:F can be attributed to improved ADG for the HIGH heifers, which was approximately 1.5 times greater than CON heifers. However, CON heifers had increased RFI based on diet NE ($P < 0.01$) compared with HIGH and LOW heifers.

Gestation length, Julian calving date, calf birth BW, and calving ease were similar among treatments ($P \geq 0.34$). Previous studies have also reported similar findings with regard to calving data (Bolze et al., 1985; Wiley et al., 1991; Martin et al., 2005; Stalker et al., 2007; Engel et al., 2008; Larson et al., 2009) based on maternal nutrition. However, some reports indicate alterations in calving difficulty, gestation length, calf birth BW, and calf vigor based on maternal nutrition during late gestation (Stalker et al., 2006; Gunn et al., 2011; Winterholler et al., 2012). Corah et al. (1975) reported heifers fed 65% of their energy requirement gave birth to calves 2 kg lighter compared with calves born to heifers fed 100% of their energy requirements. In the current study, calves born to CON dams tended ($P = 0.06$) to have improved calf vigor scores compared with calves born to LOW dams. Lammoglia et al. (1999) reported improved calf vigor for calves born to fat
supplemented dams. Gunn et al. (2011) utilized DDGS as an energy source in primiparous heifer diets during late gestation. These authors reported similar calf BW and vigor, but increased gestation length and dystocia for heifers fed DDGS compared with heifers fed a control diet of corn silage and haylage. Similarly, Wiley et al. (1991) reported similar birth BW and vigor scores for heifers fed a low nutrient diet compared with heifers fed to maintenance levels during late gestation.

There was no difference ($P = 0.58$) in proportion of cows pregnant with a second calf based on late gestation nutrition. The impact of late gestation nutrition on the following breeding season reproductive performance in beef cattle has been mixed (Bader et al., 2000; Sletmoen-Olsen et al., 2000; Hess et al., 2005; Martin et al., 2005; Winterholler et al., 2012). Patterson et al. (2003) reported increased pregnancy rates for heifers supplemented with RUP during late gestation to meet MP requirements. Also, Engel et al. (2008) indicated feeding DDG to primiparous heifers during late gestation improved reproductive performance, although proportions of animals initiating estrous cycles prior the breeding season was similar for DDGS and SBH heifers. Martin et al. (2007) reported similar proportions of heifers displaying estrus prior to the first breeding season, but heifers supplemented distillers grains during development had improved AI conception and pregnancy rates compared with heifers supplemented with a dried corn gluten feed based supplement during development.

**Preweaning and weaning calf performance**

There was a significant difference ($P < 0.05$) for steer and heifer performance with regard to BW, feedlot performance and carcass characteristics, thus data will be presented by gender. Previous literature also report different performance for calves.
based on gender. Rolfe et al. (2011) report a 17 kg increase in steer BW across treatments compared with heifer BW at steer initial feedlot entry. Bailey (2006) reported a 26 kg increase ($P < 0.01$) in BW at feedlot entry for steers compared with heifers. Furthermore, steers had greater DMI, ADG, HCW, and LM area compared with heifers (Bailey, 2006). Previous data also indicate similar differences in steer and heifer performance, as well as, alterations in the efficiency in which they deposit tissue (Hedrick et al., 1969; Ray et al., 1969; Fox and Black, 1984; Brown and Lawrence, 2010; Long et al., 2010).

Data for steer and heifer progeny performance preweaning and in the feedlot are summarized in Tables 5 and 6, respectively. Calf BW for steer or heifers based on maternal diet at 2 mo of age ($P \geq 0.39$) was not different. Similarly, Wiley et al. (1991) reported no difference in calf BW at 30, 60, or 90 d of age based on maternal late gestation nutrient intake. Conversely, Winterholler et al. (2012) reported 60 and 90 d calf BW tended to increase ($P = 0.06; 0.07$) as maternal DDGS supplementation increased during late gestation. However, Winterholler et al. (2012) also reported a linear increase in calf birth BW based on maternal diet during late gestation.

Weaning BW tended to be greater ($P = 0.09$) for steer calves born to HIGH dams compared with steers born to CON dams. Previous reports document improved weaning BW for calves born to dams offered DDGS during late gestation (Larson et al., 2009; Gunn et al., 2011). Stalker et al. (2006, 2007) and Larson et al. (2009) reported a 6-12 kg increase in calf weaning BW for calves born to protein supplemented dams. Similarly, Underwood et al. (2010) demonstrated calves born to cows grazing improved pasture from d 120 to 180 of gestation resulted in increased weaning BW. Corah et al. (1975) reported a 13 kg increased in weaning BW for calves born to heifers fed 100% of their
energy requirements during late gestation, and Beaty et al. (1994) reported an increase in calf weaning BW as the amount of CP fed to dams during gestation increased. Rolfe et al. (2011) utilized a 2 × 4 factorial design to determine the effect of weaning date and maternal supplementation during late gestation on calf performance. Calves born to cows grazing dormant winter range and receiving no supplement had reduced BW at approximately 7 mo of age compared with calves from cows receiving either 0.45 or 0.91 kg/d (32% CP, DM basis) of supplement during late gestation and grazing winter range, or cows grazing corn residue. Conversely, Engel et al. (2008) reported no differences in weaning BW or ADG for calves born to DDG or SBH fed primiparous heifers. Interestingly, there was no difference in heifer calf weaning BW based on maternal diet ($P = 0.86$). Martin et al. (2007) and Funston et al. (2010) reported an increase in heifer adjusted 205-d weaning BW and actual weaning BW, respectively, for heifers born to cows supplemented with a DDGS based supplement during late gestation.

Previous data suggests increasing RUP levels in the diet during late gestation may increase milk production (Moorby et al., 1996; Greenfield et al., 1998). Van Saun et al. (1993) reported an increase in milk protein but not yield when dietary RUP levels were increased 3 wk prepartum. However, studies supplementing or feeding DDGS during late gestation in beef cattle have reported no improvements in subsequent lactation milk production. Gunn et al. (2011) reported heifers fed DDGS as an energy source had similar milk production compared with control cohorts fed an isocaloric, isonitrogenous diet. Similarly, Winterholler et al. (2012) reported no differences in milk yield based on DDGS supplementation, regardless of the level of DDGS supplement offered. Radunz et al. (2010) reported cows fed hay, limit fed corn, or limit fed DDGS beginning on d 209 of
gestation had similar milk production levels when measured at early, mid, and late lactation. Milk production was not measured in the current study; however, the data previously mentioned would suggest no differences in milk production. Increased weaning BW in steers born to HIGH dams compared with CON would suggest a fetal programming mechanism allowing for improved calf performance after birth. In a recent review, Vonnahme and Lemley (2012) stated uterine blood flow was increased nearly two fold within 30 d if cows were offered a protein supplement during late gestation compared with non-supplemented cows. These data would suggest increased nutrient availability to the fetus due to improved uterine blood flow.

**Calf feedlot performance**

Calf feedlot data and carcass characteristics are based on the first 2 yr of the study. At feedlot entry, BW was 37 kg (± 23 kg) greater for calves born to HIGH dams compared with CON dams. However, there were no differences ($P \geq 0.42$) in re-implant BW or end BW among treatments. Similarly, ADG among treatments for the initial, re-implant, or total feedlot period for steer or heifer calves was not different ($P \geq 0.48$). Rolfe et al. (2011) reported similar findings for steers during the feedlot phase with no differences in ADG, G:F, or DMI based on maternal nutrition during late gestation. Conversely, Stalker et al. (2007) and Larson et al. (2009) reported steer ADG tended to be greater for steers born to protein supplemented dams. Underwood et al. (2010) reported improving maternal nutrition during the gestation resulted in increased ADG and tended to increase final BW.

In yr 2, RFI was calculated for the initial to re-implant period and from re-implant to slaughter. Previous literature reports steer and heifer RFI can change over time,
especially when steers are moved from a growing to finishing diet (Durunna et al., 2011, 2012). Steers born to CON dams had a tendency \((P = 0.14)\) for increased RFI in the initial period compared with steers born to HIGH dams. However, there was no difference in RFI for the re-implant to slaughter period. Interestingly, initial RFI tended \((P = 0.08)\) to be lower for heifers from HIGH dams compared with heifers born to CON dams. Furthermore, there was a tendency \((P = 0.12)\) for a greater difference in RFI from the initial to re-implant for heifers from HIGH dams compared with heifers from CON dams. Change in RFI from the initial to the re-implant period was 0.103, -1.220, and 0.245 kg for CON, HIGH, and LOW born steers, respectively, and -1.995, 1.058, and -.300 kg for CON, HIGH and LOW born heifers. Durunna et al. (2011) suggested 58% of steers had a 0.24 to 0.38 kg/d change in RFI when switched from a growing to finishing diet. Similarly, Durunna et al. (2012) reported RFI of 51% of heifers was re-ranked from period 1 to period 2, although diet did not change between the two periods. Similar to the report by Durunna et al. (2011), 27% of the heifers re-ranked between periods had a change in RFI of approximately 0.37 to 0.44 kg of DM/d (Durunna et al., 2012).

Although the periods measured in this study do not reflect the time in diet change from a growing to finishing diet, the time frame in which implant is changed also seems to impact steer and heifer RFI.

**Carcass characteristics**

Carcass characteristics are reported in Tables 7 and 8 for steers and heifers, respectively. Hot carcass weight did not differ for steers or heifers based on maternal diet during late gestation \((P \geq 0.49)\). Similarly, Stalker et al. (2006) reported no difference in HCW for calves born to supplemented or non-supplemented dams. However, Stalker et
al. (2007) and Larson et al. (2009) reported increased HCW for steers born to protein-supplemented dams and Underwood et al. (2010) reported improving maternal nutrition during gestation increased progeny HCW. Marbling score tended ($P = 0.06$) to be greater for CON born steers compared with LOW born steers (688 vs. 591 ± 52). Larson et al. (2009) reported an increase in marbling score for calves born to protein supplemented dams. In that study the protein supplement was a DDGS-based supplement, similar to the HIGH supplement in the current study. Furthermore, Larson et al. (2009) reported improved calf health in the feedlot if steers were born to supplemented dams. Previous literature reports a correlation between respiratory disease episodes in the feedlot and marbling score (Gardner et al., 1999; Busby et al., 2004). Radunz (2009) reported an increase in marbling score for calves born to cows fed hay compared with cows limit fed corn during late gestation. However, there was no difference in marbling score for calves from hay fed and DDGS limit-fed cows.

Heifers born to LOW supplemented dams tended ($P = 0.08, 0.09$) to have reduced EBF and yield grade compared with CON born heifers. Steers born to CON dams tended ($P = 0.08$) to have improved tenderness compared with LOW born steers. Also, ether extract levels were greater ($P < 0.01$) from both CON and HIGH born steers compared with LOW born steers. Underwood et al. (2010) reported a trend for increased ether extract in LM samples from steers born to cows grazing improved pastures compared with native range during gestation. Radunz (2009) reviewed the site-specific preferences for adipocytes. Subcutaneous adipocytes prefer acetate whereas intramuscular adipocytes prefer glucose as a substrate (Smith and Crouse, 1984; Rhoades et al., 2007). Furthermore, Radunz (2009) suggested increased marbling for calves born to hay fed...
dams in late gestation result from increased insulin sensitivity during gestation for hay compared with corn fed cows, altering fetal adipocyte development and formation. Also, plasma insulin secretion has been correlated to carcass adiposity and insulin stimulates glucose uptake and lipogenesis in adipocytes (Trenkle and Topel, 1978; Radunz, 2009).

Although the proportion of steers and heifers grading USDA Choice or greater was similar among maternal treatments, the proportion of steers grading USDA modest or greater tended to be greater ($P = 0.10$) for steers born to HIGH dams compared with LOW. Larson et al. (2009) reported a 19% increase in the proportion of steers grading USDA modest or greater for steers born to protein supplemented dams compared with steers born to non-supplemented dams. Similarly, Radunz (2009) reported calves born to protein supplemented cows had increased proportions grading USDA Choice or greater. However, Stalker et al. (2006) did not report any differences in proportion of steers grading USDA Choice based on maternal protein supplementation. Remember, Larson et al. (2009) fed a distillers based supplement high in RUP, whereas Stalker et al. (2006) supplemented cows with a sunflower seed meal/cottonseed meal supplement with approximately 31% RUP. These data would suggest an increased USDA quality grade for steers born to dams supplemented with a greater proportion of RUP. However, it should also be noted the current study did not report an improvement in the proportion of steers grading USDA modest or greater for HIGH compared with CON born steers, as reported by Larson et al. (2009).

Findings in the current study differ from previously reported data for late gestation protein supplementation of multiparous cows. Studies conducted by Stalker et al. (2006, 2007), Martin et al. (2007), Larson et al. (2009), and Funston et al. (2010)
utilized mature cows grazing winter range or corn residue, with maternal supplementation provided during late gestation. The stage of gestation in which supplementation occurred was similar to the time of supplementation in the current study. However, differences in calf performance and carcass characteristics could likely be related to control diet quality in those studies compared with the current study. The current study offered CON heifers ad libitum grass hay consumption. As previously mentioned this diet was not deficient in energy, MP, or RDP (Table 2). Grazing cattle on dormant winter range does not meet the MP requirement for late gestating cattle, thus the non-supplemented dams in the previous studies would have been in a negative energy balance, unlike CON heifers in the current study. It is likely that reducing CON heifer intake to create a negative nutrient intake, similar to that previously observed by our group in multiparous studies would result in a greater fetal programming effect.

**IMPLICATIONS**

Primiparous heifer performance was not different when comparing the two levels of RUP supplemented during late gestation. However HIGH heifers had increased final BW, ADG and G:F compared with CON heifers. Steer calves from HIGH supplemented dams had greater weaning BW and calves born to supplemented dams during late gestation had increased feedlot entry BW compared with calves from CON heifers. Furthermore, feedlot production and carcass characteristics were impacted by maternal nutrition. Additional research is warranted to determine the effects differing RUP supplements may have on primiparous heifers grazing low quality forages during late gestation.
LITERATURE CITED


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<td><strong>LOW</strong>²</td>
<td>-</td>
<td>72.4</td>
<td></td>
</tr>
<tr>
<td>DDGS³</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>CGF⁴</td>
<td>-</td>
<td>-</td>
<td>72.4</td>
</tr>
<tr>
<td>Corn germ</td>
<td>-</td>
<td>-</td>
<td>24.5</td>
</tr>
<tr>
<td>Urea</td>
<td>-</td>
<td>-</td>
<td>2.1</td>
</tr>
<tr>
<td>Trace minerals and vitamins</td>
<td>1.0</td>
<td>1.0</td>
<td></td>
</tr>
</tbody>
</table>

Nutrient analysis⁵

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CP, %</td>
<td>28.5</td>
<td>28.0</td>
<td></td>
</tr>
<tr>
<td>RUP, % CP</td>
<td>59.0</td>
<td>34.0</td>
<td></td>
</tr>
<tr>
<td>TDN</td>
<td>79.4</td>
<td>77.3</td>
<td></td>
</tr>
<tr>
<td>Crude Fat, %</td>
<td>11.9</td>
<td>11.9</td>
<td></td>
</tr>
</tbody>
</table>

¹Heifers offered 0.82 kg/d (DM) distillers grain based supplement.
²Heifers offered 0.82 kg/d (DM) dried corn gluten feed based supplement.
³Dried distillers grains with solubles.
⁴Dried corn gluten feed.
⁵Wet Chemistry, Ward Laboratories Inc., Kearney, NE; RUP based on NRC (1996).
Table 2. Nutrient balance for primiparous heifers during late gestation

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CON</th>
<th>HIGH</th>
<th>LOW</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diet supplied NE Mcal/d</td>
<td>11.5</td>
<td>12.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Required NE Mcal/d</td>
<td>7.9</td>
<td>7.9</td>
<td>7.9</td>
</tr>
<tr>
<td>Difference Mcal/d</td>
<td>3.6</td>
<td>4.6</td>
<td>4.4</td>
</tr>
<tr>
<td>Diet supplied MP, g/d</td>
<td>631</td>
<td>817</td>
<td>733</td>
</tr>
<tr>
<td>Required MP, g/d</td>
<td>535</td>
<td>571</td>
<td>565</td>
</tr>
<tr>
<td>MP from RUP, g/d</td>
<td>243</td>
<td>352</td>
<td>275</td>
</tr>
<tr>
<td>Difference MP, g/d</td>
<td>96</td>
<td>247</td>
<td>168</td>
</tr>
<tr>
<td>Diet supplied RDP, g/d</td>
<td>606</td>
<td>727</td>
<td>716</td>
</tr>
<tr>
<td>Required RDP, g/d</td>
<td>709</td>
<td>759</td>
<td>839</td>
</tr>
<tr>
<td>RDP balance, g/d</td>
<td>103</td>
<td>32</td>
<td>123</td>
</tr>
</tbody>
</table>

1 Based on NRC model application.
2 Primiparous heifers individually fed meadow hay and no supplement (CON), 0.82 kg/d (DM) distillers grain based supplement (HIGH), or 0.82 kg/d (DM) dried corn gluten feed based supplement (LOW) during late gestation.
3 Based on DMI.
Table 3. Composition of backgrounding and finishing diets fed in the feedlot to progeny of primiparous heifers fed either no, 0.82 kg/d high RUP or 0.82 kg/d low RUP supplement during the last trimester of gestation.

<table>
<thead>
<tr>
<th>Item</th>
<th>Backgrounding</th>
<th>Finishing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dry rolled corn</td>
<td>15</td>
<td>48</td>
</tr>
<tr>
<td>Corn gluten feed</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Prairie hay</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Supplement(^3)</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^1\)Contained 20% CP, 1.1% Ca, 0.58% P, and 1.2% K (DM basis).
\(^2\)Contained 16% CP, 0.67% Ca, 0.56% P, and 0.9% K (DM basis).
\(^3\)Provided dietary concentration of 28 g/t of monensin and 10 g/t of tylosin (DM basis, Elanco Animal Health, Indianapolis, IN).
Table 4. Effects of supplementation on primiparous heifer late gestation performance, calving results and subsequent breeding season reproductive efficiency

<table>
<thead>
<tr>
<th>Item</th>
<th>CON</th>
<th>HIGH</th>
<th>LOW</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>37</td>
<td>39</td>
<td>38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td>451</td>
<td>446</td>
<td>451</td>
<td>9</td>
<td>0.44</td>
</tr>
<tr>
<td>Final BW, kg</td>
<td>501&lt;sup&gt;a&lt;/sup&gt;</td>
<td>519&lt;sup&gt;b&lt;/sup&gt;</td>
<td>513&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ADG, kg</td>
<td>0.61&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.75&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.14</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DMI, kg/d</td>
<td>4.68&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.15&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>RFI&lt;sup&gt;2&lt;/sup&gt;</td>
<td>-0.213&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.102&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.078&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.138</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>G:F</td>
<td>0.061&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.086&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.073&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>0.013</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Calving date, Julian d</td>
<td>60</td>
<td>60</td>
<td>62</td>
<td>1</td>
<td>0.34</td>
</tr>
<tr>
<td>Gestation length, d</td>
<td>276</td>
<td>276</td>
<td>277</td>
<td>0.8</td>
<td>0.86</td>
</tr>
<tr>
<td>Calf birth BW, kg</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>0.9</td>
<td>0.89</td>
</tr>
<tr>
<td>Calving ease</td>
<td>1.4</td>
<td>1.4</td>
<td>1.5</td>
<td>0.1</td>
<td>0.67</td>
</tr>
<tr>
<td>Calf vigor</td>
<td>1.3</td>
<td>1.5</td>
<td>1.9</td>
<td>0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Pre-breeding BW, kg</td>
<td>444&lt;sup&gt;a&lt;/sup&gt;</td>
<td>458&lt;sup&gt;b&lt;/sup&gt;</td>
<td>461&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13</td>
<td>0.03</td>
</tr>
<tr>
<td>Cow BW at weaning, kg</td>
<td>469</td>
<td>487</td>
<td>493</td>
<td>17</td>
<td>0.23</td>
</tr>
<tr>
<td>Cows pregnant with second calf, %</td>
<td>89</td>
<td>91</td>
<td>83</td>
<td>12</td>
<td>0.58</td>
</tr>
</tbody>
</table>

<sup>1</sup>Primiparous heifers individually fed meadow hay and no supplement (CON), 0.82 kg/d (DM) distillers grain based supplement (HIGH), or 0.82 kg/d (DM) dried corn gluten feed based supplement (LOW) during late gestation.

<sup>2</sup>RFI calculated based on NE DMI.
Table 5. Effect of late gestation supplementation on steer progeny preweaning and feedlot performance

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>CON</th>
<th>HIGH</th>
<th>LOW</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>17</td>
<td>16</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May calf BW, kg</td>
<td></td>
<td>101</td>
<td>107</td>
<td>101</td>
<td>5</td>
<td>0.46</td>
</tr>
<tr>
<td>Weaning Wt, kg</td>
<td></td>
<td>247</td>
<td>270</td>
<td>259</td>
<td>9</td>
<td>0.09</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td></td>
<td>266a</td>
<td>303b</td>
<td>292ab</td>
<td>23</td>
<td>0.03</td>
</tr>
<tr>
<td>Reimplant BW, kg</td>
<td></td>
<td>412</td>
<td>433</td>
<td>430</td>
<td>21</td>
<td>0.42</td>
</tr>
<tr>
<td>End BW, kg&lt;sup&gt;2&lt;/sup&gt;</td>
<td></td>
<td>608</td>
<td>621</td>
<td>622</td>
<td>21</td>
<td>0.80</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial</td>
<td></td>
<td>1.87</td>
<td>1.75</td>
<td>1.81</td>
<td>0.11</td>
<td>0.56</td>
</tr>
<tr>
<td>Re-implant</td>
<td></td>
<td>1.91</td>
<td>1.84</td>
<td>1.85</td>
<td>0.10</td>
<td>0.79</td>
</tr>
<tr>
<td>Total ADG</td>
<td></td>
<td>1.90</td>
<td>1.79</td>
<td>1.84</td>
<td>0.08</td>
<td>0.48</td>
</tr>
<tr>
<td>Yr 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI&lt;sup&gt;3&lt;/sup&gt;, kg</td>
<td></td>
<td>8.60</td>
<td>8.43</td>
<td>8.47</td>
<td>0.23</td>
<td>0.83</td>
</tr>
<tr>
<td>G:F</td>
<td></td>
<td>0.220</td>
<td>0.213</td>
<td>0.216</td>
<td>0.007</td>
<td>0.72</td>
</tr>
<tr>
<td>RFI</td>
<td></td>
<td>-0.009</td>
<td>0.007</td>
<td>-0.014</td>
<td>0.020</td>
<td>0.71</td>
</tr>
<tr>
<td>Yr 2&lt;sup&gt;4&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Initial&lt;sup&gt;5&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, kg</td>
<td></td>
<td>10.15</td>
<td>11.20</td>
<td>10.50</td>
<td>1.30</td>
<td>0.75</td>
</tr>
<tr>
<td>G:F</td>
<td></td>
<td>0.182</td>
<td>0.152</td>
<td>0.172</td>
<td>0.015</td>
<td>0.26</td>
</tr>
<tr>
<td>RFI</td>
<td></td>
<td>-0.068</td>
<td>1.198</td>
<td>0.346</td>
<td>0.552</td>
<td>0.15</td>
</tr>
<tr>
<td>Re-implant&lt;sup&gt;6&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DMI, kg</td>
<td></td>
<td>10.97</td>
<td>9.35</td>
<td>11.12</td>
<td>0.76</td>
<td>0.23</td>
</tr>
<tr>
<td>G:F</td>
<td></td>
<td>0.176</td>
<td>0.183</td>
<td>0.174</td>
<td>0.015</td>
<td>0.87</td>
</tr>
<tr>
<td>RFI</td>
<td></td>
<td>-0.024</td>
<td>-0.546</td>
<td>0.355</td>
<td>0.896</td>
<td>0.73</td>
</tr>
<tr>
<td>RFI difference&lt;sup&gt;7&lt;/sup&gt;</td>
<td></td>
<td>0.103</td>
<td>-1.220</td>
<td>0.245</td>
<td>1.090</td>
<td>0.55</td>
</tr>
</tbody>
</table>

<sup>1</sup>Dams individually fed meadow hay and no supplement (CON), 0.82 kg/d (DM) distillers grain based supplement (HIGH), or 0.82 kg/d (DM) dried corn gluten feed based supplement (LOW) during late gestation.

<sup>2</sup>Calculated from HCW and adjusted to a common dressing percent (63.0%).

<sup>3</sup>DMI calculated in yr 1 using the prediction formula presented by Tedeschi et al. (2006) where DMI = 4.18 + (1.98 x ADG) + (0.0013 x (MBW<sup>0.75</sup>) + (0.019 x EBF).

<sup>4</sup>Steer calves from yr 2 were placed in a Grow-Safe feeding system and individual intakes recorded daily to calculate DMI, G:F and RFI.

<sup>5</sup>Period from feedlot initial BW to re-implant (75 d).

<sup>6</sup>Period from re-implant to slaughter (97 d).

<sup>7</sup>Difference in RFI between initial and re-implant periods.
Table 6. Effect of late gestation supplementation on heifer progeny preweaning and feedlot performance

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>CON</th>
<th>HIGH</th>
<th>LOW</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td></td>
<td>20</td>
<td>23</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>May calf BW, kg</td>
<td></td>
<td>98</td>
<td>103</td>
<td>101</td>
<td>6</td>
<td>0.39</td>
</tr>
<tr>
<td>Weaning Wt, kg</td>
<td></td>
<td>250</td>
<td>252</td>
<td>253</td>
<td>5</td>
<td>0.86</td>
</tr>
<tr>
<td>Initial BW, kg</td>
<td></td>
<td>270</td>
<td>282</td>
<td>280</td>
<td>10</td>
<td>0.28</td>
</tr>
<tr>
<td>Reimplant BW, kg</td>
<td></td>
<td>394</td>
<td>407</td>
<td>400</td>
<td>8</td>
<td>0.36</td>
</tr>
<tr>
<td>End BW, kg²</td>
<td></td>
<td>558</td>
<td>569</td>
<td>574</td>
<td>10</td>
<td>0.51</td>
</tr>
<tr>
<td>ADG, kg/d</td>
<td>Initial</td>
<td>1.59</td>
<td>1.59</td>
<td>1.57</td>
<td>0.06</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Re-implant</td>
<td>1.60</td>
<td>1.63</td>
<td>1.67</td>
<td>0.06</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>Total ADG</td>
<td>1.60</td>
<td>1.62</td>
<td>1.64</td>
<td>0.04</td>
<td>0.76</td>
</tr>
<tr>
<td>Yr 1</td>
<td>DMI³, kg</td>
<td>8.12</td>
<td>7.94</td>
<td>8.08</td>
<td>0.12</td>
<td>0.57</td>
</tr>
<tr>
<td></td>
<td>G:F</td>
<td>0.203</td>
<td>0.197</td>
<td>0.203</td>
<td>0.004</td>
<td>0.49</td>
</tr>
<tr>
<td></td>
<td>RFI</td>
<td>0.012</td>
<td>0.015</td>
<td>-0.017</td>
<td>0.012</td>
<td>0.15</td>
</tr>
<tr>
<td>Yr 2²</td>
<td>Initial³</td>
<td>10.28</td>
<td>9.85</td>
<td>9.57</td>
<td>0.36</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>DMI, kg</td>
<td>0.147</td>
<td>0.160</td>
<td>0.160</td>
<td>0.009</td>
<td>0.42</td>
</tr>
<tr>
<td></td>
<td>RFI</td>
<td>2.152</td>
<td>-1.085</td>
<td>0.221</td>
<td>1.474</td>
<td>0.10</td>
</tr>
<tr>
<td>Re-implant⁶</td>
<td>DMI, kg</td>
<td>9.98</td>
<td>9.98</td>
<td>9.63</td>
<td>0.35</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>G:F</td>
<td>0.157</td>
<td>0.166</td>
<td>0.171</td>
<td>0.008</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>RFI</td>
<td>0.146</td>
<td>-0.022</td>
<td>-0.362</td>
<td>0.299</td>
<td>0.38</td>
</tr>
<tr>
<td>RFI difference⁷</td>
<td></td>
<td>-1.995</td>
<td>1.058</td>
<td>-0.300</td>
<td>1.161</td>
<td>0.14</td>
</tr>
</tbody>
</table>

¹Dams individually fed meadow hay and no supplement (CON), 0.82 kg/d (DM) distillers grain based supplement (HIGH), or 0.82 kg/d (DM) dried corn gluten feed based supplement (LOW) during late gestation.
²Calculated from HCW and adjusted to a common dressing percent (63.0%).
³DMI calculated in yr 1 using the prediction formula presented by Tedeschi et al. (2006) where DMI = 4.18 + (1.98 x ADG) + (0.0013 x (MBW⁰.⁷⁵)) + (0.019 x EBF).
⁴Heifer calves from yr 2 were placed in a Grow-Safe feeding system and individual intakes recorded daily to calculate DMI, G:F and RFI.
⁵Period from feedlot initial BW to re-implant (75 d).
⁶Period from re-implant to slaughter (97 d).
⁷Difference in RFI between initial and re-implant periods.
Table 7. Effect of late gestation protein supplementation on steer progeny carcass characteristics

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment</th>
<th>SEM</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HIGH</td>
<td>LOW</td>
</tr>
<tr>
<td>n</td>
<td>17</td>
<td>13</td>
<td>12</td>
</tr>
<tr>
<td>HCW, kg</td>
<td>383</td>
<td>391</td>
<td>392</td>
</tr>
<tr>
<td>Empty body fat, %&lt;sup&gt;2&lt;/sup&gt;</td>
<td>28.7</td>
<td>29.5</td>
<td>27.9</td>
</tr>
<tr>
<td>Marbling score&lt;sup&gt;3&lt;/sup&gt;</td>
<td>688</td>
<td>664</td>
<td>591</td>
</tr>
<tr>
<td>12-th rib fat, cm</td>
<td>1.89</td>
<td>2.03</td>
<td>1.75</td>
</tr>
<tr>
<td>LM area, cm&lt;sup&gt;2&lt;/sup&gt;</td>
<td>87.94</td>
<td>88.73</td>
<td>90.94</td>
</tr>
<tr>
<td>Yield grade</td>
<td>3.35</td>
<td>3.62</td>
<td>3.22</td>
</tr>
<tr>
<td>WBSF, kg&lt;sup&gt;4&lt;/sup&gt;</td>
<td>3.35</td>
<td>3.61</td>
<td>3.99</td>
</tr>
<tr>
<td>Crude fat, %</td>
<td>6.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>6.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.71&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quality grade, % Sm&lt;sup&gt;5&lt;/sup&gt; or greater</td>
<td>100</td>
<td>97</td>
<td>85</td>
</tr>
<tr>
<td>Quality grade, % Md&lt;sup&gt;6&lt;/sup&gt; or greater</td>
<td>75</td>
<td>88</td>
<td>38</td>
</tr>
</tbody>
</table>

<sup>1</sup>Dams individually fed meadow hay and no supplement (CON), 0.82 kg/d (DM) distillers grain based supplement (HIGH), or 0.82 kg/d (DM) dried corn gluten feed based supplement (LOW) during late gestation.

<sup>2</sup>Empty body fat (EBF) calculated using the prediction formula presented by Guiroy et al. (2001) where EBF = 17.76107 + (11.8908 x 12th rib fat depth) + (0.0088 x HCW) + (0.81855 x [(marbling score/100) + 1] – (0.4356 x LM area).

<sup>3</sup>Where 500 = small<sup>0</sup>.

<sup>4</sup>WBSF= Warner-Bratzler shear force.

<sup>5</sup>Sm = small quality grade, USDA low Choice.

<sup>6</sup>Md = modest quality grade, USDA average Choice.
Table 8. Effect of late gestation protein supplementation on heifer progeny carcass characteristics

<table>
<thead>
<tr>
<th>Item</th>
<th>Treatment 1</th>
<th></th>
<th></th>
<th></th>
<th>SEM</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>HIGH</td>
<td>LOW</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>18</td>
<td>22</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCW, kg</td>
<td>351</td>
<td>358</td>
<td>362</td>
<td>6</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Empty body fat, %²</td>
<td>29.5</td>
<td>28.5</td>
<td>27.6</td>
<td>0.7</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Marbling score³</td>
<td>668</td>
<td>650</td>
<td>681</td>
<td>36</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>12-th rib fat, cm</td>
<td>0.86</td>
<td>0.79</td>
<td>0.72</td>
<td>0.05</td>
<td>0.13</td>
<td></td>
</tr>
<tr>
<td>LM area, cm²</td>
<td>87.52</td>
<td>90.16</td>
<td>93.34</td>
<td>2.29</td>
<td>0.19</td>
<td></td>
</tr>
<tr>
<td>Yield grade</td>
<td>3.41</td>
<td>3.16</td>
<td>2.89</td>
<td>0.28</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>WBSF, kg⁴</td>
<td>3.85</td>
<td>4.28</td>
<td>4.40</td>
<td>0.57</td>
<td>0.25</td>
<td></td>
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<tr>
<td>Crude fat, %</td>
<td>6.88</td>
<td>6.29</td>
<td>6.76</td>
<td>0.58</td>
<td>0.51</td>
<td></td>
</tr>
<tr>
<td>Quality grade, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sm⁵ or greater</td>
<td>91</td>
<td>100</td>
<td>100</td>
<td>5</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Quality grade, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Md⁶ or greater</td>
<td>83</td>
<td>72</td>
<td>85</td>
<td>13</td>
<td>0.63</td>
<td></td>
</tr>
</tbody>
</table>

¹Dams individually fed meadow hay and no supplement (CON), 0.82 kg/d (DM) distillers grain based supplement (HIGH), or 0.82 kg/d (DM) dried corn gluten feed based supplement (LOW) during late gestation.
²Empty body fat (EBF) calculated using the prediction formula presented by Guiroy et al. (2001) where EBF = 17.76107 + (11.8908 x 12th rib fat depth) + (0.0088 X HCW) + (0.81855 x [(marbling score/100) + 1] – (0.4356 x LM area).
³Where 500 = small⁷.
⁴WBSF= Warner-Bratzler shear force.
⁵Sm = small quality grade, USDA low Choice.
⁶Md = modest quality grade, USDA average Choice.
CHAPTER IV: Bovine females with thecal cell androgen excess result in altered oocyte maternal effect gene expression

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ABSTRACT

In Nebraska, 1.9 million cows calve annually; however, 550,000 more (30%) are bred but fail to have a calf. Thus, if markers to identify reduced fertility females identified even 5% of infertile females this would reduce costs associated with development and breeding due to removal from the herd. Within the UNL physiology herd we have identified two sub-populations of cows, one that inefficiently converts androgens (A4) to estrogen (E2; low granulosa efficient; LGE) and a population that efficiently converts E2 to A4 (HGE; high fertility group). These classifications are 87% repeatable over multiple estrous cycles/ years suggesting intrinsic differences in steroidogenic capability. Therefore, the hypothesis was androgen excess is a result of altered theca cell gene expression and abundance of mRNAs affecting oocyte competence. The objective of this study was to identify differences in mRNA abundance of theca steroidogenic enzymes and oocyte maternal effect genes collected from these two cow subpopulations. Beef cows (4.7 ± 0.3 yr) were synchronized (modified Co-Synch + CIDR) protocol and ovariectomies were performed 36 h after PGF₂α injection and CIDR removal. Follicular fluid, theca cells, granulosa cells and cumulus-oocyte
complex (COC) from dominant follicles were collected. Androstenedione production was 19-fold greater ($P = 0.0004$) for LGE ($n = 53$) compared with HGE ($n = 28$) cows. In LGE cows, expression of $CYP11A1$ was 3.3-fold greater ($P = 0.02$) and $CYP17A1$ 15.5-fold greater ($P = 0.03$) compared with HGE cows. Abundance of $ZAR1$ was decreased ($P = 0.05$) while conversely, $NLPR5$ had a tendency ($P = 0.12$) to be increased 13.5-fold in COCs from LGE cows. Interestingly, LGE cows display characteristics similar to androgen excess and polycystic ovary syndrome (PCOS) in women. Taken together, increased androgen production in LGE cows alters gene expression and/or mRNA stability during oocyte growth and maturation, which may reduce fertility success.

**Key words:** steroidogenesis, theca, androgen excess, fertility

**INTRODUCTION**

Beef cattle production is the largest sector of animal agriculture in the United States (USDA-NASS, 2007). Profitability is directly related to the ability of a cow to maintain a 365-d calving interval and wean a marketable calf each yr (Hohenboken, 1988). Consequently, longevity affects profitability with the main reason cows are removed from the production herd is the inability to maintain pregnancy (Renquist et al., 2006; Cushman et al., 2009). Early embryonic mortality results in loss of 20-44% of pregnancies in beef cattle (Humbolt, 2001). Thus development of tools or markers to help predict fertility in beef cattle could decrease the number of low fertility heifers developed and placed in the herd.
Over the past several decades, scientists have developed the reproductive tract scoring (RTS) system and collect antral follicle count (AFC) via ultrasonography to help predict fertility in heifers (Martin et al., 1992; Ireland et al., 2008; Cushman et al., 2009; Mossa et al., 2009). Unfortunately, these predictor tools are typically utilized prior to the first breeding season, after retention and development of weaned heifers has occurred. In an effort to identify fertility markers in beef cattle our lab has reported two unique sub-populations of cows with differing androstenedione (A4; high; mean = 113 ng/mL vs. low; mean = 6 ng/mL) follicular fluid concentrations. Cows with high A4 concentrations cannot efficiently convert A4 to estradiol (E2) in the granulosa cell, resulting in altered steroidogenesis and an increased androgenic environment in the ovary. Interestingly our data indicates that although A4 concentration differs, E2 concentration is similar, suggesting altered efficiency of CYP19A1 in converting A4 to E2 and thus inefficient granulosa cell function. The inability to convert A4 to E2 appears to be intrinsic owing to cows classified as high granulosa cell efficient (HGE; E2/A4 ratio > 100) or low granulosa cell efficient (LGE; E2/A4 ratio < 100) similarly classified 87% of the time over several estrous cycles and multiple years (McFee et al., unpublished data).

Infertility is increasing among people of reproductive age in the United States. One specific disorder affecting approximately 5-10 % of reproductive aged women is polycystic ovary syndrome (PCOS; Franks, 2005). Polycystic ovary syndrome is characterized by anovulatory infertility due to arrested follicle maturation, hyperandrogenism, and/or polycystic ovaries (Ehrmann, 2005). Furthermore, polycystic ovaries are reported to have an accumulation of small follicles containing hypertrophied thecal cell layers (Legro et al., 1998). Several studies have identified alterations in
steroidogenic pathway enzymes as key contributors to the PCOS phenotype (Barbieri, 1992; Gilling-Smith et al., 1994; Franks, 1995; Nelson et al., 1999; Nelson et al., 2001; Wood et al., 2003). Increased mRNA abundance of CYP11A1, CYP17A1, and GATA6 reported previously (Nelson et al., 1999; Wood et al., 2005), in concert with reports of increased testosterone and androstenedione (Barbieri, 1992; Gilling-Smith et al., 1994) in women with PCOS suggest impairment or inefficiency of estrogen production in these individuals.

To improve understanding of PCOS several non-human models have been created (reviewed in Dumesic et al., 2007; Steckler et al., 2009; Hogg et al., 2012). These models utilize exposure to androgen during fetal development to produce PCOS phenotypes after birth. Non-human primates treated with testosterone prior to birth have ovarian morphology similar to PCOS women (Dumesic et al., 2007). Furthermore, sheep treated with testosterone propionate from d 30 to 90 of gestation display ovarian, hormonal, and metabolic characteristics of PCOS (Veiga-Lopez et al., 2008; Steckler et al., 2009). However, it should be noted these models are created via the administration of exogenous androgen. Identification of a naturally occurring PCOS model in domestic livestock could help us gain a better understanding of PCOS by increasing the amount of available tissue for study. The objective of the current study was to establish an altered theca cell molecular phenotype in a subset of cows with reduced fertility. Furthermore, the effect of a follicular environment of androgen excess on the oocyte molecular phenotype was verified.
MATERIAL AND METHODS

The University of Nebraska-Lincoln Institutional Animal Care and Use Committee approved the procedures and facilities used in this experiment.

Animals

Non-lactating composite beef cows [25% MARC III (1/4 Angus, 1/4 Hereford, 1/4 Pinzgauer, 1/4 Red Poll) and 75% Red Angus] from the beef physiology herd located at the University of Nebraska Agricultural Research and Development Center (ARDC) were used in this study. The cows utilized had been culled from the herd due to failure to become pregnant. Average age was 4.7 ± 0.3 yr and average weight was approximately 550 kg (n = 81).

Estrous was synchronized utilizing a Co-Synch + CIDR protocol for timed artificial insemination (Figure 1) except ovariectomy was performed after synchronization rather than timed AI. Cows received a single injection (100 μg/cow; i.m.) of gonadotropin-releasing hormone (GnRH; Cystorelin, Merial Limited, Duluth, GA) on treatment day 0 to induce ovulation and thus, initiate a new follicular wave. Also on day 0, an intravaginal insert (Controlled Internal Drug Release device: CIDR; EAZI-BREED CIDR, Pfizer Animal Health, New York, NY) containing 1.38 g of progesterone (P4) was inserted.

Approximately 84 h prior to ovariectomy cows were transported approximately 53 km to the University of Nebraska-Lincoln Animal Science building for holding and surgeries. The CIDR was removed on day 7 and cows administered a single injection (25 mg/cow; i.m.) of prostaglandin F$_{2α}$ (PGF$_{2α}$; ProstaMate, AgriLabs, St. Joseph, MO) (Figure 1). Cows were offered *ad libitum* access to grass hay (90% DM) until 12 h prior
to surgery. Cows were offered *ad libitum* access to fresh water during the fasting period. After surgery cows were placed in individual stalls and monitored. Cows monitored every 3 h after surgery for the first 24 h and were offered *ad libitum* access to fresh water. Hay was offered beginning 4-6 h after surgery. Approximately 24 h after surgery cows were given a single injection of Flu-Nix D (3 mL, im; AgriLabs, St. Joseph, MO) and penicillin (10 mL, i.m.; Twin Pen, AgriLabs, St. Joseph, Mo) and moved to a small group confinement pen until transported back to ARDC.

**Ovarian tissue collection**

Ovariectomy was performed on cows approximately 36 h after the day 7 PGF2α injection (Figure 1). Prior to surgery, transrectal ultrasonography (ALOKA SSD-500V and UST-5821-7.5, ALOKA Co. Ltd., Tokyo, Japan) was performed by a single technician to evaluate the presence and size of any large follicles or CLs. Both ovaries were removed via a right flank laparotomy using aseptic techniques (Youngquist et al., 1995). Local anesthesia was induced using an inverted L-block with 2% lidocaine (approximately 60-80 ml/cow i.m.) and an additional 30-60 ml of lidocaine per cow was administered topically to the muscle layers and peritoneum prior to incision.

Ovarian phenotypic measurements were recorded after removal. Each ovary was measured (length and width), weighed, and a surface antral follicle count performed. The dimensions of the largest (dominant) and second largest (subordinate) follicles were also measured and recorded. The follicular fluid was then aspirated from these follicles, cumulus oocyte complex retrieved, and their theca cells removed via microdissection. Granulosa cells were collected following centrifugation and decanting of the follicular fluid and washing theca cells. Granulosa and theca cell weights and volume were
recorded (approximated by Eppendorf tube volume) and samples placed in 0.5 mL Tri-
reagent (Sigma-Aldrich, St. Louis, MO) for tissue homogenization. The volume of
follicular fluid aspirated from each follicle was also documented. Follicular fluid and
cells were stored at -80°C until further use.

**Hormone assays**

Radioimmunoassays were conducted to determine follicular fluid E2 and P4
concentrations. Estradiol concentrations were determined using a previously established
RIA protocol (Kojima et al., 1992). Intra- and inter-assay coefficients of variation for E2
were 22.8% and 14.40%, respectively. To determine P4 concentration a Coat-a-Count
assay kit (Diagnostic Products Corporation, Los Angeles, CA) was utilized. Follicular
fluid samples were diluted 1:500. Intra- and inter-assay coefficients of variation for P4
were 26.9% and 16.1%, respectively. Follicular fluid androstenedione (A4)
concentrations were determined through utilizing a human A4 ELISA kit (Alpha
Diagnostics International, San Antonio, TX). Samples were initial diluted 1:100; however
samples that produced A4 concentrations above or below the standard curve were
adjusted. Raw data was utilized to calculate the A4 concentration for undiluted follicular
fluid and this adjusted data was used for analysis. The intra- and inter-assay coefficients
of variation for A4 were 5.729% and 6.49%, respectively.

**Granulosa cell efficiency (GCE) classification**

After hormone assay completion sample E2 and P4 concentrations were compared
and follicles classified as either E2-active (E2:P4 > 1) or as E2-inactive (E2:P4 ≤ 1;
Echternkamp et al., 2004). Previously, our group has reported altered A4 production in
two sub-populations of the UNL physiology herd. Herd average A4 concentration is 43
ng/mL and ratio of converting A4 to E2 (E2/A4) 103. Thus sample E2 and A4 concentrations were compared to determine the E2:P4 ratio and cows classified as either high granulosa cell efficiency (HGE; > 100) or low granulosa cell efficiency (LGE; < 100).

**RNA extraction and RT**

Total RNA was extracted from each COC utilizing the Amibion® MicroPoly(A) Purist kit (Life Technologies Corp., Carlsbad, CA). After RNA extraction, linear amplification (1 round) and conversion to cDNA was completed utilizing WT Ovation RNA amplification system (NuGen Technologies Inc., San Carlos, CA). Total RNA was extracted from theca cells using Tri Reagent (Sigma-Aldrich). The extracted RNA was subsequently converted to cDNA using SuperScript III and random primers (Invitrogen, Carlsbad, CA) following standard procedures previously reported (McFee et al., 2009).

**Quantitative RT-PCR**

Quantitative RT-PCR (QRT-PCR) was performed to evaluate mRNA abundance in theca cells and COC as previously reported (Mack et al., 2012). A complete list of genes and primer sequences investigated summarized in Table 1 and Table 2 for theca cells and COC, respectively. The constitutively expressed genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), ribosomal protein L 15 (RPL-15), and ribosomal protein L 19 (RPL-19) were tested to be utilized as controls for RNA amplification. Primers and probes were developed in either Primer Express 1.5 (software that accompanied the 7700 Prism sequence detector; Applied Biosystems, Foster City, CA), or Integrated DNA Technologies; Table 1). The probes and primers were synthesized by Integrated DNA Technologies.
QRT-PCR was performed in 384 well plates (Axygen Scientific, Union City, CA) with samples run in triplicate in separate consecutive wells. Each well contained 0.5 μL sample and 9.5 μL master mix. Master mix for vascular endothelial growth factor A 164 (VEGFA_164), vascular endothelial growth factor A 164b (VEGFA_164B), and GAPDH contain 5 μL 2x TaqMan (Applied Biosystems, Foster City, CA), 3 μL Millipore water, 1 μL, 2 μM probe, as well as 0.25 μL, 20 μM each forward and reverse primers. Prior to utilization of primers requiring Power SYBR Green (Applied Biosystems) within the master mix, primers were tested empirically to determine maximal concentration to produce specific amplification of the target sequence. Master mix for ribonuclease type III (RNASEN), luteinizing hormone/choriogonadotropin receptor (LHCGFR), 17α-hydroxylase/17,20 lyase (CYP17A1), cholesterol side chain cleavage enzyme (CYP11A1), steroidogenic acute regulatory protein (StAR), GATA6, DNA methyltransferases-1 (DNMT1), developmental pluripotency associated protein 3 (DPPA3), RPL-19, kinase insert domain receptor (KDR), and neuropilin1 (NRP-1) contained 5 μL Power SYBR Green, 4.2 μL Millipore water, and 0.15 μL, 20 μM each forward and reverse primers. Master mix for RPL-15, insulin-like growth factors-1 (IGF-1), Maternal antigen embryos require (NLRP5), and bone morphogenetic protein 6 (BMP6) contained 5 μL Power SYBR Green, 4.35 μL Millipore water, and 0.075 μL, 20 μM each forward and reverse primers. Master mix for DiGeorge syndrome critical region 8 (DGCR8) and zygote arrest-I (ZARI) contained 5 μL Power SYBR Green, 4.41 μL Millipore water, and 0.045 μL, 20 μM each forward and reverse primers. Master mix for endoribonuclease (DICER), WEE-1, exportin 5 (XPO5), eukaryotic translation initiation factor 2C (EIF2C2), and MYC contained 5 μL Power SYBR Green, 3.7 μL Millipore water, and 0.40 μL, 20 μM
each forward and reverse primers. Reactions were carried out with the Applied Biosystems Model 7900 sequence detector using standard cycling conditions. Samples utilizing Power SYBR Green were also plotted on a dissociation curve to ensure the absence of primer dimerization. Analysis of the QRT-PCR results was performed as previously described (McFee et al., 2009). Briefly, the stability of the normalizers was calculated using normfinder. Based on this analysis, candidate gene mRNA abundance was normalized using the geometric mean of GAPDH and RPL-15. The geometric mean for GAPDH and RPL-15 was calculated for each sample and served as a control based on methods previously described (Vandesompele et al., 2002; Huggett et al., 2005). After individual gene values were calculated against the genomic means, gene expression data was calculated on a fold change basis with individual values divided by the mean for the HGE group (control).

**Statistical analysis**

Ovariectomies were preformed over a 5 yr period with approximately 10-14 cows ovariectomized each replication. Thus each surgery was considered a replication and animals considered the experimental unit. Ovarian phenotypic measurements, including surface and ultrasound derived AFC, ovary size and weight, theca cell weight, follicle size and follicular fluid volume were analyzed utilizing the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Furthermore, hormone concentrations and gene expression in both theca and COC were analyzed using the MIXED procedure of SAS. The model included granulosa cell efficiency (GCE) as the main effect with replication as the random effect. The original model included GCE and age as fixed effects with replication as the random effect. Age was not different among treatments, and thus was
removed from the model. Data for NLRP5, WEE-1, EIF2C2, DICER, DPPA3, NRP-1, VEGFA_164 and VEGFA_164B were log transformed in the COC to meet normal distribution assumptions. Furthermore, CYP17A1, CYP11A1, GATA6, LHCGR, VEGFA_164, VEGFA_164B, and VEGFA_164/VEGFA_164B were log transformed in the theca cells to meet normal distribution assumptions. A \( P \)-value \( \leq 0.05 \) was considered significant.

**RESULTS**

*Ovarian phenotypic data and hormone concentrations*

Ovarian phenotypic data, follicle characteristics and hormone concentrations are summarized in Table 2. There was no difference \( (P = 0.28) \) in age for cows classified HGE compared with LGE \( (4.16 \text{ vs. } 4.90 \pm 0.65 \text{ yr}) \). After removal, ovaries were measured (length and width) and area calculated to determine the effect of GCE on ovarian size. Similarly, there was no difference \( (P \geq 0.20) \) in follicle diameter, ovarian weight, theca cell weight, or AFC. Ovarian area also tended \( (P = 0.09) \) to be greater for LGE compared with HGE cows. Furthermore, LGE cows tended \( (P = 0.09) \) to have increased follicular fluid volume compared with HGE cows \( (1.31 \text{ vs. } 1.10 \pm 0.11 \text{ mL}) \).

Estradiol concentrations in the follicular fluid tended \( (P = 0.07) \) to be greater for LGE compared with HGE cows. However, there was no difference \( (P = 0.15) \) in P4 concentration based on cow GCE. Androstenedione concentration was approximately 19-times greater \( (P = 0.0004) \) in the follicular fluid of LGE cows with the E2/A4 12.4 times greater \( (P < 0.0001) \) for HGE cows. Similarly, A4/P4 was greater \( (P = 0.0001) \) in the LGE cows, but E2/P4 was similar \( (P = 0.89) \) among classifications.
**Theca cell RT-PCR gene expression**

Theca cells are important in the regulation of steroidogenesis in the ovary. Steroidogenic enzyme gene expression, \( LHCGR \) and growth factors regulating angiogenesis were determined. The reaction of steroid acute regulatory protein (\( StAR \)) is the rate limiting step in steroidogenesis and is required to transport cholesterol into the mitochondria. There was no difference in \( StAR \) mRNA expression between LGE cows \( (P = 0.28, \text{Fig. 2A}) \) and HGE. However, enzymes in the theca cells responsible for conversion of cholesterol to pregnenolone \( (CYP11A1) \) and conversion of pregnenolone to 17-OH pregnenolone and ultimately dehydroepiandrosterone (DHEA) were upregulated. Abundance of \( CYP11A1 \) mRNA was 3.3-fold greater \( (P = 0.02, \text{Fig 2B}) \) in LGE cows compared with controls and \( CYP17A1 \) mRNA abundance increased \( (P = 0.03, \text{Fig. 2C}) \) 15.5-fold compared with HGE cows. Expression of \( GATA6 \) has previously been reported to increase promoter activities of \( CYP11A1 \) and \( CYP17A1 \) (Wood et al., 2004). We report LGE cows tend to have an 11.6-fold increase in expression of \( GATA6 \) mRNA in theca cells compared with HGE cows \( (P = 0.08, \text{Fig. 2E}) \). Furthermore, \( LHCGR \) mRNA expression tended to be approximately 6-fold greater in LGE \( (P = 0.14, \text{Fig. 2D}) \) compared with HGE cows.

A member of the transforming growth factor-\( \beta \) (TGF-\( \beta \)) family, \( BMP6 \) is reportedly expressed in the theca and granulosa cells of follicles and inhibits the production of LH-induced androgens (Glister et al., 2005). Abundance of mRNA tended to be increased in LGE cows \( (1.3 \pm 0.1, P = 0.10, \text{Fig. 3D}) \) compared with HGE cows \( (1.0 \pm 0.3) \). Furthermore, we determined the expression of \( VEGFA_164 \) and \( VEGFA_164B \) in theca cells. Although there was no difference \( (P \geq 0.45) \) in mRNA
expression for either the angiogenic isoform (VEGFA_164, Fig. 3A) or anti-angiogenic isoform (VEGFA_164B, Fig. 3B), there was a trend for the ratio of VEGFA_164/VEGFA_164B to be reduced 10-fold in LGE cows compared with HGE cows. Finally, mRNA abundance of IGF-1 was similar in theca cells of LGE and HGE classified cows ($P = 0.38$, Fig. 3E).

**COC RT-PCR gene expression**

Expression of angiogenic promoting growth factors and their receptors (VEGFA_164, VEGFA_164B, KDR, and NPL-1), maternal effect (ZAR1, NLRP5, DPPA3, and DNMT1), microRNA processing (DGCR8, RNASEN, XPO5, DICER, and EIF2C2), and meiotic resumption (MYC and WEE-1) genes were investigated in the COC. Binding of VEGFA occurs through tyrosine kinase co-receptors. Expression of KDR (Fig. 4A) and NRP-1 (Fig. 4B) mRNA were similar among LGE and HGE cows. Furthermore, there was no difference in mRNA abundance of VEGFA_164 (Fig. 4C), VEGFA_164B (Fig. 4D), or the ratio of VEGFA_164/VEGFA_164B (Fig. 4E).

Expression of miRNA processing gene abundance has previously been reported in bovine COCs based on follicle size and cell type (Miles et al., 2012). Although there was a tendency for DGCR8 expression to be reduced 5-fold in LGE cows ($P = 0.14$, Fig. 5A), there was no difference in expression of RNASEN (Fig. 5B), XPO5 (Fig. 5C), DICER (Fig. 5D), or EIF2C2 (Fig. 5E) based on GCE classification.

Abundance of ZAR1 mRNA was reduced 11-fold in LGE ($P = 0.05$, Fig. 6A) compared with HGE cows. Similarly, DNMT1 mRNA abundance tended to be reduced 2.5-fold in LGE ($P = 0.12$, Fig. 6D) cows compared with HGE cows. Conversely, expression of NLRP5 mRNA in LGE cows tended to be increased 13.5-fold compared
with HGE cows ($P = 0.14$, Fig. 6B). Whereas there was no difference among classification groups for the expression of $DPPA3$ (Fig. 6C, $P = 0.58$). Additionally we looked at the expression of meiotic resumption gene mRNA. Abundance of mRNA for both $MYC$ (Fig. 6E) and $WEE-1$ (Fig. 6F) was similar among GCE classifications.

**DISCUSSION**

From these data we report a subpopulation of beef cows from our herd contain a similar phenotype to women with PCOS. Women with PCOS are characterized to have chronic anovulation due to abnormal E2 secretion (Franks, 1995). Although E2 concentrations in serum are similar in PCOS and normal women during the early and mid-follicular phases of the menstrual cycle, the pattern of estrogen secretion is altered in PCOS women due to the lack of midluteal E2 increases (Franks, 1995). Furthermore, PCOS is characterized by increased androgen production. Nelson et al. (1999) reported 20-, 22- and 4-fold increases in P4, 17-OH pregnenolone, and testosterone (T) in cultured theca cells. These data agree with previous literature reporting increased T and A4 concentrations in women diagnosed with PCOS (Barbieri, 1992; Gilling-Smith et al., 1994). Our findings report a 19-fold increase in A4 production in LGE cows, with similar production of P4 and a trend for increased E2 production in LGE cows.

As previously mentioned, it is hypothesized hyperandrogenemia is attributed to the increased secretion of LH (Franks, 1995). Several reports indicate an increase in both pulse amplitude and frequency in women with PCOS (Burger et al., 1985; Waldstreicher et al., 1988; Berga et al., 1993). Furthermore, reports have indicated *in vivo* mRNA expression of LHCGR is increased in theca cells from PCOS follicles compared with size
matched control follicles (Jakimiuk et al., 2001). Similarly, in the current study there was a tendency for LHCGR mRNA expression to be increased in LGE animals.

Taylor et al. (1997) reported 75% of PCOS patients an increase in pooled LH concentration and pooled LH correlated positively to 17-OH progesterone. One of the steroidogenic pathway intermediates, 17-OH progesterone is produced via the enzymatic action of \textit{CYP17A1} on pregnenolone in the theca cell. We reported increased expression of \textit{CYP11A1} and \textit{CYP17A1} in LGE compared with HGE cows. These data are in agreement with previous findings in PCOS women and models (Nelson et al. 1999; Wood et al., 2003, Hogg et al., 2012). The conversion of cholesterol to pregnenolone is accomplished via the enzymatic activity of \textit{CYP11A1}. Cattle utilize the $\Delta^5$ steroidogenic pathway (Conley and Bird, 1997) thus pregnenolone is converted to 17-OH pregnenolone via \textit{CYP17A1} followed by the production of DHEA, the precursor for A4.

Similarly, the expression of \textit{GATA6} tended to be increased in LGE cows. Previous data report \textit{GATA6} can influence promoter activity of both \textit{CYP11A1} and \textit{CYP17A1} (Wood et al., 2003). Thus it is likely the increased \textit{GATA6} expression reported in the current study, albeit as a trend, increases regulation of the steroidogenic factors previously mentioned. Increased expression of \textit{CYP11A1} and \textit{CYP17A1} seems counter intuitive due to the increased concentrations of A4 reported in PCOS patients, as well as in LGE cows in the current study. It is likely the inefficient production of E2 in the PCOS patient and LGE cows results in increased steroidogenic enzyme activity and thus increased production of A4. Furthermore, previous reports suggest in women T concentrations increase to a greater extent than A4 levels (Barbieri, 1992). These data
would suggest the inefficiency in E2 production could be related to impaired aromatase activity in the granulosa cells.

Studies involving human and sheep theca cells report discrepancies regulation of Star in PCOS women or PCOS like phenotypes in sheep. Nelson et al. (1999) and Wickenheisser et al. (2009) reported no differences in Star expression in PCOS theca cells. Conversely, Jakimiuk et al (2001) reported increased expression of Star in human theca from PCOS follicles and Hogg et al. (2012) reported similar increases for theca cells from progeny sheep born to ewes treated with testosterone propionate from d 62 to 102 of a 147 d gestation. Steroid acute regulatory protein is essential for the movement of cholesterol into the cell mitochondria to allow for steroidogenesis (Stocco and Clark, 1996). Increased Star expression would suggest an increased amount of cholesterol is being presented to the steroidogenic pathway. Furthermore, it would suggest the alterations in enzymes and increased intermediates reported further down the pathway in PCOS women results from increased cholesterol levels in the ovary compared with inefficiency in enzymatic conversion of cholesterol to its resulting metabolites. In the current study we reported no difference in Star expression in cows categorized by GCE, suggesting inefficiency in the production of E2 and not increased cholesterol levels present in LGE animals.

Bone morphogenetic protein 6 tended to be upregulated in the LGE group. It has been reported BMPs, are produced in both the theca and granulosa cells and can alter the expression of LH-induced androgens and increase E2 production in cultured cells (Glister et al., 2005). Glister et al. (2005) reported BMP-4, -6, and -7 suppressed both basal and LH-induced A4 secretion in a dose-dependent manner in cultured bovine thecal cells.
Perhaps the increased A4 produced in the LGE groups stimulated intrinsic ovarian factors increasing expression of *BMP6* to inhibit further LH secretion and LH-induced androgen production.

The VEGFA family plays an important role in stimulating vascularization and follicle growth and development (Berisha et al., 2000; Dor et al., 2001; Grazul-Bilska et al., 2007). Expression of VEGFA isoforms have been reported to increase as follicle size increases (Wulff et al., 2001; Einspainer et al., 2002). Furthermore, hormones responsible for ovulation have been reported to increase VEGFA expression in the preovulatory follicle (Reynolds and Redmer, 1998). Previous literature reports VEGF concentrations are higher in the follicular fluid of PCOS patients compared with controls (Agrawal et al., 1998; Artini et al., 2009). Similarly, Kamat et al. (1995) reported strong immunohistochemical staining for VEGF in the theca and ovarian stroma of individual with PCOS. In a review of the literature on PCOS and VEGF, Peitsidis and Agrawal (2010) suggested this increased staining for VEGF may explain the presence of the hypervascularized stroma typically presented in women with PCOS and this increased vascularization likely leads to the alteration in theca cell growth. There was no difference in expression of angiogenic VEGFA or antiangiogenic VEGFA in the current study. We also examined the ratio of angiogenic/antiangiogenic VEGFA isoform ratio in both theca and COC. Previous reports do not quantify the relationship of angiogenic vs. antiangiogenic isoforms of VEGFA in PCOS patients (Agrawal et al., 1998; Artini et al., 2009). We report LGE classified cows tend to have reduced VEGFA_164/VEGFA_164B compared with HGE cows. Perhaps the increased level of antiangiogenic VEGA is inhibiting or altering the action of angiogenic VEGFA altering vascularization and blood
flow to the ovary in the LGE animals. Further work is needed to understand the potential influence the ratio of angiogenic and antiangiogenic VEGFA isoforms have on ovarian and follicular histology.

Previous reports in the literature suggest women with PCOS have altered oocyte health, with differential expression of maternal effect and meiotic regulatory genes (Wood et al., 2007). Maternal effect genes are important in promoting survival during early embryogenesis. The embryonic block coincides with the moment maternal genome activation is transferred to embryo genome activation (De Sousa et al, 1998). Cattle encounter the embryo block at the fourth cell cycle, similar to humans (third to fourth cell cycle. Maternal antigen that embryos require (Mater, NLRP5) is transcribed specifically in the oocytes, accumulates during oogenesis, and persists through the blastocyst stage (Tong et al., 2002). Furthermore, NLRP5 plays a critical role in the progression of embryos beyond the 2-cell stage (Tong et al., 2000). Wood et al. (2007) reported a 3-fold increase in NLRP5 expression for PCOS oocytes; whereas in the current study we report oocytes from LGE cows tend to have increased NLRP5 expression. Although NLRP5-null mice have similar ovarian morphology, exhibit normal oocyte maturation, and estrous cycles compared with wild type mice, they are sterile. Tong et al. (2000) reported although fertilization occurs in NLRP5-null mice, embryos arrest at the 2-cell stage, which coincides with the activation of the embryonic genome. This would suggest arrestment of bovine embryos would occur at the fourth cell cycle due to the later onset of embryonic genome activation if NLPR5 activity was reduced.

Unlike NLRP5 expression, we reported a decrease in ZAR1 in LGE cows and a tendency for DNMT1 to decrease in LGE cows. These data are in contrast to the
hypothesized expression of maternal effect genes in PCOS oocytes. Zygote arrest-1-null female mice are sterile, although no abnormalities in ovarian phenotype, ovulation, or fertilization are observed (Wu et al., 2003). Interestingly, although expression of ZAR1 has been reported in the male, ZAR1-null males are fertile. Thus, perhaps the role of ZAR1 in the male may not be as significant as in the female. Unlike NLRP5-null mice, most embryos from ZAR1-null mice fail to progress to the 2-cell stage suggesting arrestment shortly after fertilization (Wu et al., 2003). The reduction of mRNA expression for ZAR1 and tendency for increased NLRP5 expression in LGE cows would suggest if embryos progress past the first cell cycle to the second cell cycle maternal effect gene activity increases to help preserve the pregnancy in the impaired environment.

The DNA methyltransferase family of enzymes plays an important role in DNA methylation and, ultimately, embryonic development and survival. Previous reports indicate DNMT-null mice die in early gestation (Lei et al., 1996), and methyltransferase mutations can cause not only abnormal fetal growth but also immunodeficiency and brain abnormalities in humans (Clouaire and Stancheva, 2008).

The bovine model can be appropriately used to better understand reproductive inefficiencies in humans. Similar to humans, cows are monovular and polycyclic (Adams et al., 2012). Also similarities in ovarian size and morphology exist between humans and cattle. Ovarian follicle size is similar between species (15 to 20 mm in diameter) and similar pathologic conditions can occur in both species (Adams and Pierson, 1995). Also, primordial follicle assembly occurs during gestation in humans as well as cattle and sheep, compared with rodents in which primordial follicle assembly begins after birth (Kezele and Skinner, 2003).
In the current study ovarian area and follicular fluid volume tends to be increased in LGE cows. Although follicle size was similar among GCE classifications, previous data show an obvious relationship between follicular fluid volume and follicle size in dominant follicles (Mihm et al., 1999). Furthermore, follicle size can be affected by LH concentration (Savio et al., 1993). Kinder et al. (1996) reported use of progestins in estrus synchronization programs for beef cows results in increases in LH pulses and the formation of large persistent follicles, reducing reproductive success. Similarly, Taft et al. (1996) reported persistence of follicles in beef cows treated with exogenous LH compared with saline treated cows.

**IMPLICATIONS**

Cows removed from the herd were similar in age, but differed in steroidogenic hormone production and efficiency of E2 production. Similar to individuals with PCOS we reported alterations in *CYP11A1* and *CYP17A1* production in LGE cows. Furthermore, androgen production was 19 times greater in LGE compared with HGE cows. Expression of mRNA in the COC was similar to that previously reported. Cows classified as LGE had altered regulation of maternal effect genes. Taken together these data suggest classifying cows based on E2/A4 as in the current study results in a naturally occurring model for PCOS in the bovine.
FIGURE LEGENDS:

Figure 1. Estrous was synchronized in cows using the Co-Synch + CIDR protocol. Cows received an intramuscular injection of gonadotropin-releasing hormone (GnRH) on day 0 and a controlled drug release intravaginal insert (CIDR) was inserted. The CIDR was removed on day 7 and an intramuscular injection of prostaglandin F2α (PGF) was administered. Ovariectomy was performed approximately 36 after the injection of PGF.

Table 1. Quantitative RT-PCR primers and probes used for theca cells.

Table 2. Quantitative RT-PCR primers and probes used for cumulus oocyte complexes.

Table 3. Phenotypic measurements and follicular fluid hormone concentrations of high granulosa cell efficient (HGE) and low granulosa cell efficient (LGE) cows. Where AFC represents antral follicle count. A P-value ≤ 0.05 is considered significant.

Figure 2. Quantitative RT-PCR results for steroid acute regulatory protein (StAR; A), cholesterol side chain cleavage enzyme (CYP11A1; B), 17α-hydroxylase/17,20 lyase (CYP17A1; C), luteinizing hormone/choriogonadotropin receptor (LHCGR; D), and transcription factor GATA6 (E) in theca cells from dominant follicles from high and low granulosa cell efficiency classified animals (HGE and LGE, respectively). The geometric mean of GAPDH and RPL-15 was used as an endogenous control to account for differences in starting material. Data for CYP11A1, CYP17A1, LHCRG, and GATA6 were log transformed to meet normal distribution assumptions. Graphs were represented as a fold change with HGE set as control (1). The mean ± SEM normalized values are presented from HGE n ≥ 12 and LGE n ≥ 27. A P ≤ 0.05 was considered significant.
Figure 3. Quantitative RT-PCR results for the proangiogenic VEGFA isoform, 164 (VEGFA_164; A) and the antiangiogenic VEGFA isoform, 164B (VEGFA_164B; B); along with the VEGFA_164:VEGFA_164B ratio (C), bone morphogenetic protein 6 (BMP6, D), and insulin like growth factor I (IGF-1, E) in theca cells from dominant follicles from high and low granulosa cell efficiency classified animals (HGE and LGE, respectively). The geometric mean of GAPDH and RPL-15 was used as an endogenous control to account for differences in starting material. Graphs were represented as a fold change with HGE set as control (1). Data for VEGFA_164, VEGFA_164B, and VEGFA_164:VEGFA_164B ratio were log transformed to meet normal distribution assumptions. The mean ± SEM normalized values are presented from HGE n ≥ 4 and LGE n ≥ 10. P ≤ 0.05 was considered significant.

Figure 4. Quantitative RT-PCR results for kinase insert domain receptor (KDR; A), neuropilin1 (NRP-1; B), the proangiogenic VEGFA isoform, 164 (VEGFA_164; C) and the antiangiogenic VEGFA isoform, 164B (VEGFA_164B; D); along with the VEGFA_164:VEGFA_164B ratio (E) in theca cells from dominant follicles from high and low granulosa cell efficiency classified animals (HGE and LGE, respectively). The geometric mean of GAPDH and RPL-15 was used as an endogenous control to account for differences in starting material. Graphs were represented as a fold change with HGE set as control (1). Data for NRP-1, VEGFA_164, and VEGFA_164B were log transformed to meet normal distribution assumptions. The mean ± SEM normalized values are presented from HGE n ≥ 3 and LGE n ≥ 4. P ≤ 0.05 was considered significant.
**Figure 5.** Quantitative RT-PCR results for DiGeorge syndrome critical region 8 (DGCR8; A), ribonuclease type III (RNASEN; B), exportin 5 (XPO5; C), endoribonuclease (Dicer; D), eukaryotic translation initiation factor 2C (EIF2C2; E) in theca cells from dominant follicles from high and low granulosa cell efficiency classified animals (HGE and LGE, respectively). The geometric mean of GAPDH and RPL-15 was used as an endogenous control to account for differences in starting material. Graphs were represented as a fold change with HGE set as control (1). Data for DICER and EIF2C2 were log transformed to meet normal distribution assumptions. The mean ± SEM normalized values are presented from HGE n ≥ 2 and LGE n ≥ 6. P ≤ 0.05 was considered significant.

**Figure 6.** Quantitative RT-PCR results for zygote arrest-1 (ZAR1; A), and maternal antigen embryos require (NLRP5; B), developmental pluripotency associated protein 3 (DPPA3; C), DNA methyltransferases-I (DNMT1; D); MYC (E), and WEE1 kinase (WEE-1; F) in theca cells from dominant follicles from high and low granulosa cell efficiency classified animals (HGE and LGE, respectively). The geometric mean of GAPDH and RPL-15 was used as an endogenous control to account for differences in starting material. Graphs were represented as a fold change with HGE set as control (1). Data for NLRP5, DPPA3, and WEE-1 were log transformed to meet normal distribution assumptions. The mean ± SEM normalized values are presented from HGE n ≥ 5 and LGE n ≥ 3. P ≤ 0.05 was considered significant.
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\(^1\)Dominant follicle follicular fluid concentration of E2/A4 ratio > 100.  
\(^2\)Dominant follicle follicular fluid concentration of E2/A4 < 100.

**Table 3.**
Figure 2.
Figure 3.
Figure 4.
Figure 5.
Figure 6.
LITERATURE CITED


