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Effects of Bovine Maternal Nutrient Restriction on Offspring MicroRNA and mRNA Expression and Muscle Fiber Type

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EFFECTS OF BOVINE MATERNAL NUTRIENT RESTRICTION ON OFFSPRING MICRORNA AND MRNA EXPRESSION AND MUSCLE FIBER TYPE

by

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ABSTRACT

Effects of bovine maternal nutrient restriction on offspring microRNA and mRNA expression and muscle fiber type

by

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Spring calving cows raised in certain parts of the US often experience a mid-gestation nutrient restriction due to seasonal changes in forage availability and nutrient composition. However, little is currently known about the effects this has on growth of the resultant offspring. We investigated whether calves from cows restricted during mid-gestation differentially expressed microRNAs (miRNA) affiliated with myogenesis and adipogenesis and their messengerRNA (mRNA) targets. We also analyzed expression of MRNA for the various myosin heavy chain (MHC) isoforms as a measure of possible impact on muscle fiber type. Cows were bred by the same sire, stratified by weight (P=0.80) and allocated to one of two treatments: maintenance (n=16) or restricted (n=18). Restricted cows received lower forage biomass (1662 kg/ha, dry matter (DM)) compared to maintenance (2309 kg/ha, DM) during the second trimester, and the restricted cows had BCS 1.55 lower (P=0.001) than maintenance cows and weight difference of 188 kg (P = 0.02) at the end of the second trimester. All cows were comingled for the third

trimester with saw no significant difference in BCS by the end ($P<0.05$). Skeletal muscle biopsies were collected from calves at weaning, beginning of the feedlot, and harvest. Compared to offspring from maintenance cows, offspring of restricted cows expressed more ($P < 0.05$) miR-133a, -133b, -181d, -214, -424 and -486 in their *longissimus lumborum* (LD) at weaning; more ($P < 0.05$) miR-133a, -133b, -206 -214, -424 and -486 in their *biceps femoris* (BF) at the beginning of the feedlot phase; and more ($P < 0.05$) miR-133a and less ($P < 0.01$) miR-486 in the LD at harvest. No differences ($P \geq 0.27$) were observed in expression of *Pax3, Pax7, Cdc25A, MamL1, Ezh2, IGF-1R* or the mRNAs for MHC within muscles due to treatment or sampling time. These data demonstrate that a nutritional insult during mid-gestation can alter postnatal expression miRNA in skeletal muscle of offspring, but more research is needed to determine the effect this has on phenotype and skeletal muscle growth.

(91 pages)

PUBLIC ABSTRACT

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For producers in more temperate areas, such as the Intermountain West, poor nutrition during the second trimester of gestation is common due to seasonal changes in forage and nutrient availability. The majority of muscle fibers are formed and adipogenesis is initiated in the second trimester, making it a critical time for skeletal muscle and adipose development in beef cattle. However, the extent to which these changes persist in the offspring postnatally is unknown. In this study, maternal nutrition was restricted during the second trimester in order to analyze the effects of maternal nutrient restriction on offspring skeletal muscle growth. Offspring were monitored throughout production postnatally and skeletal muscle samples were taken at weaning, the beginning of the feedlot phase, and at harvest. We investigated whether calves from cows restricted in the second trimester had a different expression of microRNA (miRNA) or messengerRNA (mRNA) known to be downstream targets of those miRNA. We also analyzed mRNA expression of myosin heavy chain (MHC) isoforms to determine whether maternal nutrition in the second trimester impacts muscle fiber type. There were no changes observed in mRNA or MHC expression between the two different treatments at either time point. Differences in expression of several miRNAs important in development of adipose and skeletal muscle were observed between the treatment groups. The findings of this research indicate that maternal nutrition during the second trimester

of gestation alters miRNA expression in the skeletal muscle. However, more research is needed to determine exactly how these miRNA impact growth of skeletal muscle postnatally.

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INTRODUCTION

Fetal programming is the prenatal influence on the development of the fetus and the response that persists in the offspring throughout life (Barker and Clark, 1997). Alterations in maternal nutrition during gestation can have direct effects on adipogenesis and myogenesis, which may affect production performance of the offspring. The most common prenatal influence seen in cattle in the Western United States is maternal nutrient restriction due to the way that cows are reared in that area. Cattle in the West typically experience nutrient deficiency during their second trimester of gestation, often in late fall, when they will be calving in the spring months. Nutrient restriction during the second trimester is a result of consumption of lower quality feed and decreased forage availability. Decreased nutrition during the second trimester of gestation is believed to cause alterations in the efficiency of adipose deposition in offspring of livestock species (Bispham et al., 2005; Edwards et al., 2005).

The growth and development of both skeletal muscle and adipose tissue are integral processes in production of livestock animals, primarily those used to produce meat. Proportions of muscle and adipose tissue within the skeletal muscle are important in determining quantity and quality of meat. Mesenchymal stem cells give rise to muscle, fat, and connective tissues during fetal development through the processes of myogenesis, adipogenesis, and fibrogenesis, respectively (Du et al., 2010). Myogenesis and adipogenesis occur at the same time prenatally. These process compete for nutrients, which makes them simultaneously sensitive to alterations in maternal nutrition.

The early stages of this study performed by Gardner (2017) and Quarnberg (2019), analyzed the effects of maternal nutrient restriction on offspring growth, feedlot

performance, carcass measurements, and meat quality. The results of the previous stages of this study indicate that a restriction in maternal nutrition during mid-gestation results in offspring that perform similarly through the feedlot phase of production and also have similar carcass quality and meat quality when compared to offspring whose dam did not experience a mid-gestation nutrient restriction (Table 1 and Table 2; Gardner, 2017; Quarnberg, 2019). These results led to further investigation of how fetal programming affected the development of offspring and how the offspring were able to perform similarly following different conditions during development *in utero*. The goal of this research is to determine the effects of maternal nutrient restriction during the second trimester on microRNA (miRNA) expression and expression of some of the messengerRNA (mRNA) downstream of the miRNA that are known to be related to adipose and/or muscle growth in the skeletal muscle of the offspring. In addition, mRNA expression of myosin heavy chain (MHC) isoforms will also be analyzed.

The working hypothesis is that offspring from dams that experience nutrient restriction during the second trimester of gestation will have decreased expression of miRNA that promote adipogenesis, resulting in increased expression of mRNA involved in adipogenesis. Additionally, we hypothesize these same offspring will have increased expression of miRNA that promote myogenesis, resulting in decreased expression of mRNA involved in myogenesis. Furthermore, offspring from restricted dams will have decreased expression of MHC-IIa and –IIx when compared to offspring from maintenance dams.

LITERATURE REVIEW

Fetal Programing

Gestational nutrition is believed to impact the prenatal, and subsequently, the postnatal deposition of adipose tissue and skeletal muscle in the offspring of several different livestock species via the phenomena of fetal programing. Fetal programing is defined as a response to a challenge during a critical time of development that has an effect on the offspring's overall development and results in persistent effects (Nathanielsz et al., 2007). Fetal programing was first investigated following the Dutch Hunger Winter that lasted from September 1944 to May 1945 (Stein et al., 1975). During this time of famine, individuals including pregnant females, were not able to meet their nutrient requirements. Most of their provisions consisted of just potatoes and bread, totaling approximately 500 kcal per day (Lumey et al., 2007). The famine had direct effects on maternal weight gain during gestation, fertility, maternal blood pressure, infant birth weight, and development of the central nervous system, among other things (Lumey et al., 2007). Decreased maternal nutrition caused offspring to have decreased birth weight and increased incidence of metabolic disease as adults, which caused researchers to speculate that they had been maternally programmed to develop a thrifty phenotype (Barker et al., 2002).

A thrifty phenotype is a change that is made in the offspring due to the environment the dam endured during gestation. The thrifty phenotype is thought to prepare the offspring to enter an environment in which inadequate levels of nutrition would be available. Seeing these results, researchers had peaked interest in how nutrient

restriction throughout the fetal stage affects the growth and development of offspring relative to production of livestock species. Fetal programing has since been studied in various livestock species as a way to learn more about the effects of maternal nutrient restriction on production and the cellular mechanisms responsible for these observed differences.

In livestock species, the phenomenon of fetal programing is of interest to researchers because of the effects it has on the development of tissues that directly impact the carcass of meat producing animals. Since meat is primarily composed of muscle, bone, connective tissue, and fat, any alterations to development of these tissues can have long term consequences on the quality of the meat produced. During the second trimester skeletal muscle and fat are competing for nutrients because they develop simultaneously in many species, including cattle (Du et al., 2010). Manipulation of maternal nutrition during this time period can lead to changes in offspring skeletal muscle and adipose development, efficiency, and overall carcass quality (Du et al., 2010).

Studies using both cattle and sheep as ruminant models for fetal programing have analyzed muscle and adipose development. Ewes receiving 60% of their calculated metabolizable energy requirements from d 28 to 80 of gestation had increased deposition of adipose tissue in fetuses collected at 140 d of gestation (Bispham et al., 2005; Edwards et al., 2005). In cattle, restricting nutrition to 60% of NRC requirements for the first 85 d of gestation resulted in larger fetal muscle fibers than offspring from those that did not experience maternal gestational nutrient restriction (Gonzalez et al., 2013). Another study performed in cattle showed that a maternal nutrient restriction to 80% of maintenance requirements during mid-gestation increased the efficiency of adipose deposition in the

offspring (Mohrhauser et al., 2015). These previous studies demonstrate that maternal nutrient intake can have an impact on development of skeletal muscle and adipose tissue of the offspring which ultimately impacts production performance.

Fetal Programing and Feedlot Performance

Most beef cattle in the United States typically enter into a feedlot setting after being weaned from their dam. The main purpose of a feedlot is to allow the animals to grow quickly and efficiently in the first few months they are at the feedlot. Later on, the main goal of the feedlot is to promote adipose deposition in order to increase quality of the resultant beef. It is important to understand how changes in maternal nutrition during gestation alter the ability of these offspring to perform in a feedlot setting.

In a study performed with sheep, a nutrient restriction of 50% during early to mid-gestation led to increased growth in the male offspring postnatally when compared to male offspring from non-restricted ewes (Ford et al., 2007). No differences were observed in birthweight or feedlot performance in calves following a mid-gestation nutrient restriction (Taylor et al., 2016). Similarly, in the first phase of this research project performed by Gardner (2017), no differences were observed in birthweights or feedlot performance in calves born to cows that were nutrient restricted during the second trimester of gestation (Table 1 and Table 2). The previous research demonstrated that changes in gestational nutrition can have effects on the performance of the offspring throughout the life of the offspring.

The severity and timing of gestational nutrient restriction alters the effects the restriction has on the offspring later in life. In beef cattle, a nutrient restriction from d 80-

90 of gestation until parturition was great enough to cause a 26% reduction in birth weight and caused the offspring to have decreased compensatory growth and growth potential (Greenwood et al., 2005). Nutrient restriction during early and late gestation can have more adverse effects on the offspring that persist throughout life.

During early gestation fetal organ development is occurring (Funston et al., 2010). Decreasing the amount of available nutrients during early gestation can causes a decrease in primary muscle fibers because the available energy is instead portioned to the development of fetal organs (Funston et al., 2010). Nutrient restriction during late gestation resulted in decreased birthweights that correlated with an increase in offspring susceptibility to disease and health issues following birth (Funston et al., 2010). Restriction during late gestation can also cause a decrease in nutrient uptake by the tissues important in growth and reproduction postnatally (Funston et al., 2010). Changes in maternal nutrition at varying times of gestation change the development of tissues important to maintain health and reproductive function of the offspring.

Although moderate nutrient restriction during mid-gestation does not negatively affect growth and performance of the resultant offspring during the feedlot phase, it is also important to understand how it will effect overall product yield and quality at harvest. Different measurements are used to evaluate the quality and yield of beef carcasses. Carcass yield grade is a value of 1-5 used as an estimate of the total amount of boneless, closely trimmed retail cuts that can be harvested from a carcass (Hale et al., 2013). When carcasses are assigned a lower value that is an indicator of lower costs for consumers and producers (Bass et al., 2016). The lower yield grade value means the animals are producing larger amounts of muscle with lower quantities of excess fat that

would have to be removed from the carcass such as back fat, kidney, pelvic, and heart fat (Bass et al., 2016). Quality grade is a measurement used to predict overall eating experience and palatability of a young beef carcass for the consumer (USDA, 1997). Quality grade is evaluated on a scale ranging from the highest grade, USDA Prime, to the lowest grade, USDA Select (USDA, 1997). Previous studies have evaluated the effects of mid-gestation nutrient restriction on carcass characteristics.

A study performed on calves that were born to dams that experienced a nutrient restriction by receiving 68.1% of net energy requirements during mid-gestation, had a decrease in muscle mass and an increase in adipocyte size (Long et al., 2012). Steers from nutrient restricted cows had an increased yield on a carcass weight basis, indicating that the restriction during gestation did not cause the animals to have an increased amount of excess carcass fat at 30 months of age (Funston et al., 2010). Mohrhauser (2015) observed an improved USDA yield grade in calves from dams that were nutrient restricted to lose one BCS during mid-gestation when compared to calves born to dams managed to maintain a BCS of 5.0 to 5.5. Along with improved yield grade, calves had no differences in hot carcass weight, dressing percent, or kidney, pelvic, and heart fat when their dams were nutrient restricted during mid-gestation (Mohrhauser et al., 2015). When ewes were restricted during mid-gestation the offspring had increased fat deposition with no alteration in lean muscle mass (Zhu et al., 2006). The study by Zhu (2006) indicates that mid-gestation nutrient restriction may allow the offspring to increase quality without decreasing the amount of lean muscle. In the previous phase of this study performed by Quarnberg (2019), carcass measurements were not significantly different when comparing offspring from dams nutrient restricted during mid-gestation to

those that were not restricted (Table 3). This same research project also showed a tendency (P = 0.10) for calves from restricted dams to have an increase marbling to back fat ratio, indicating that these animals were more efficient at depositing adipose (Quarnberg, 2019). However, studies observed that calves from dams that were nutrient restricted from mid to late-gestation by decreasing crude protein by in the diet by 5% compared to the non-restricted group, had a decrease in hot carcass weight and yield grade (Greenwood et al., 2009; Underwood et al., 2010). Although there is evidence that nutrient restriction during gestation has effects on offspring performance, the mechanisms through which it is occurring are still unclear.

Myogenesis and Adipogenesis

During fetal development, mesenchymal stem cells give rise to muscle, fat, and connective tissues. Development of these fetal tissues occurs through competition for progenitor cells during the early stages of gestation in beef cattle (Du et al., 2010). A portion of mesenchymal stem cells commit to becoming myogenic cells after they receive signals from neighboring tissues (Kollias and McDermott, 2008). These cells are pluripotent prenatally and can differentiate into myocytes, adipocytes or other cell types (Aguiari et al., 2008; Kuang et al., 2008; Yablonka-Reuveni et al., 2008). The order in which cell type the mesenchymal stem cells differentiate into depends on their priority to development. The development of skeletal muscle is less of a priority when compared to the heart and brain during fetal development (Zhu et al., 2006). As such, more nutrients are partitioned to the development of vital organs such as heart, liver, and brain which makes skeletal muscle more vulnerable to the effects of decreased nutrient availability

(Zhu et al., 2006). Due to the complexity of the signaling pathways involved in regulating the differentiation of mesenchymal stem cells, changes in maternal nutrition can affect the amount of cells that differentiate to adipocytes or myocytes. The main pathway thought to be involved in regulating differentiation of adipocytes or myocytes prenatally is the Wnt signaling pathway.

When the Wnt pathway is activated, Wnt binds to Frizzled proteins which activates the Disheveled family of proteins (Johnson et al., 2006). The activation of the Disheveled proteins leads to an accumulation of β-catenin, which inhibits a complex of proteins including axin, glycogen synthesis kinase-3 β, and anaphase-promoting complex (Katanaev et al., 2005, Polesskaya et al., 2003). The inhibition of these proteins allows β-catenin to enter the nucleus of stem cells and act as a transcription factor, ultimately leading to the differentiation of stem cells into myocytes while simultaneously causing a decrease in adipocyte development (Du et al., 2010b). In the absence of Wnt signaling, β-catenin is unable to enter the nucleus because it is phosphorylated by glycogen synthesis kinase-3 β (Du et al., 2010b). Whether or not the Wnt signaling pathway is activated can be affected by maternal nutrition (Figure 1). When maternal nutrition is decreased, the Wnt pathway is activated resulting in an increase in adipogenesis. Intramuscular fat deposition or marbling is determined by the number and size of stem cells that differentiate into adipocytes (Du et al., 2010b).

Myogenesis begins prenatally and is divided into two different phases: primary myogenesis and secondary myogenesis (Figure 3). Primary myogenesis occurs during the beginning of gestation, or the embryonic stage, around d 21 and continues until d 90 of

gestation (Robelin et al., 1991). During primary myogenesis, a small number of primary fibers arise to serve as a template for the formation of secondary muscle fibers. Secondary myogenesis occurs during the fetal stage and is most prominent during the second trimester of gestation. At this stage of development, secondary muscle fibers are formed allowing for growth of the fetus. Secondary muscle fibers account for the majority of skeletal muscle fibers the fetus will be born with (Beermann et al, 2008). Primary and secondary fibers develop simultaneously at the beginning of mid-gestation, and then secondary fibers continue to form throughout mid and late gestation (Du et al., 2010). At parturition, calves are ultimately born with a set number of muscle fibers (Picard et al., 1995). The fibers that are present at birth no longer undergo hyperplasia, but will continue to growth through hypertrophy. Previous studies have shown that *in utero* alterations to muscle fiber development impact final muscle fiber number, characteristics, and growth potential.

A study by Zhu (2004) showed a nutrient restriction of 50% of NRC requirements (NRC, 1985) from d 28 to 78 of gestation in sheep reduced the total number of secondary fibers and the ratio of secondary to primary muscle fibers in the fetal *longissimus dorsi* (Zhu et al., 2004). A later study showed that, under the same conditions, lambs at eight months of age born to nutrient restricted ewes had a decreased number of muscle fibers when compared to the control lambs (Zhu et al., 2006). Unlike the nutrient restriction in early gestation, maternal nutrient restriction during late gestation has not been shown to impact muscle fiber number (Du et al., 2010b), but has been shown to reduce muscle fiber size in sheep (Greenwood et al., 1999). Restricted maternal nutrition during late gestation typically results in decreased calf birth weight, likely due to the reduced muscle

fiber size (Freetly et al., 2000). Previous research performed on the effects of nutrient restriction in gestation on muscle development has shown that restriction at varying times through gestation has different effects on muscle development. Less is known about the effects of nutrient restriction specifically during mid-gestation in beef cattle on muscle and adipose development later in life.

Adipogenesis begins around mid-gestation during secondary myogenesis and continues at an increasing rate until parturition and throughout life (Du et al., 2010b, Figure 2). Similar to myogenesis, changes in maternal nutrition during gestation can have long-term physiological effects on adipogenesis (Godfrey and Barker, 2000). Since adipocytes and myocytes both originate from mesenchymal stem cells to create the basic structure of skeletal muscle, regulating their differentiation is important for growth and development (Du et al., 2010). Adipocyte differentiation occurs under the regulation of several key transcription factors and signaling pathways (Hausman et al., 2009).

In beef production specifically, adipose is important to overall production and quality of meat products. Marbling plays a marketable role in the palatability of beef carcasses by contributing to both juiciness and flavor (Du et al., 2010b). Marbling is a main component of flavor and juiciness in beef products. The United States Department of Agriculture (USDA) uses a grading system in order to measure the amount of marbling in a beef carcass that ranges from abundant to practically devoid. A study of consumer acceptance by Platter (2003) suggests that consumer acceptance of steaks increases by 10% with each marbling score ranging from slight to slightly abundant.

Nutritional management of the offspring during early stages of development can increase marbling (Du et al., 2010b). Marbling can be increased easily during this stage

because multipotent stem cells are abundant prenatally and decrease gradually as an animal ages (Du et al., 2010). Marbling can also be increased during a prominent period of growth for bovine animals, known as the feedlot stage. During this stage of development, skeletal muscle growth occurs through hypertrophy while an increased amount of fat is deposited through adipogenesis by feeding a higher energy diet that consists primarily of concentrates. At this time, it is unclear what effects maternal nutrition can have on this period of growth and development in the offspring and what effects it will have on marbling.

Muscle Fiber Type

Skeletal muscle is comprised of a combination of different muscle fiber types. The proportion of different skeletal muscle fiber types within a muscle effect the overall quantity and quality of the meat that is produced (Picard et al., 1998). Three different muscle fiber types have been identified in bovine muscle: type I, type IIa, and type IIx (Thornton et al., 2012). These muscle fibers differ in metabolism, size, color, and function. Type I muscle fibers are smaller in diameter, red in color, have an aerobic metabolism, and are sometimes referred to as slow oxidative fibers. Type IIx fibers or fast glycolytic fibers, are larger in diameter, white in color, and have anaerobic metabolism. Type IIa are classified as intermediate due to their intermediate size, red to white color, and a metabolism that is a combination of both anaerobic and aerobic (Kirchofer et al., 2002). Type IIa fibers are also referred to as fast oxidative-glycolytic fibers.

The impact that maternal plane of nutrition has on muscle fiber characteristics of the offspring postnatally is not currently well understood. Adjustments in maternal nutrition can directly impact muscle development (Gonzalez et al., 2013). Alterations in muscle fiber number and type during fetal stages can carry over and have direct effects on growth and performance (Du et al., 2010). Since secondary myogenesis is occurring during the second trimester of gestation, nutrient restriction during this period may cause a decrease in the number of muscle fibers that are developed.

Different skeletal muscle fiber types have corresponding myosin heavy chain (MHC) isoforms. Identification of specific MHC isoforms can be achieved using their nucleotide sequence (Chikuni et al., 2004). Myosin is the most recognized contractile protein and plays a role in the shape and motion of muscle cells (Montowska et al., 2011). Myosin molecules are characterized into two regions: the two globular heads and an α-helical coiled-coil rod or tail. The head consists of approximately 900 amino acids and is the catalytic site for ATP hydrolysis and the binging binding site for actin (Choi and Kim, 2009). The rod region is approximately 150 nm long and 2 nm in diameter, (Figure 3). The backbone of myosin is the rod that is composed of almost 1000 amino acids (Levitsky, 2004).

In different species there are different MHC isoforms that are grouped into a total of 15 classes (Choi and Kim, 2009). Myosin heavy chain I is expressed in all species (Chikuni et al., 2004), but in cattle, only MHC-slow, -IIa, and -IIx are expressed (Picard et al., 1999). Variations in MHC isoforms can result in large variation in shortening velocity, peak power, optimum efficiency at shortening, and the rate of ATP splitting in isometric conditions (Choi and Kim, 2009). The shortening varies between the slow and

fast-twitch fibers that contain their related MHC isoform, but also within the fast-twitch fibers themselves. The fast-twitch fibers containing the fast-twitch MHC have a higher shortening velocity, with type IIB having the highest contraction velocity in those species expressing a type IIB fiber (Choi and Kim, 2009). The consumption of ATP is also higher in fibers containing the fast MHC isoforms when compared to the fibers containing slow MHC isoforms (Choi and Kim, 2009).

Although calves are ultimately born with a set number of muscle fibers (Picard et al., 1995), muscle fiber type can shift throughout life in response to neural signals, endocrine factors, and functions demands (Brandstetter et al., 1998). These changes permit each muscle to develop a fiber type composition suited to very specific tasks. Most commonly, the muscle fiber type shift in cattle happens between the type IIa and IIx fibers, while the proportion of type I muscle fibers remains relatively consistent (Brandstetter et al., 1998).

Multiple studies on nutrient restriction of ewes have shown that maternal nutrient restriction can have effects on muscle fiber type (Zhu et al., 2004; Zhu et al., 2006). Although ovine muscles stain differently than bovine muscles, the studies conducted in sheep demonstrate that maternal nutrition has effects on ultimate muscle fiber type proportions (Zhu et al., 2004; Zhu et al., 2006). When ewes were nutrient restricted by 50% of their requirements from d 30 to 70, the offspring had more type I fibers and fewer type II fibers in the *semitendinosus* and the *longissimus muscle* 14 d after parturition (Fahey et al., 2005). Muscle of eight month-old lambs from ewes restricted by 50% of their requirements from d 28 to 78 of gestation, had a decreased number of muscle fibers and an increased ratio of type II muscle fibers (Zhu et al., 2004). Although there is

evidence that fetal muscle ultrastructure is altered during fetal programing, there is considerably less information available regarding the long term effects of maternal plane of nutrition on muscle ultrastructure of the offspring. By evaluating the muscle fiber development using MHC isoform analysis throughout different stages of production, we hope to provide more detail to the long term effects of maternal nutrition on muscle development.

MicroRNA and Messenger RNA

MicroRNAs are single-stranded RNA molecules that are 21-23 nucleotides in length (Jin et al., 2010). MicroRNA are involved in many physiological processes, such as differentiation, proliferation, apoptosis and development (Catalanotto et al., 2016). The pathway of miRNA biogenesis (Figure 4) starts when miRNAs are transcribed by RNA polymerase II (RNApolII) to long primary miRNA (pri-miRNA) in the nucleus (Catalanotto et al., 2016). The pri-miRNA is made up of approximately 30 base pairs, a terminal loop and two flanking unstructured single stranded tails (Catalanotto et al., 2016). A protein complex consisting of Drosha and Di George syndrome critical region 8 gene (DGCR8), processes pri-miRNAs into short 70 nucleotide structures called precursor miRNAs (pre-miRNA; Catalanotto et al., 2016). Following the processing in the nucleus, pre-miRNAs are exported to the cytoplasm by exportin 5 (Kim et al., 2009).

Once in the cytoplasm, a protein called Dicer cleaves the pre-miRNA near the terminal loop to create non-hairpin miRNA duplexes to be loaded onto an Argonaute (AGO) protein (Kim et al., 2009). The AGO protein unwinds the duplex and the guide strand of mature miRNA is loaded on to the RNA-induced silencing complex (RISC;

Kim et al., 2009). The RISC complex then directs the miRNA to its target mRNA (Kim et al., 2009).

Studies in animals demonstrate that only the seed sequence (sequence from position 2 to 8 at the 5' end) is important for the recognition of target genes (Bartel, 2009). The seed sequence is able to pair fully to its responsive element mainly at the 3' untranslated region (UTR) of the specific target mRNA (Bartel, 2009). The binding of miRNA to mRNA can lead to degradation of the target mRNA and/or translational suppression (Huang, 2014). Many miRNAs can target an mRNA, just as a miRNA can target many mRNAs.

The miRNAs that have been studied inhibit gene expression by blocking translation and decreasing stability of the target gene. The majority of mRNA decay starts when the poly(A) tail is removed by the 3'-5' exoribonucleases. The mRNA is either degraded in the 3'-5' direction, or the decapping enzyme first removes the 5' terminal cap, and the body of the RNA is degraded by a 5'-3' exonuclease (Fabian et al., 2009).

Although there are many miRNAs with varying targets, there are some that have been identified to have specific roles in the regulation of myogenesis and/or adipogenesis. Some of these specific miRNAs are miR-1, -133, -206, -181, -27b, -424, -486, and -214, (Table 1; Güller et al., 2010; Yan et al., 2013). These miRNAs regulate myogenesis and adipogenesis by targeting specific mRNAs that play a role in differentiation and proliferation of mesenchymal stem cells in early life.

MiR-133, miR-1, and miR-206 are often referred to as the muscle specific miRNAs due to their roles in regulation of development of skeletal muscle tissue. MiR-133 increases the proliferation of myoblasts by repressing expression of serum response

factor, a transcription factor (Chen et al., 2006). Expression of miR-133 is increased in C2C12 cells during myogenic differentiation (Kato et al., 2009). MiR-1 stimulates myoblast differentiation by inhibiting histone deacetylase 4 (HDAC4), which is a transcriptional repressor of muscle gene expression (Chen et al., 2006). When miR-1 is expressed at high levels, there is an increased expression of α-actin, sarcomeric myosin, and creatine kinase (Nakajima et al., 2006). Myocyte enhancer factor-2, which functions as a transcription factor in regulating myogenesis, was increased by miR-1 and miR-133 (Liu et al., 2007). In a study performed in zebra fish, miR-1 and miR-133 controlled the expression of muscle genes and regulated the organization of the fundamental contractile unit of a muscle fiber (Mishima et al., 2009). Insulin-like Growth Factor-1 Receptor (IGF-1R) and miR-133 and miR-1 have an inverse relationship, as miR-133 and miR-1 increase they block expression of IGF-1R (Huang et al., 2011). Since IGF-1R is so important in the regulation of muscle cell differentiation and proliferation and it is repressed by the two must abundant miRNAs found in skeletal muscle, the abundance of IGF-1R may be a deciding factor in myogenesis (Huang et al., 2011). The miR-133 also targets mastermind like transcriptional coactivator 1 (*MamL1)* Luo et al., 2013).

Although the role of *MamL1* is not well documented in cattle, it has been more heavily studied in mice and humans. *MamL1* and *MEF2C* (myocyte enhancer factor 2C) work together to activate several genes that are required for the development and function of skeletal muscle (Cesan et al., 2011). *MamL1* also plays a role in regulating Notch signaling, a pathway critical in the cell fate determination (Cesan et al., 2011; Shen et al., 2006). Notch-signaling pathways are involved in the development of neural tissues, blood

vessels, heart, pancreas, mammary gland, T lymphocytes, hematopoietic lineages, and other cell types (Miller et al., 2017). In *in vitro* studies using mouse C2C12 cells, over expression of *MamL1* dramatically enhanced myotube formation and increased the expression of genes related specifically to muscle (Shen et. al., 2006). In order to influence muscle differentiation, *MamL1* appears to mediate cross talk between Notch signaling and *MEF2C*, demonstrating the importance of *MamL1* in muscle development (Shen et al., 2006).

MiR-206 influences differentiation through indirect down-regulation of the helix–loop–helix protein Id, a suppressor of myogenic differentiation factor 1 (*MyoD*; Kim et al. 2006). Myogenic differentiation factor 1, a protein that has been shown to play a role in regulation of muscle differentiation (Davis et al., 1987), is then able to induce the transcription of miR-206. The induced transcription of miR-206 leads to promotion of myogenic differentiation (Yan et al., 2013). MiR-206 also plays a role in skeletal muscle development through the regulation of the expression connexin43, a gap junction protein that is required for skeletal myoblast fusion (Anderson et al., 2006).

Both miR-206 and miR-486 have been shown to induce the differentiation of myoblasts by down regulating paired-box transcription factor 7 (Pax7; Dey et al., 2011). Pax7 is a transcription factor that is expressed in proliferating myoblasts, but is down regulated during differentiation (Dey et al., 2011). When Pax7 was upregulated, it had inverse effects on MyoD, meaning that higher expression of Pax7 results in cells with lower expression of MyoD leading to less cell differentiation (Dey et al., 2011). In a recent study, bovine fetuses from dams that were nutrient restricted for the first 85 d of

gestation had a reduction in Pax7 immunopositive nuclei in the *infraspinatus* when collected at 85 d (Gonzalez et al., 2013). Additionally, mRNA coding for the potent growth factor IGF-1, known to alter myogenesis, was lower in the skeletal muscle of fetuses from dams experiencing nutrient restriction during the first 85 d of gestation (Gonzalez et al., 2013). However, a second study performed when the dams were nutritionally restricted for 91 d during mid-gestation, revealed few differences in expression of genes important to adipogenesis and myogenesis in mature offspring at harvest (Mohrhauser et al., 2015).

MiR-181 is up-regulated during the process of muscle differentiation. Similarly to miR-206, miR-181 can promote differentiation by inhibiting the homeobox protein Hox-A1, which is also a protein that can inhibit MyoD expression (Yamamoto & Kuroiwa, 2003). An increase in the expression of miR-214 also promotes the proliferation and differentiation of myoblasts. In a study performed with C2C12 cells, decreased expression of miR-214 inhibited muscle cell proliferation and differentiation (Feng et al., 2011. The changes in proliferation and differentiation caused by mir-214 is possibly because of the relationship with Enhancer of Zeste 2 Polycomb Repressive Complex 2 (*Ezh2*; Luo et al., 2013).

Polycomb group proteins contribute to cell commitment and differentiation through their ability to repress developmental regulators in skeletal muscle cells (Juan et al., 2009). Differentiation coincides with polycomb group disengagement, recruitment of the developmental regulators, and activation of miR-214 transcription (Juan et al., 2009). Following transcription miR-214 has negative feedback on polycomb proteins by

targeting Ezh2, allowing for muscle cell differentiation to be accelerated (Juan et al., 2009). Thus demonstrating how the relationship between miR-214 and Ezh2 works to regulate polycomb dependent gene expression during skeletal muscle differentiation.

The expression of miR-322/424 and miR-503 is stimulated during muscle cell differentiation and arrests the cell cycle through the down-regulation of cell division cycle 25 A (*Cdc25A*; Yan et al., 2013). Cdc25A is the phosphatase responsible for removing inhibitory phosphorylation of cyclin-dependent-kinase 2 (cdk2; Sarkar et al., 2010). A down regulation of Cdc25A increases the inhibition of cdk2 which is important in the differentiation of myoblasts into myotubes (Sarkar et al., 2010).

Paired box transcription factor 3 (*Pax3*) is important in skeletal muscle myogenesis (Du et al., 2010). MiR-27b downregulates *Pax3* and increases the early differentiation of muscle cells (Crist et al., 2009). Inhibiting miR-27b allows for levels of *Pax3* to be maintained. More *Pax3* allows for more cell proliferation and delayed onset of cell differentiation (Güller et al., 2010). Studies have also shown that miR-27 plays a role in the regulation of adipogenesis (Yan et al., 2013). When miR-27 is over expressed before the initiation of adipogenesis, there was an inhibition of adipogenesis (Yan et al., 2013). MiR-27 is able to inhibit adipogenesis by preventing the expression of transcription factors that are important in pathways leading to adipose tissue development (Lin et al., 2009).

Various miRNAs along with their target genes regulate myogenesis and adipogenesis. Researchers estimate that 60% of all protein-coding genes are regulated by miRNAs (Kim et al., 2009). These miRNAs may play an important role in fetal programming because of their ability to alter gene expression. Nutrient restriction in the

dams could cause changes to expression of certain miRNAs and lead to changes in the offspring. Although a fair amount of research has been completed regarding how miRNA expression impacts skeletal muscle growth, little work has been completed in this area focusing specifically on changes in skeletal muscle throughout production.

Previous studies have shown that maternal nutrient restriction can have effects on the development of the offspring of various species (Gonzolez et al., 2013; Zhu et al., 2006; Du et al., 2010; Morhauser et al., 2015). The research varies though, and not much has been performed specifically on beef cattle in the second trimester of gestation. The hope of this study is to provide further insight into how maternal nutrient restriction during the second trimester in cattle effects MHC isoform, mRNA, and miRNA expression.

MATERIALS AND METHODS

Cow Management

Animal procedures were reviewed and approved by the Institutional Animal Care and Use Committee as required by federal law and Utah State University Policy (IACUC-2373). Calves were produced from 34 commercial cows, of heavy Angus influence, naturally bred to the same pure bred Angus sire. The cows were allocated to one of two treatment groups: maintenance (MAIN, n=16) or restricted (REST, n=18) prior to the second trimester of gestation. The MAIN group was managed to maintain a body condition score (BCS) of 5.0-5.5, while the REST group was managed to lose one BCS over an 84-d period during the second trimester (Table 5; Gardner, 2017). The MAIN groups grazed on approximately 54 acres of irrigated pasture and were supplemented with alfalfa hay when needed in order to maintain a BCS according to the nutrient requirements of beef cattle (NRC, 2000). The REST group grazed on 6.4 acres of non-irrigated pasture and did not receive any extra supplementation until the beginning of the third trimester when the animals were again comingled. During the third trimester dams from both treatment groups were comingled and fed to meet maintenance requirements until parturition. Body weight and BCS were evaluated at 0, 28, 56, and 84 d of mid-gestation. Seven weeks following comingling, weights and BCS were collected again to measure compensatory gain during the third trimester. The weights were collected using a Digistar SW300 indicator, Stockweigh load cells, and Wrangler alleyway platform (Digi-star LLC, Fort Atkinson, WI).

Maternal Feedstuff Nutrient Content

Nutrient availability was measured in all pastures during the restriction and recovery phases. In each pasture plant cover was measured by taking five readings using a 0.1-m^2 Daubenmire frame following previously described methods (Bonham, 2013). Samples collected each month were placed in paper bags and dried in a forced-air oven at 60°C for 48 hours. The samples were then ground in a Wiley mill with a 1-mm screen and analyzed for dry matter, neutral detergent fiber, acid detergent fiber, and crude protein (CP) as previously described (Van Soest et al., 1991). Total digestible nutrients was calculated using the CP and fiber concentrations following previously described methods (Table 6; Swift, 1957; Weiss et al., 1992; NRC, 2000).

Postpartum Offspring Management

All calves' birthdate and heart girth measurements were recorded. The heart girth was measured using a tape measure (beef weight tape, Nasco, Fort Atkinson, WI) drawn snug around the calves' girth just behind the shoulders to determine the approximate weight. The cow-calf pairs continued to be comingled on the same dietary treatment as they were during the third trimester until weaning. The bull calves were castrated within three months following birth. At approximately 75 d of age, the calves were vaccinated (Piliguard Pinkeye-1 Trivalent, Intervet Inc., Madison, NJ; Ultrabac 8, Zoetis Inc., Florham Park, NJ; Bovi-Shield Gold 5, Zoetis Inc.; and a Multimin 90 shot, Multimin North America Inc., Fort Collins, CO). Blood samples were collected at this time. Serum and plasma were collected from the blood, aliquoted and stored at -20°C until further analysis. Calves received another dose of Bovi-Shield Gold 5 and Ultrabac 8 at weaning.

Feedlot Management

Weaning occurred when the calves were an average of 206 d old. The calves were transported to the Utah State University Research Feedlot (Wellsville, UT) where upon arrival they received a sequential Ralgro Implant (Merck Animal Health, Summit, NJ) to resemble typical feedlot practices. For seven weeks the calves were co-mingled and fed a backgrounding ration. They were then sorted into individual pens and switched to a grower ration for the first 85 d of the feedlot phase. Calves were then stepped up to a final feedlot ration by increasing the amount of barley in the ration by 10% until they reached a final finishing ration (Table 7).

Feed intake was measured by weighing feed offered and feed refused each day. The management of the feed bunk was done using the clean-bunk management system as previously described (Prictchard and Burns, 2003). The date that the calves were sorted to their individual pens was considered d 0. The cattle were weighed and shipped to a commercial JBS harvest facility (Hyrum, UT) on d 196, once an average backfat thickness of 7.0 mm was reached. The calves were weighed at 0, 28, 56, 84, 111, 139, 168, and 186 d on feed. Additionally, an Exago Ultra Portable ultrasound with 5 cm muscle probe (Universal Imaging, Bedford Hills, NY) was used to take predictive measurements of back fat thickness on the days that the calves were weighed. Readings for backfat thickness were taken between the 12th and 13th rib as previously described (Greiner et al., 2003). Blood samples were collected at approximately 75 d of age, 7 d before starting the grower ration and then again 84 d following the grower ration. The blood samples were collected from the jugular vein to collect both plasma and serum and stored at -20°C for further analysis. Skeletal muscle biopsies were collected from three

different time points. The first skeletal muscle biopsy was taken at weaning following previously described procedures from the *longissimus lumborum* and immediately snap-frozen in liquid nitrogen and stored at -80°C for further analysis (Schneider et al., 2010). The second skeletal muscle biopsy was taken from the *biceps femoris* (BF) right before the calves began their step-up feedlot ration following the same procedures (Schneider et al., 2010). The third skeletal muscle biopsy was collected from the *longissimus lumborum* (LD) following previously described procedures and within 20 min of exsanguination following harvest and immediately snap frozen in liquid nitrogen and stored at -80°C for further analysis (Thornton et al., 2017).

Myosin Heavy Chain Analysis

Skeletal muscle samples collected from the BF at the beginning and the LD at the end of the feedlot phase (LD), were ground under liquid nitrogen, and total RNA was extracted using TriZol following the manufacturer's protocol (Invitrogen, Carlsbad, CA). The RNA was quantified using a Take3 plate and Synergy H1 hybrid multi-mode microplate reader (Biotek). The TaqMan high capacity RNA to cDNA kit was used to convert mRNA to cDNA (Life Technologies). Using TaqMan advanced assays (Life Technologies) and 7500 Fast Real-Time PCR (Applied Biosystems) instrument, the expression of the MHC isoforms MHC-slow, IIa, and IIx were measured and 18S was used for the housekeeping gene (Table 8).

mRNA Expression in Skeletal Muscle:

Skeletal muscle samples collected from the BF at the beginning and the LD at the end of the feedlot phase (LD), were ground under liquid nitrogen, and total RNA was

extracted using TriZol following the manufacturer's protocol (Invitrogen, Carlsbad, CA). The RNA was quantified using a Take3 plate and Synergy H1 hybrid multi-mode microplate reader (Biotek). The TaqMan high capacity RNA to cDNA kit was used to convert mRNA to cDNA (Life Technologies). Using TaqMan advanced assays (Life Technologies) and 7500 Fast Real-Time PCR (Applied Biosystems) instrument, the expression of *Pax3, Pax7, IGF-1R, MamL1, Cdc25A, and Ezh2* was measured. The gene 18S was again used as the housekeeping gene (Table 9).

miRNA Expression in Skeletal Muscle

Samples collected from all three time points were ground under liquid nitrogen. MicroRNA were extracted using the MirVana miRA isolation kit following the manufacturer's protocol (Life Technologies, Waltham, MA). The miRNA was quantified using a Take3 plate and Synergy H1 hybrid multi-mode microplate reader (Biotek). The conversion of miRNA to cDNA was performed following the manufacturer's protocol using the TaqMan advanced miRNA cDNA synthesis kit (Life Technologies). TaqMan advanced miRNA assays (Life Technologies) and 7500 Fast Real-Time PCR (Applied Biosystems) were used to measure the expression of miR-1, -133a, -133b, -206, -181d, -27b, -424, -486, -214, and let-7g. Let-7g was used as the housekeeping miRNA.

Statistical Analysis

All miRNA and mRNA expression data used each individual calf as the experimental unit, and comparisons were made within each individual time point. The data were all analyzed using the general linear mixed model procedure of SAS® version 9.4 (SAS Institute, Cary, NC). No significant ($P > 0.05$) differences were observed

between calf, sex, birthdate, and pen location; as such, these parameters were included as random effects in the final model. All miRNA and mRNA expression data is shown as $2^{-\Delta CT}$ (ΔCt = Ct (gene of interest) – Ct (housekeeping gene)). Least square means of mRNA and miRNA expression were calculated using the general linear mixed model of procedure of SAS version 9.4 (SAS Institute Differences). The data met assumptions for normality and equal variance. Differences due to the main effect of maintenance vs. restriction were considered significant at $P \leq 0.05$. Correlations were determined using Pearson correlations.

RESULTS

Myosin Heavy Chain Expression

The expression of MHC isoforms MHC-slow, -IIa, and –IIx were measured and there was no difference ($P \geq 0.14$) in expression of the MHCs between offspring from the two treatment groups from the *biceps femoris* at the beginning or *longissimus lumborun* at the end of the feedlot phase, (Table 10).

mRNA Expression:

The expression of six different mRNA were measured from the *biceps femoris* at the beginning and the *longissimus lumborum at the* end of the feedlot phase. There was no change in expression ($P \geq 0.27$) of *Pax3, Pax7, Cdc25A, MamL1, Ezh2, and IGF-1R* between offspring from the two treatment groups at either of the time points, (Table 11).

MicroRNA Expression

The expression of nine miRNAs, miR-1, -27b, -133a, -133b, -181d, -206, -214, -424, and -486 were analyzed at all three time points; weaning, the beginning of feedlot, and the end of the feedlot. At weaning in the *longissimus lumborun*, there was an increase ($P < 0.01$) in miR-27b in the MAIN offspring when compared to the REST offspring, (Table 12). Expression of miR-133a, -133b, -181d, -214, -424, and -486 were all increased ($P < 0.05$) in the skeletal muscle of REST offspring at weaning, (Table 12). There was no change ($P > 0.30$) in expression of miR-1 or -206 in the skeletal muscle when comparing calves from the two different treatments at weaning, (Table 12).

There was an increase ($P < 0.05$) in expression of miR-133a, -133b, -206, -214, -424, and -486 in the REST offspring at the beginning of the feedlot phase in the *biceps*

femoris, (Table 13). MicroRNAs miR-1, -27b, and -181d had no change ($P \geq 0.12$) in expression between the two treatments at the beginning of the feedlot phase, (Table 12).

At the end of the feedlot phase, in the *longissimus lumborum*, expression of miR-486 was increased ($P < 0.05$) in the MAIN offspring when compared to the REST offspring, (Table 14). In addition, offspring from MAIN dams tended to have increased expression of miR-27b ($P = 0.06$) when compared to REST offspring, (Table 14). There was no change ($P > 0.44$) in expression of miR-1, -27b, -181d, -206, -214, and -424, (Table 14). An increase ($P <0.05$) in expression of miR-133a was found in the REST offspring at the end of the feedlot phase, (Table 14).

miRNA and mRNA Correlations

Pearson correlations were performed to compare miRNA and mRNA at the beginning and the end of the feedlot phase, (Table 15 and Table 16). At the beginning of the feedlot phase there was a positive ($P = 0.02$, $R = 0.47$) correlation between the expression of *Pax3* and miR-133b, (Table 15). The expression of miR-206 was positively ($P = 0.02$, $R = 0.47$) correlated with the expression of *Pax3*, and had a tendency to be negatively ($P = 0.08$, $R = -0.37$) correlated with *Cdc25A*, (Table 15). The expression of miR-27b, -133a, -181d, -214, -424, -1, and -486 had no significant ($P \geq 0.14$) correlations with any of the miRNA, (Table 15).

At the end of the feed lot phase there were multiple miRNA that had a correlation with mRNA. The expression of miR-27b was negatively correlated with *Pax7* ($P = 0.01$, $R = -0.58$), *Ezh2* ($P = 0.03$, $R = -0.48$), *MamL1* ($P = 0.02$, $R =-0.53$), and *IGF-1R* ($P = 0.01$, $R =-.060$), (Table 16).The miR-181d was negatively ($P = 0.05$, $R = -0.47$)

correlated with the expression of *Cdc25A* and had a tendency (P =0.11, R = -0.40) to be negatively correlated with *IGF-1R*, but showed no correlations with any of the other mRNAs (P ≥ 0.22), (Table 16). The miR-206 had a tendency (P =0.06, R =-.045) to be negatively correlated with *Cdc25A*, but no other significant correlations (P ≥ 0.17). MiR-486 had a positive (P < 0.0001, R = 0.99) correlation with the expression of *MamL1*, and a negative (P = 0.02, R = -0.51) correlation with *Pax7*, (Table 16). There was no significant (P ≥ 0.16) correlation between miR-486 and any of the other mRNAs, (Table 16). A group of miRNAs consisting of miR-1 (P ≥ 0.27), miR-424 (P ≥ 0.32), miR-214 (P ≥ 0.13) and miR-133a (P ≥ 0.23) showed no significant correlation with any of the mRNAs, (Table 16).

DISCUSSION

Maternal nutrition was restricted during the second trimester for an 84 d period in order to analyze the effects of maternal nutrient restriction on offspring performance postnatally. Mid-gestation is an essential period for development of tissues that are economically important as fat and muscle are developing simultaneously. In the Intermountain West, the second trimester of gestation often occurs at the same time dams are receiving lower quality and quantity of forage in the late fall. Since mid-gestation nutrient restriction is occurring due to the way cattle are reared in the West, it is important to understand the effects the restriction has on the offspring. This study was designed to mirror the restriction that is happening to cattle in the West by placing cows in a smaller, non-irrigated pasture as compared to the maintenance cows that were placed in a larger, irrigated pasture for the second trimester (Gardner, 2017). When comparing offspring born to the two treatment groups, Gardner (2017) and Quarnberg (2019) saw no differences in growth, feedlot performance, or carcass measurements. The lack of phenotypic change documented by the previous researchers led to further investigation into whether maternal mid-gestation nutrient restriction effected MHC isoform, mRNA, and miRNA expression in skeletal muscle of offspring from both treatment groups.

No significant differences in the expression of myosin heavy chains MHC-slow, IIa, and -IIx between the two treatment groups at both the beginning and end of the feedlot phases were reported. Although no differences were found, other studies analyzing the effects of maternal nutrition at varying time points in gestation saw changes in muscle fiber composition in multiple species. In cattle, a nutrient restriction of 60% NRC requirements for the first 85 d of gestation resulted in larger fetal muscle fibers than

those that did not experience maternal gestational nutrient restriction (Gonzalez et al., 2013). In pigs, an increase in nutrient intake during gestation caused an increased number of muscle fibers and an increase in the proportion of secondary to primary muscle fibers in the offspring than in those not experiencing a change in gestational nutrition (Dwyer et al., 1994). A study performed in sheep experiencing a nutrient restriction from d 30-70, left the offspring with a significantly lower number of fast fibers and significantly more slow fibers at 14 d of age (Fahey et al., 2005). Muscle fiber type has the ability to shift throughout life (Brandsetter et al., 1998). Most commonly, the muscle fiber type shift in cattle happens between the type IIa and IIx fibers, while the proportion of type I muscle fibers remains relatively consistent (Brandstetter et al., 1998). When comparing to previous studies in cattle, muscle fiber type was analyzed in fetuses that experienced nutrient restriction during gestation rather than adult offspring as was done in this study (Gonzales et al., 2013). The ability of muscle fiber type to shift may be a cause as to why there was not a phenotypic change in muscle fiber type that persisted throughout the feedlot phase and to harvest. More research needs to be conducted to determine the impacts of time of restriction and severity of restriction in order to increase our understanding of how maternal plane of nutrition may impact muscle fiber type of the offspring while in the feedlot.

Although we did not see a change in phenotypic outcomes, we did see a change in miRNA expression. To the knowledge of the authors, this is the first report detailing how a decreased plane of nutrition during mid-gestation impacts miRNA expression in the skeletal muscle of offspring through weaning, feedlot growth, and at harvest. The miRNAs that were analyzed consisted of miR-1, -27b, -133a, -133b, -181d, -206, -214, -

424, and -486, all of which have been previously shown to play a role in adipogenesis and/or myogenesis. At weaning, offspring from restricted mothers had an increased expression of miR-133a, -133b, -181d, -214, -424, and -486 when compared to non-restricted mothers. There was a decrease in expression of miR-27b in the restricted offspring.

The miRNA that had an increase in expression in the restricted offspring each play an important role in muscle development. MiR-133a is a highly conserved muscle specific miRNA that plays a role in myoblast proliferation in mice (Chen et al., 2006). The miR-206 and -486 are expressed in skeletal muscle and are up regulated during myoblast differentiation in a study performed in mice (Dey et al., 2011). Lambs from ewes that received 70% of the control diet from mating to six days after mating saw changes in the expression of miR-206 in comparison to control lambs (Lei et al., 2014). MiR-181 is upregulated during muscle differentiation. An increase in the expression of miR-214 promotes the proliferation and differentiation of mouse myoblasts (Feng et al., 2011). The miR-424 is also stimulated during muscle cell differentiation (Yan et al., 2013). At this time point, we see increased expression of six different miRNAs relating specifically to proliferation and differentiation of skeletal muscle in the offspring from restricted mothers. At weaning, the observations were consistent with the hypothesis that miRNA relating to adipogenesis were decreased in the offspring from restricted mothers, while there was and increased expression of miRNA related to myogenesis in the same offspring. This demonstrates that the nutrient restriction may have influenced the Wnt signaling pathway that plays a role in the differentiation of cells to either myoblasts or adipocytes.

While cattle are ultimately born with a set number of muscle fibers, a population of pluripotent stem cells are still present to allow for muscle growth and adipogenesis postnatally (Du et al., 2010). As the animal ages the pool of multipotent stem cells decreases in abundance. Having an increased amount of pluripotent cells would allow for an increased amount of proliferation and differentiation in muscle cells. At weaning, the offspring in the study are between the birth and 250 d stage, where there is still an abundance of pluripotent cells (Figure 5). The increase in more miRNA related to differentiation and proliferation of muscle cells in offspring from restricted dams may be because they have a higher amount of pluripotent cells remaining that did not differentiate during the second trimester of gestation due to the nutrient restriction. It may also be that the analysis at this time point was closer to the inflicted nutrient restriction so any changes that were made during gestation could still be present. However, more research needs to be conducted in order to fully understand the effects that these changes in miRNA might have on production of the animal.

Analysis at the second time point, the beginning of the feedlot phase, resulted in an increased expression of six miRNAs in samples collected from the *biceps femoris* in offspring from restricted mothers when compared to non-restricted mothers. The miRNAs that had increased expression in offspring from restricted mothers at this time point were miR-133a, -133b, - 206, -214, -424, and -486 when compared to non-restricted mothers. Again, miRNAs with roles in myogenesis were up regulated during this time point. During this time point the offspring are still experiencing a period of muscle growth, but there is less muscle cell differentiation occurring. The miRNA that had increased expression in the offspring from nutrient restricted dams at this time point

all play a role in decreasing muscle cell differentiation (Luo et al.,2013). The increases may be because the restricted offspring are experiencing decreased muscle cell differentiation as they continue to grow and the amount of adipose that is being deposited is increasing. As they transition farther into the finishing phases in the feedlot the deposition of adipose will increase as muscle development decreases. Fewer miRNA were significantly different between the two treatment groups as the offspring aged. This may be due to the animals being in the same environment for an extended period of time. Future studies could take samples more frequently to more thoroughly understand how maternal nutrition effects miRNA expression and how it changes through the life of the offspring.

At the end of the feedlot phase only two miRNAs differed in expression in samples from the *longissimus lumborum* when comparing the two treatment groups. When comparing offspring from restricted mothers to those from non-restricted mothers, miR-133a and -486 were the only ones that were significantly different between treatments. The miR-133a was significantly higher in the offspring from restricted mothers, while miR-486 was significantly lower. There was a tendency for offspring from non-restricted mothers to have an increased expression of miR-27b.

Previous research analyzing the periconceptional period in sheep experiencing undernutrition demonstrates that in fetal offspring, expression of several different miRNAs were altered, including miRNA-27b and miRNA-206 (Lei et al., 2014). In the previously described study, miR-27b had a decreased expression in lambs from ewes experiencing nutrient restriction for 60 d prior to mating compared to lambs from ewes receiving no nutrient restriction (Lei et al., 2014). At this stage in production very little

muscle cell proliferation would be occurring, as muscle cell growth is slowing and mainly adipose tissue is being developed. Decreased expression of muscle specific miRNAs in restricted offspring at this time point could be due to the amount of increased adipogenesis occurring. During this time in growth, more nutrients are being used for the development of adipose tissue, as nutrients are partitioned away from the development of skeletal muscle. Previous studies showed that offspring expiring a nutrient restriction during gestation had an increased amount of fat deposition compared to those not experiencing a gestational nutrient restriction (Mohrhauser et al., 2015). Seeing the changes in miRNA, led to the expectation that there would be changes in the expression of mRNA that are known targets of these miRNA in the offspring.

The expression of six different mRNA, *Pax3, Pax7, Cdc25A, Ezh2, MamL, and IGF-1R,* were analyzed at the beginning and the end of the feedlot phases from the *biceps femoris* and *longissimus lumborum,* respectively. No significant differences were observed in any of the genes when comparing the two treatment groups. These specific genes were chosen for analysis due to their previously studied relationship as targets of the analyzed miRNA (Luo et al., 2013). Some research has been conducted to look at how mid-gestation nutrient restriction changes mRNA expression in bovine fetal skeletal muscle (Jennings et al., 2016). The study by Jennings (2016) observed changes in the expression of mRNA involved in skeletal muscle and adipose development between offspring from dams that were nutrient restricted for d 85 to 180 of gestation compared with non-nutrient restricted dams (2016). A study by Mohrhauser (2015) analyzed the mRNA expression of several different genes known to be involved in the growth of skeletal muscle in offspring from nutritionally restricted dams. The study looked at

mRNA expression in the *longissimus lumborum* and the *semitendinosus* at weaning and harvest (Mohrhauser et al., 2015). Mohrhauser's (2015) research concluded that there were no differences in mRNA expression in the *longissimus lumborum* at either time point. The findings of the present study agree with those that have been previously reported by Mohrhauser (2015). Due to the transient nature of mRNA any changes that may have been made initially during gestation, may not have persisted into maturity. The expression of these genes may have been more heavily changed closer to the time of the nutrient restriction. There may have been more postnatal and environmental influences that had effects on the expression of mRNA that could account to the lack of differences in expression. Although there were no significant changes in genes expression, there were some significant correlations between the mRNA and miRNA.

At the beginning of the feedlot phase there were only significant positive correlations between *Pax3* and miR-133b and miR-206. The expression of *Pax3* is typically down regulated by miR-206 and not affected by miR-133b (Luo et al., 2013). In mice, miR-206 and miR-133b are clustered together on the same chromosome, but have different transcription and expression (Luo et al., 2013). Some of the reason we see correlations with both of these miRNA may be because of their close relationship with each other. The increased expression of both miRNAs and *Pax3* at this time point could also be effected by a decrease in another miRNA such as miR-486 that plays a role in down regulating *Pax3.* Since the pathways that miRNA are able to affect proliferation and differentiation through are so complex it is hard to know exactly why we see these correlations. However, further research in this area is needed before more conclusions are drawn about the correlations between the expression of these specific miRNA and mRNA

and how they are effected by maternal nutrient restriction during mid-gestation. An improved understanding of the role between miRNA and mRNA in cattle during the feedlot phase of production would provide important insight into the molecular mechanism through which skeletal muscle growth and adipose deposition occur within our feedlot cattle.

At the end of the feedlot phase, five of the mRNA were correlated with expression of miRNA. There was a negative correlation between *Pax7* and both miR-27b and miR-486. Previous research has demonstrated a correlation between *Pax7* and miR-27b where miR-27b down regulates the expression of *Pax7* leading to decreased muscle cell differentiation (Luo et al., 2013). Both miR-27b and miR-206 were negatively correlated with *Ezh2*. Again, miR-27b was negatively correlated with *MamL1* and I*GF-1R. MamL1* and *IGF-1R* are both promoters of skeletal muscle differentiation, while miR-27b is down regulated muscle cell differentiation (Luo et al., 2013). Their opposing roles could be why we see a negative correlation at this stage in production. The expression of *MamL1* was highly correlated with miR-486. MiR-181d and miR-206 were negatively correlated with *Cdc25A* which could be because miR-181d and miR-206 both have roles in promoting skeletal muscle differentiation and *Cdc25A* works to block muscle cell differentiation (Luo et al., 2013). Although not all of these correlations are recorded as being biologically relevant, it is important to note them since multiple miRNA can interact with multiple mRNA. There may be more relevance to the interactions that has not been previously noted. These data show us that miRNA have a relationship with their specific target mRNA and other mRNA as well, again demonstration the complexity of their pathways.

Throughout the different phases of development, we analyzed the expression of MHC isoform, miRNA, and mRNA expression from different muscles. The weaning and end of feedlot samples were collected from the *longissimuns lumborum*, while the beginning of the feedlot samples were collected from the *biceps femoris.* Collecting samples from different muscles did not allow for analysis across time points. The analysis from the two muscles does demonstrate that mid-gestation nutrient restriction is having effects on multiple muscles within the carcass.

Although there were differences in the expression of miRNA between the two treatment groups, all the offspring had similar feedlot performance and no differences in the expression of MHC isoforms. The carcass data from the two treatment groups also showed no significant differences (Quarnberg, 2019). The offspring from the restricted group did however have a tendency to have a higher marbling to back fat ratio (Quarnberg, 2019). Although no significant changes were observed, previous studies observed an improved USDA yield grade in calves from dams that were nutrient restricted during mid-gestation (Mohrhauser et al., 2015). Along with improved yield grade, calves had no differences in hot carcass weight, dressing percent, or kidney, pelvic, and heart fat when their dams were nutrient restricted during mid-gestation (Mohrhauser et al., 2015). The differing expression of some miRNA could be the reason offspring from restricted are dams able to perform similarly to those from non-restricted dams. The offspring may have some changes developed during gestation that allowed them to utilize the nutrients available to them following parturition more efficiently to account for any decreases in growth or development they may have encountered in

gestation. Further analysis of mid-gestation nutrient restriction in beef cattle is needed to fully understand the effects it can have on the offspring.

CONCLUSION

No changes were observed in phenotype (i.e. growth or carcass), and accordingly no changes were seen in MHC or mRNA expression. Surprisingly, significant differences were observed in miRNA expression within the skeletal muscle. However, the function of these miRNA in postnatal skeletal muscle growth is currently unknown. As such, more research is needed to determine not only the role of these miRNA in postnatal skeletal muscle function, but also how they may relate to a decreased plane of nutrition during the second trimester of gestation.

IMPLICATIONS

Gaining a better understanding of how fetal programing effects offspring development in cattle will help to broaden this area of research. Currently there is not a lot of information on how fetal programming during the second trimester of gestation in cattle is effecting offspring development throughout life. Research varies by time of restriction, severity of restriction, and species. More documentation about fetal programming will lead to more areas of research.

Furthering the understanding of fetal events, growth, and development may impact recommendations for livestock management. By understanding how nutrient restriction during gestation effects the performance of offspring, producers could implement production practices that allow them to reach the most economic gain.

LIMITATIONS

As all studies do, this study had some limitations and areas that could have been improved. One thing that could have been performed differently is that the dams were group housed and fed. Individual feed intake data for the dams could have given us more insight to the severity of the nutrient restriction during gestation for each individual animal.

Another thing that could have had effects on the study is that the offspring were a mixed group of both male and female cattle. It has been well established that heifers and steers have a very different hormone profile, which has an impact on overall physiology of the animals. While it is not uncommon for heifers to end up in a feedlot, it is more likely that there is a higher percentage of steers entering a feedlot program. If there had been enough animals of each sex born in each treatment group, we could have divided them up and analyzed how the females performed as replacement heifers for another generation, and how the males performed in the feedlot setting. Separating the sexes and analyzing them that way may be more accurate to what would occur in normal production practices and take the unknowns of the different hormonal profiles out of the equation.

Skeletal muscle biopsies were collected from different muscles at the three different time points. Collecting muscle biopsies from the same muscle consistently through the study would have allowed for the time points to all be compared. Being able to compare the time points would have allowed for collection of data that looked at how the animals' expression of miRNA, mRNA, and MHC isoforms changed over time. Along with collecting biopsies from the same muscle, if muscle biopsies were collected at birth as well there would be more accurate representation of the effects of the maternal

nutrient restriction on the offspring without the effects of the environment. This would also allow for a better analysis on how expression of miRNA, mRNA, and MHC isoforms changed over time.

Muscle fiber type was analyzed by looking at the expression of different MHC isoforms. Performing histochemical staining would allow for a more accurate assessment of muscle fiber type. With histochemical staining, fiber type proportion and size could be analyzed as well. Similarly to most studies, if this study was performed again, there are ways it could be improved.

REFERENCES

Aguiari, P., Leo, S., Zavan, B., Vindigni, V., Rimessi, A., Bianchi, K., Franzin, C., Cortivo, R., Rossato, M., Vettor, R., Abatangelo, G., Pozzan, T., Pinton, P., Rizzuto, R., 2008. High glucose induces adipogenic differentiation of muscle-derived stem cells. Proc. Natl. Acad. Sci. U.S.A. 105, 1226–1231. https://doi.org/10.1073/pnas.0711402105

Ailhaud, G., 2006. Adipose tissue as a secretory organ: from adipogenesis to the metabolic syndrome. Comptes Rendus Biologies 329, 570–577. https://doi.org/10.1016/j.crvi.2005.12.012

Anderson, C., Catoe, H., Werner, R., 2006. MIR-206 regulates connexin43 expression during skeletal muscle development. Nucleic Acids Research 34, 5863–5871. https://doi.org/10.1093/nar/gkl743

Barker, D., Eriksson, J., Forsén, T., Osmond, C., 2002. Fetal origins of adult disease: strength of effects and biological basis. International Journal of Epidemiology 31, 1235–1239. https://doi.org/10.1093/ije/31.6.1235

Barker, D.J., Clark, P.M., 1997. Fetal undernutrition and disease in later life. Rev. Reprod. 2, 105–112.

Bartel, D.P., 2009. MicroRNAs: Target Recognition and Regulatory Functions. Cell 136, 215–233. https://doi.org/10.1016/j.cell.2009.01.002

Bass, P.D., n.d. The scientific basis of the Certified Angus Beef® Brand Carcass Specifications. [WWW Document]. URL https://www.cabpartners.com/articles/news/3027/Scientific_bases_of_CAB_carcass_specs-Phil_Bass.pdf

Bispham, J., Gardner, D.S., Gnanalingham, M.G., Stephenson, T., Symonds, M.E., Budge, H., 2005. Maternal Nutritional Programming of Fetal Adipose Tissue Development: Differential Effects on Messenger Ribonucleic Acid Abundance for Uncoupling Proteins and Peroxisome Proliferator-Activated and Prolactin Receptors. Endocrinology 146, 3943–3949. https://doi.org/10.1210/en.2005-0246

Bonham, C.D., 2013. Measurements for terrestrial vegetation, Second Edition. ed. Wiley-Blackwell, Chichester, West Sussex ; Hoboken, NJ.

Brandstetter, A.M., Picard, B., Geay, Y., 1998. Muscle fibre characteristics in four muscles of growing bulls. Livestock Production Science 53, 15–23. https://doi.org/10.1016/S0301-6226(97)00149-8

Bruns, K.W., Pritchard, R.H., 2003. Controlling variation in feed intake through bunk management1. Journal of Animal Science 81, E133–E138. https://doi.org/10.2527/2003.8114_suppl_2E133x

Canani, R.B., Di Costanzo, M., Leone, L., Bedogni, G., Brambilla, P., Cianfarani, S., Nobili, V., Pietrobelli, A., Agostoni, C., 2011. Epigenetic mechanisms elicited by nutrition in early life. Nutrition Research Reviews 24, 198–205. https://doi.org/10.1017/S0954422411000102

Catalanotto, C., Cogoni, C., Zardo, G., 2016. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. International Journal of Molecular Sciences 17, 1712. https://doi.org/10.3390/ijms17101712

Cesana, M., Cacchiarelli, D., Legnini, I., Santini, T., Sthandier, O., Chinappi, M., Tramontano, A., Bozzoni, I., 2011. A Long Noncoding RNA Controls Muscle Differentiation by

Functioning as a Competing Endogenous RNA. Cell 147, 358–369. https://doi.org/10.1016/j.cell.2011.09.028

Chen, J.-F., Mandel, E.M., Thomson, J.M., Wu, Q., Callis, T.E., Hammond, S.M., Conlon, F.L., Wang, D.-Z., 2006. The role of microRNA-1 and microRNA-133 in skeletal muscle proliferation and differentiation. Nature Genetics 38, 228–233. https://doi.org/10.1038/ng1725

Chikuni, K., Muroya, S., Nakajima, I., 2004. Absence of the Functional Myosin Heavy Chain 2b Isoform in Equine Skeletal Muscles. Zoological Science 21, 589–596. https://doi.org/10.2108/zsj.21.589

Choi, Y.M., Kim, B.C., 2009. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. Livestock Science 122, 105–118. https://doi.org/10.1016/j.livsci.2008.08.015

Cooper, G.M., 2000. The cell: a molecular approach, 2. ed. ed. ASM Press [u.a.], Washington, DC.

Council, N.R., 2000. Nutrient Requirements of Beef Cattle: Seventh Revised Edition: Update 2000. The National Academies Press, Washington, DC. https://doi.org/10.17226/9791

Crist, C.G., Montarras, D., Pallafacchina, G., Rocancourt, D., Cumano, A., Conway, S.J., Buckingham, M., 2009. Muscle stem cell behavior is modified by microRNA-27 regulation of Pax3 expression. Proceedings of the National Academy of Sciences 106, 13383–13387. https://doi.org/10.1073/pnas.0900210106

D. Hale, n.d. USDA beef quality and yield grades [WWW Document]. URL Department of Animal Science Texas A&M AgriLife Extension Service College Station TX 77843, 2471

Dauncey, M.J., Harrison, A.P., 1996. Developmental regulation of cation pumps in skeletal and cardiac muscle. Acta Physiologica Scandinavica 156, 313–323. https://doi.org/10.1046/j.1365-201X.1996.188000.x

Davis, R.L., Weintraub, H., Lassar, A.B., 1987. Expression of a single transfected cDNA converts fibroblasts to myoblasts. Cell 51, 987–1000. https://doi.org/10.1016/0092-8674(87)90585-X

Dey, B.K., Gagan, J., Dutta, A., 2011. miR-206 and -486 Induce Myoblast Differentiation by Downregulating Pax7. Molecular and Cellular Biology 31, 203–214. https://doi.org/10.1128/MCB.01009-10

Du, M., Tong, J., Zhao, J., Underwood, K.R., Zhu, M., Ford, S.P., Nathanielsz, P.W., 2010. Fetal programming of skeletal muscle development in ruminant animals1. Journal of Animal Science 88, E51–E60. https://doi.org/10.2527/jas.2009-2311

Du, Min, Yan, X., Tong, J.F., Zhao, J., Zhu, M.J., 2010a. Maternal Obesity, Inflammation, and Fetal Skeletal Muscle Development1. Biology of Reproduction 82, 4–12. https://doi.org/10.1095/biolreprod.109.077099

Du, Min, Yin, J., Zhu, M.J., 2010b. Cellular signaling pathways regulating the initial stage of adipogenesis and marbling of skeletal muscle. Meat Science 86, 103–109. https://doi.org/10.1016/j.meatsci.2010.04.027

Dwyer, C.M., Stickland, N.C., Fletcher, J.M., 1994. The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. J. Anim. Sci. 72, 911–917.

Edwards, L.J., McFarlane, J.R., Kauter, K.G., McMillen, I.C., 2005. Impact of periconceptional nutrition on maternal and fetal leptin and fetal adiposity in singleton and

twin pregnancies. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 288, R39–R45. https://doi.org/10.1152/ajpregu.00127.2004

Fabian, M.R., Sonenberg, N., Filipowicz, W., 2010. Regulation of mRNA Translation and Stability by microRNAs. Annual Review of Biochemistry 79, 351–379. https://doi.org/10.1146/annurev-biochem-060308-103103

Fahey, A.J., Brameld, J.M., Parr, T., Buttery, P.J., 2005. The effect of maternal undernutrition before muscle differentiation on the muscle fiber development of the newborn lamb1,2. Journal of Animal Science 83, 2564–2571. https://doi.org/10.2527/2005.83112564x

Feng, Y., Cao, J.-H., Li, X.-Y., Zhao, S.-H., 2011. Inhibition of miR-214 expression represses proliferation and differentiation of C2C12 myoblasts: PROLIFERATION OF C2C12 MYOBLASTS. Cell Biochemistry and Function 29, 378–383. https://doi.org/10.1002/cbf.1760

Ford, S.P., Hess, B.W., Schwope, M.M., Nijland, M.J., Gilbert, J.S., Vonnahme, K.A., Means, W.J., Han, H., Nathanielsz, P.W., 2007. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring1. Journal of Animal Science 85, 1285–1294. https://doi.org/10.2527/jas.2005-624

Freetly, H.C., Ferrell, C.L., Jenkins, T.G., 2000. Timing of realimentation of mature cows that were feed-restricted during pregnancy influences calf birth weights and growth rates. Journal of Animal Science 78, 2790. https://doi.org/10.2527/2000.78112790x

Funston, R.N., Larson, D.M., Vonnahme, K.A., 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production1. Journal of Animal Science 88, E205–E215. https://doi.org/10.2527/jas.2009-2351

Funston, R.N., Summers, A.F., 2013. Epigenetics: Setting Up Lifetime Production of Beef Cows by Managing Nutrition. Annual Review of Animal Biosciences 1, 339–363. https://doi.org/10.1146/annurev-animal-031412-103649

Gardner, J., 2017. Effects of Gestational Dietary Intake on Calf Growth and Early Feedlot Performance of Offspring (Masters Thesis). Utah State University.

Godfrey, K.M., Barker, D.J., 2000. Fetal nutrition and adult disease. The American Journal of Clinical Nutrition 71, 1344S-1352S. https://doi.org/10.1093/ajcn/71.5.1344s

Gonzalez, J.M., Camacho, L.E., Ebarb, S.M., Swanson, K.C., Vonnahme, K.A., Stelzleni, A.M., Johnson, S.E., 2013. Realimentation of nutrient restricted pregnant beef cows supports compensatory fetal muscle growth1. Journal of Animal Science 91, 4797–4806. https://doi.org/10.2527/jas.2013-6704

Greenwood, P.L., Cafe, L., Hearnshaw, H., Hennessy, D., 2005. Consequences of nutrition and growth retardation early in life for growth and composition of cattle and eating quality of beef. Recent Advances in Animal Nutrition in Australia 15, 183–195.

Greenwood, P.L., Cafe, L.M., Hearnshaw, H., Hennessy, D.W., Morris, S.G., 2009. Consequences of prenatal and preweaning growth for yield of beef primal cuts from 30-month-old Piedmontese- and Wagyu-sired cattle. Anim. Prod. Sci. 49, 468. https://doi.org/10.1071/EA08160

Greenwood, P.L., Slepetis, R.M., Bell, A.W., Hermanson, J.W., 1999. Intrauterine growth retardation is associated with reduced cell cycle activity, but not myofibre number, in ovine fetal muscle. Reproduction, Fertility and Development 11, 281. https://doi.org/10.1071/RD99054

Greiner, S.P., Rouse, G.H., Wilson, D.E., Cundiff, L.V., Wheeler, T.L., 2003. The relationship between ultrasound measurements and carcass fat thickness and longissimus muscle area in beef cattle. Journal of Animal Science 81, 676–682. https://doi.org/10.2527/2003.813676x

Güller, I., Russell, A.P., 2010. MicroRNAs in skeletal muscle: their role and regulation in development, disease and function: MicroRNAs in skeletal muscle. The Journal of Physiology 588, 4075–4087. https://doi.org/10.1113/jphysiol.2010.194175

Handel, S.E., Stickland, N.C., 1987. The growth and differentiation of porcine skeletal muscle fibre types and the influence of birthweight. J. Anat. 152, 107–119.

Hausman, G.J., Dodson, M.V., Ajuwon, K., Azain, M., Barnes, K.M., Guan, L.L., Jiang, Z., Poulos, S.P., Sainz, R.D., Smith, S., Spurlock, M., Novakofski, J., Fernyhough, M.E., Bergen, W.G., 2009. BOARD-INVITED REVIEW: The biology and regulation of preadipocytes and adipocytes in meat animals1,2. Journal of Animal Science 87, 1218–1246. https://doi.org/10.2527/jas.2008-1427

Huang, M.-B., Xu, H., Xie, S.-J., Zhou, H., Qu, L.-H., 2011. Insulin-Like Growth Factor-1 Receptor Is Regulated by microRNA-133 during Skeletal Myogenesis. PLoS ONE 6, e29173. https://doi.org/10.1371/journal.pone.0029173

Huang, Y.-Z., Sun, J.-J., Zhang, L.-Z., Li, C.-J., Womack, J.E., Li, Z.-J., Lan, X.-Y., Lei, C.-Z., Zhang, C.-L., Zhao, X., Chen, H., 2014. Genome-wide DNA Methylation Profiles and Their Relationships with mRNA and the microRNA Transcriptome in Bovine Muscle Tissue (Bos taurine). Scientific Reports 4, 6546.

Jenkinson, C.M.C., Earl, A.K., Kenyon, P.R., Blair, H.T., 2012. Effects of maternal nutrition during pregnancy on fetal growth and maternal constraint in sheep. Anim. Prod. Sci. 52, 524. https://doi.org/10.1071/AN11245

Jennings, T.D., Gonda, M.G., Underwood, K.R., Wertz-Lutz, A.E., Blair, A.D., 2016. The influence of maternal nutrition on expression of genes responsible for adipogenesis and myogenesis in the bovine fetus. animal 10, 1697–1705. https://doi.org/10.1017/S1751731116000665

Jin, W., Dodson, M.V., Moore, S.S., Basarab, J.A., Guan, L.L., 2010. Characterization of microRNA expression in bovine adipose tissues: a potential regulatory mechanism of subcutaneous adipose tissue development. BMC Molecular Biology 11, 29. https://doi.org/10.1186/1471-2199-11-29

Johnson, M.L., Rajamannan, N., 2007. Diseases of Wnt signaling. Reviews in Endocrine and Metabolic Disorders 7, 41–49. https://doi.org/10.1007/s11154-006-9003-3

Juan, A.H., Kumar, R.M., Marx, J.G., Young, R.A., Sartorelli, V., 2009. Mir-214-Dependent Regulation of the Polycomb Protein Ezh2 in Skeletal Muscle and Embryonic Stem Cells. Molecular Cell 36, 61–74. https://doi.org/10.1016/j.molcel.2009.08.008

Katanaev, V.L., Ponzielli, R., Sémériva, M., Tomlinson, A., 2005. Trimeric G Protein-Dependent Frizzled Signaling in Drosophila. Cell 120, 111–122. https://doi.org/10.1016/j.cell.2004.11.014

Kato, Y., Miyaki, S., Yokoyama, S., Omori, S., Inoue, A., Horiuchi, M., Asahara, H., 2009. Real-time functional imaging for monitoring miR-133 during myogenic differentiation. The International Journal of Biochemistry & Cell Biology 41, 2225–2231. https://doi.org/10.1016/j.biocel.2009.04.018

Kim, H.K., Lee, Y.S., Sivaprasad, U., Malhotra, A., Dutta, A., 2006. Muscle-specific microRNA miR-206 promotes muscle differentiation. The Journal of Cell Biology 174, 677–687. https://doi.org/10.1083/jcb.200603008

Kim, V.N., Han, J., Siomi, M.C., 2009. Biogenesis of small RNAs in animals. Nature Reviews Molecular Cell Biology 10, 126.

Kirchofer, K.S., Calkins, C.B., Gwartney, B.L., 2002. Fiber-type composition of muscles of the beef chuck and round. J. Anim. Sci. 80, 2872–2878.

Kollias, H.D., McDermott, J.C., 2008. Transforming growth factor-beta and myostatin signaling in skeletal muscle. J. Appl. Physiol. 104, 579–587. https://doi.org/10.1152/japplphysiol.01091.2007

Kuang, S., Gillespie, M.A., Rudnicki, M.A., 2008. Niche regulation of muscle satellite cell self-renewal and differentiation. Cell Stem Cell 2, 22–31. https://doi.org/10.1016/j.stem.2007.12.012

Kumar, M.S., Lu, J., Mercer, K.L., Golub, T.R., Jacks, T., 2007. Impaired microRNA processing enhances cellular transformation and tumorigenesis. Nature Genetics 39, 673–677. https://doi.org/10.1038/ng2003

Levitsky, D.I., 2004. Actomyosin systems of biological motility. Biochemistry (Moscow) 69, 1177–1189. https://doi.org/10.1007/s10541-005-0063-x

Lie, S., Morrison, J.L., Williams-Wyss, O., Suter, C.M., Humphreys, D.T., Ozanne, S.E., Zhang, S., MacLaughlin, S.M., Kleemann, D.O., Walker, S.K., Roberts, C.T., McMillen, I.C., 2014. Periconceptional Undernutrition Programs Changes in Insulin-Signaling Molecules and MicroRNAs in Skeletal Muscle in Singleton and Twin Fetal Sheep1. Biology of Reproduction 90. https://doi.org/10.1095/biolreprod.113.109751

Lin, Q., Gao, Z., Alarcon, R.M., Ye, J., Yun, Z., 2009. A role of miR-27 in the regulation of adipogenesis. FEBS J. 276, 2348–2358.

Liu, N., Williams, A.H., Kim, Y., McAnally, J., Bezprozvannaya, S., Sutherland, L.B., Richardson, J.A., Bassel-Duby, R., Olson, E.N., 2007. An intragenic MEF2-dependent enhancer directs muscle-specific expression of microRNAs 1 and 133. Proceedings of the National Academy of Sciences 104, 20844–20849. https://doi.org/10.1073/pnas.0710558105

Long, N.M., Vonnahme, K.A., Hess, B.W., Nathanielsz, P.W., Ford, S.P., 2009. Effects of early gestational undernutrition on fetal growth, organ development, and placentomal composition in the bovine1. Journal of Animal Science 87, 1950–1959. https://doi.org/10.2527/jas.2008-1672

Lumey, L., Stein, A.D., Kahn, H.S., van der Pal-de Bruin, K.M., Blauw, G., Zybert, P.A., Susser, E.S., 2007. Cohort Profile: The Dutch Hunger Winter Families Study. International Journal of Epidemiology 36, 1196–1204. https://doi.org/10.1093/ije/dym126

Luo, W., Nie, Q., Xiquan Zhang, 2013. MicroRNAs Involved in Skeletal Muscle Differentiation. Journal of Genetics and Genomics 40, 107–116. https://doi.org/10.1016/j.jgg.2013.02.002

McDaneld, T.G., 2009. MicroRNA: Mechanism of gene regulation and application to livestock1. Journal of Animal Science 87, E21–E28. https://doi.org/10.2527/jas.2008-1303

Miller, M.A., Zachary, J.F., 2017. Mechanisms and Morphology of Cellular Injury, Adaptation, and Death11For a glossary of abbreviations and terms used in this chapter

see E-Glossary 1-1., in: Pathologic Basis of Veterinary Disease. Elsevier, pp. 2-43.e19. https://doi.org/10.1016/B978-0-323-35775-3.00001-1

Mishima, Y., Abreu-Goodger, C., Staton, A.A., Stahlhut, C., Shou, C., Cheng, C., Gerstein, M., Enright, A.J., Giraldez, A.J., 2009. Zebrafish miR-1 and miR-133 shape muscle gene expression and regulate sarcomeric actin organization. Genes & Development 23, 619–632. https://doi.org/10.1101/gad.1760209

Mohrhauser, D.A., Taylor, A.R., Gonda, M.G., Underwood, K.R., Pritchard, R.H., Wertz-Lutz, A.E., Blair, A.D., 2015. The influence of maternal energy status during mid-gestation on beef offspring tenderness, muscle characteristics, and gene expression. Meat Science 110, 201–211. https://doi.org/10.1016/j.meatsci.2015.07.017

Mohrhauser, D. A., Taylor, A.R., Underwood, K.R., Pritchard, R.H., Wertz-Lutz, A.E., Blair, A.D., 2015. The influence of maternal energy status during midgestation on beef offspring carcass characteristics and meat quality1. Journal of Animal Science 93, 786–793. https://doi.org/10.2527/jas.2014-8567

Montowska, M., Pospiech, E., 2011. Differences in two-dimensional gel electrophoresis patterns of skeletal muscle myosin light chain isoforms between Bos taurus, Sus scrofa and selected poultry species. Journal of the Science of Food and Agriculture 91, 2449–2456. https://doi.org/10.1002/jsfa.4486

Nakajima, N., Takahashi, T., Kitamura, R., Isodono, K., Asada, S., Ueyama, T., Matsubara, H., Oh, H., 2006. MicroRNA-1 facilitates skeletal myogenic differentiation without affecting osteoblastic and adipogenic differentiation. Biochemical and Biophysical Research Communications 350, 1006–1012. https://doi.org/10.1016/j.bbrc.2006.09.153

Nathanielsz, P.W., Poston, L., Taylor, P.D., 2007. In utero exposure to maternal obesity and diabetes: animal models that identify and characterize implications for future health. Clin Perinatol 34, 515–526, v. https://doi.org/10.1016/j.clp.2007.09.005

Picard, B., Barboiron, C., Duris, M.P., Gagniére, H., Jurie, C., Geay, Y., 1999. Electrophoretic separation of bovine muscle myosin heavy chain isoforms. Meat Science 53, 1–7. https://doi.org/10.1016/S0309-1740(99)00021-2

Picard, B., Duris, M.P., Jurie, C., 1998. Classification of bovine muscle fibres by different histochemical techniques. Histochem. J. 30, 473–479.

Platter, W.J., Tatum, J.D., Belk, K.E., Chapman, P.L., Scanga, J.A., Smith, G.C., 2003. Relationships of consumer sensory ratings, marbling score, and shear force value to consumer acceptance of beef strip loin steaks. Journal of Animal Science 81, 2741–2750. https://doi.org/10.2527/2003.81112741x

Polesskaya, A., Seale, P., Rudnicki, M.A., 2003. Wnt signaling induces the myogenic specification of resident CD45+ adult stem cells during muscle regeneration. Cell 113, 841–852.

Quarnberg, S., 2019. Effects of Mid-gestation Nutrient Restriction on Carcass Measurments and meat Quality of Resultant Offspring (Masters Thesis). Utah State University.

Robelin, J., Lacourt, A., Béchet, D., Ferrara, M., Briand, Y., Geay, Y., 1991. Muscle differentiation in the bovine fetus: a histological and histochemical approach. Growth Dev Aging 55, 151–160.

Sarkar, S., Dey, B.K., Dutta, A., 2010. MiR-322/424 and -503 Are Induced during Muscle Differentiation and Promote Cell Cycle Quiescence and Differentiation by Down-

Regulation of Cdc25A. Molecular Biology of the Cell 21, 2138–2149. https://doi.org/10.1091/mbc.e10-01-0062

Sciote, J.J., Morris, T.J., 2000. Skeletal Muscle Function and Fibre Types: the Relationship Between Occlusal Function and the Phenotype of Jaw-closing Muscles in Human. Journal of Orthodontics 27, 15–30. https://doi.org/10.1093/ortho/27.1.15

Shen, H., 2006. The Notch coactivator, MAML1, functions as a novel coactivator for MEF2C-mediated transcription and is required for normal myogenesis. Genes & Development 20, 675–688. https://doi.org/10.1101/gad.1383706

Staff, N.R.C., 1984. Nutrient Requirements of Sheep: Sixth Revised Edition, 1985. National Academies Press, Washington.

Swift, R.W., 1957. The Caloric Value of TDN. Journal of Animal Science 16, 753–756. https://doi.org/10.2527/jas1957.164753x

Taylor, A.R., Mohrhauser, D.A., Pritchard, R.H., Underwood, K.R., Wertz-Lutz, A.E., Blair, A.D., 2016. The influence of maternal energy status during mid-gestation on growth, cattle performance, and the immune response in the resultant beef progeny 1 1This research was funded in part by a grant from the Agriculture and Food Research Initiative Competitive Grant number 2010-65206-20667 from the USDA National Institute of Food and Agriculture and the South Dakota Beef Industry Council. Salaries and research support were provided by state and federal funds appropriated to South Dakota State University. The Professional Animal Scientist 32, 389–399. https://doi.org/10.15232/pas.2015-01469

Thornton, K.J., Chapalamadugu, K.C., Eldredge, E.M., Murdoch, G.K., 2017. Analysis of *Longissimus thoracis* Protein Expression Associated with Variation in Carcass Quality

Grade and Marbling of Beef Cattle Raised in the Pacific Northwestern United States. Journal of Agricultural and Food Chemistry 65, 1434–1442. https://doi.org/10.1021/acs.jafc.6b02795

Thornton, K.J., Welch, C.M., Davis, L.C., Doumit, M.E., Hill, R.A., Murdoch, G.K., 2012. Bovine sire selection based on maintenance energy affects muscle fiber type and meat color of F1 progeny1. Journal of Animal Science 90, 1617–1627. https://doi.org/10.2527/jas.2011-4678

Underwood, K.R., Tong, J.F., Price, P.L., Roberts, A.J., Grings, E.E., Hess, B.W., Means, W.J., Du, M., 2010. Nutrition during mid to late gestation affects growth, adipose tissue deposition, and tenderness in cross-bred beef steers. Meat Science 86, 588–593. https://doi.org/10.1016/j.meatsci.2010.04.008

USDA, 1997. United States standards for grades of carcass beef. [WWW Document]. Livestock and Seed Program. URL https://www.ams.usda.gov/rules-regulations/united-states-standards-grades-carcass-beef-0

Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for Dietary Fiber, Neutral Detergent Fiber, and Nonstarch Polysaccharides in Relation to Animal Nutrition. Journal of Dairy Science 74, 3583–3597. https://doi.org/10.3168/jds.S0022-0302(91)78551-2

Ward, S.S., Stickland, N.C., 1991. Why are slow and fast muscles differentially affected during prenatal undernutrition? Muscle & Nerve 14, 259–267. https://doi.org/10.1002/mus.880140310

Weiss, W.P., Conrad, H.R., St. Pierre, N.R., 1992. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Animal Feed Science and Technology 39, 95–110. https://doi.org/10.1016/0377-8401(92)90034-4

Yablonka-Reuveni, Z., Day, K., Vine, A., Shefer, G., 2008. Defining the transcriptional signature of skeletal muscle stem cells1,2. Journal of Animal Science 86, E207–E216. https://doi.org/10.2527/jas.2007-0473

Yamamoto, M., Kuroiwa, A., 2003. Hoxa-11 and Hoxa-13 are involved in repression of MyoD during limb muscle development. Dev. Growth Differ. 45, 485–498.

Yan, X., Zhu, M.-J., Dodson, M.V., Du, M., 2013. Developmental Programming of Fetal Skeletal Muscle and Adipose Tissue Development. Journal of Genomics 1, 29–38. https://doi.org/10.7150/jgen.3930

Zhu, M.J., Ford, S.P., Means, W.J., Hess, B.W., Nathanielsz, P.W., Du, M., 2006. Maternal nutrient restriction affects properties of skeletal muscle in offspring: Nutrient restriction and offspring muscle. The Journal of Physiology 575, 241–250. https://doi.org/10.1113/jphysiol.2006.112110

Zhu, M.-J., Ford, S.P., Nathanielsz, P.W., Du, M., 2004. Effect of Maternal Nutrient Restriction in Sheep on the Development of Fetal Skeletal Muscle1. Biology of Reproduction 71, 1968–1973. https://doi.org/10.1095/biolreprod.104.034561

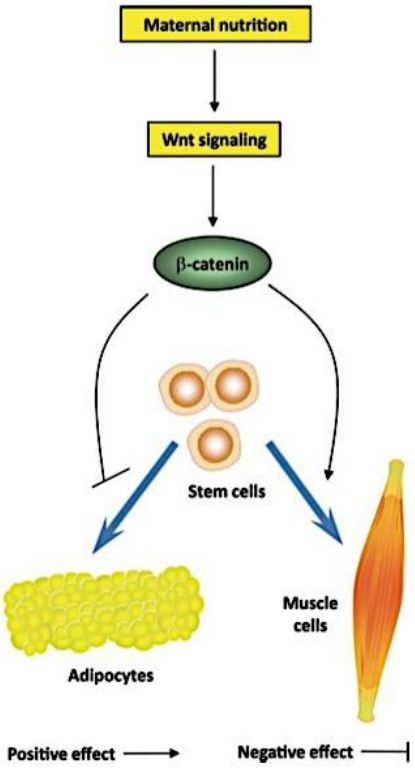

Figure 1. Wnt signaling pathway adapted from Du et al., 2010.

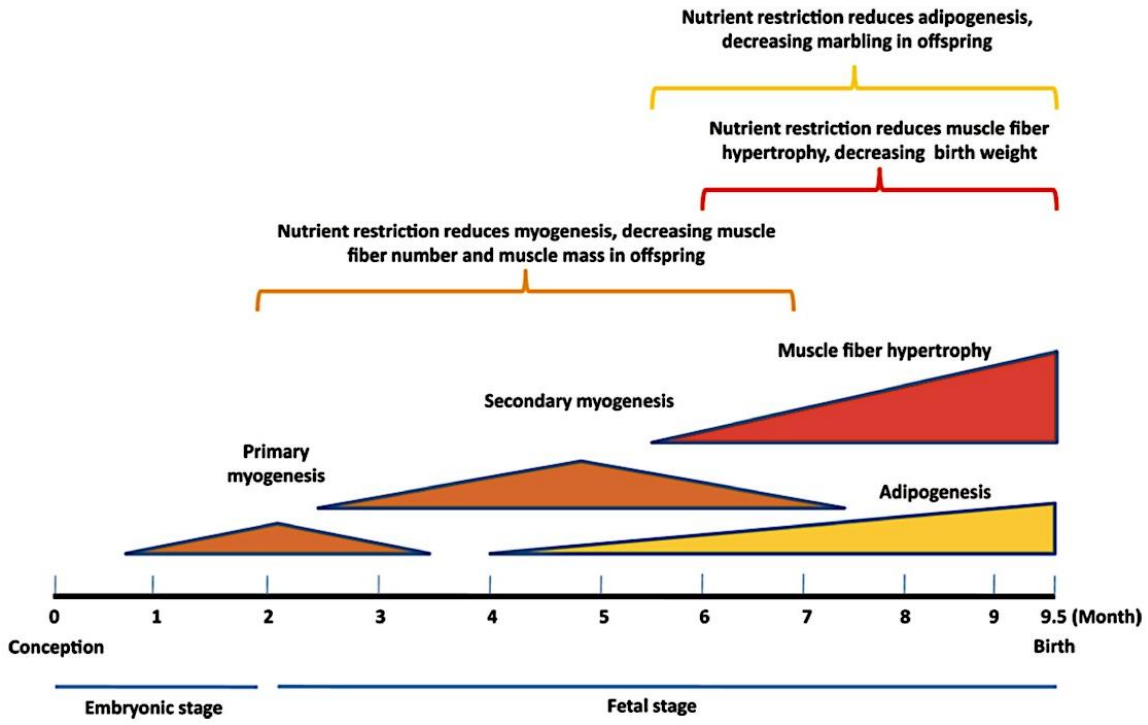

Figure 2. Phases of adipogenesis and myogenesis. Adapted from Du et al. 2010.

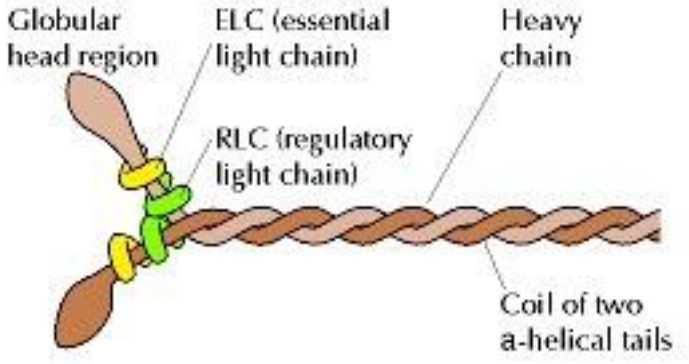

Figure 3. Structure of myosin heavy chains adapted from Cooper, 2000.

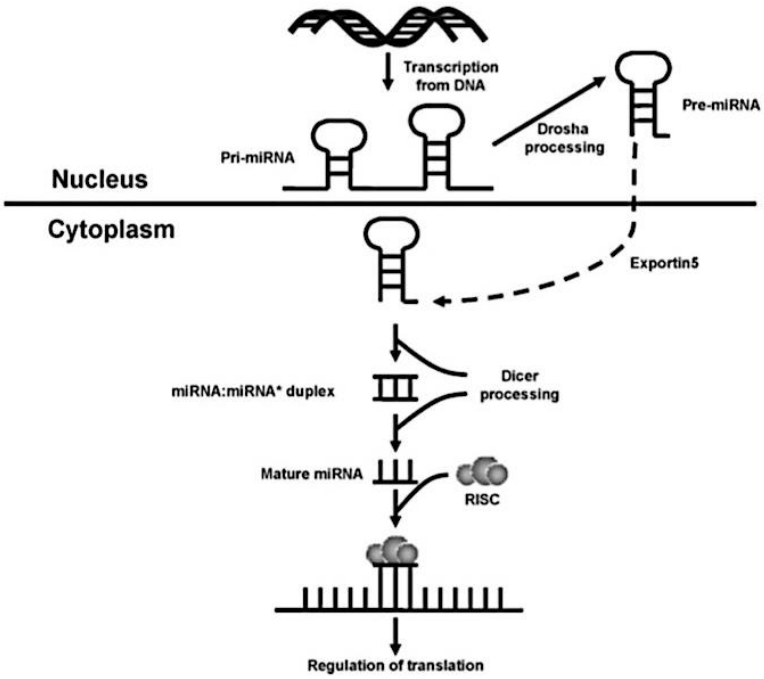

Figure 4. Biogenesis of miRNA adapted from McDaneld, 2009.

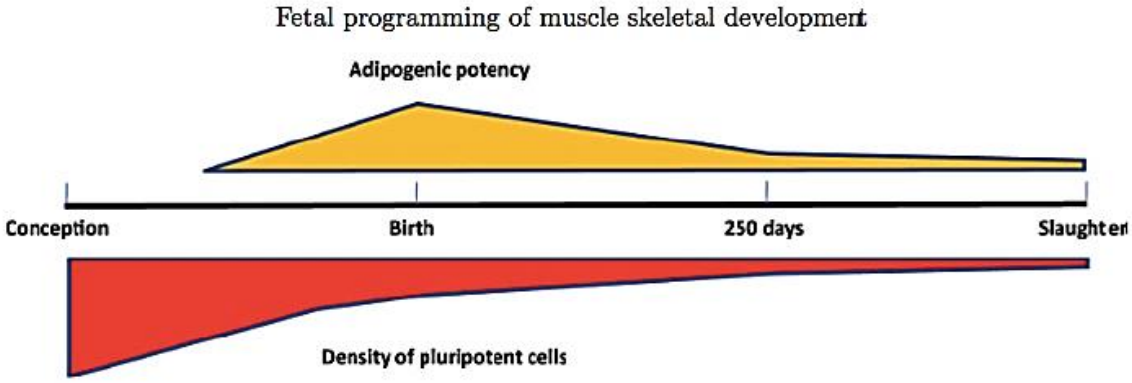

Figure 5. The fetal programming of muscle skeletal development adapted from Du et al., 2010.

Table 1. Birth and Weaning Weights of Calves

	Treatment		SEM	P-value
	Maintenance[1]	Restricted[2]		
Birth weight, kg	40.8	40.76	2.1369	0.99
Weaning weight, kg	242.1	228.01	8.664	0.25

Data adapted from Gardner, 2017.

[1]Treatment consisted of calves (n = 15) that were born from cows that did not receive a nutritional insult during the second trimester.

[2]Treatment consisted of calves (n = 17) that were born from cows that experienced a nutritional restriction during the second trimester.

Table 2. Intake, ADG, and Feed Efficiency of Offspring During the Feedlot Phase

	Treatment[1]		SEM	P-value
	Maintenance	Restricted		
Average DMI[2]				
Days 0-28	8.28	8.54	0.51	0.46
Days 29-56	9.90	10.34	0.71	0.50
Days 57-84	10.52	10.69	0.69	0.75
Days 85-112	10.89	10.84	0.69	0.92
Days 113-140	10.34	10.16	0.34	0.68
Days 141-168	11.95	12.01	0.57	0.88
Days 169-196	11.05	10.73	0.38	0.43
Days 0-196	10.39	10.50	0.49	0.78
Average daily gain[3]				
Days 0-28	1.23	1.43	0.19	0.13
Days 29-56	0.97	0.95	0.07	0.76
Days 57-84	1.33	1.29	0.08	0.72
Days 85-112	1.24	1.16	0.09	0.40
Days 113-140	1.58	1.55	0.18	0.83
Days 141-168	0.56	0.58	0.03	0.41
Days 169-196	0.91	0.62	0.45	0.38
Days 0-196	1.11	1.09	0.04	0.71
Average Gain:Feed				
Days 0-28	0.112	0.132	0.013	0.09
Days 29-56	0.094	0.097	0.010	0.79
Days 57-84	0.110	0.109	0.006	0.83
Days 85-112	0.114	0.108	0.007	0.53
Days 113-140	0.156	0.151	0.015	0.75
Days 141-168	0.046	0.049	0.001	0.19
Days 169-196	0.080	0.067	0.041	0.68
Days 0-196	0.102	0.102	0.007	0.99

Data adapted from Gardner, 2017 and Quarnberg, 2019.

[1] Maintenance treatment consisted of cows (n = 16) that did not have a nutritional insult during the second trimester while cows (n = 18) from the restricted treatment had a nutritional restriction.

[2]amount of DMI in kg

[3]average daily gain in kg

Table 3. LS means of carcass measurements of calves from maintenance and restricted cows.

	Treatment[1]		
	Maintenance	Restricted	P-value[2]
Hot carcass weight (kg)	324.64 ± 9.33	313.66 ± 9.23	0.15
Loin weight (kg)	5.56 ± 0.30	5.29 ± 0.30	0.38
Kidney, pelvic, and heart fat (%)	2.47 ± 0.30	2.58 ± 0.30	0.49
Ribeye area (cm^2)	73.86 ± 3.38	73.48 ± 3.36	0.86
USDA Yield Grade	3.08 ± 0.24	2.82 ± 0.23	0.16
Adjusted 12th rib backfat (cm)	7.78 ± 0.42	7.10 ± 0.40	0.18
Marbling Score[3]	533.38 ± 25.18	560.56 ± 23.74	0.44
Marbling to backfat Ratio[4]	-0.36 ± 0.34	0.34 ± 0.32	0.10

Data adapted from Quarnberg, 2019.

[1]Maintenance cows' calves (n = 16), nutrient restricted cows' calves (n = 18)

[2]Probability value of the F-test for treatment effect

[3]Marbling score = 9 levels of marbling category (devoid-abundant) with 100 degrees of variation (0-99) within levels

[4]Marbling to backfat ratio was determined using the calculations previously described by Mohrhauser et al., 2015a. [(observation marbling score- marbling score $\bar{x}$)/marbling SD]- [(observation backfat-backfat $\bar{x}$)/backfat SD]

Table 4. Hypothesized functions of miRNA

miRNA	**Function**	**Predicted Targets**
miR-1	Promotes myogenic differentiation	*HDAC4*
miR-27b	Regulates adipogenesis	*Pax3*
miR-133a	Promotes proliferation/differentiation of myoblasts	*MAML1, IGF-1R*
miR-133b	Promotes proliferation/differentiation of myoblasts	*MAML1,IGF-1R*
miR-181d	Important in skeletal muscle development	*Hox-A11*
miR-206	Promotes myogenic differentiation	*Pax3, Pax7*
miR-214	Promotes proliferation/differentiation of myoblasts	*Ezh2*
miR-424	Involved in skeletal muscle differentiation	*Cdc25A*
miR-486	Promotes growth of skeletal muscle	*Pax7*

Table 5. Body weight and BCS of cows during gestation

	Treatment		SEM	P-value
	Maintenance[1]	Restricted[1]		
Initial Weight[2], kg	531.81	526.36	20.71	0.85
End Weight[2], kg	552.27	462.81	20.88	0.04
BCS[3], start of second trimester	5.50	5.39	0.27	0.72
BCS[3], end of second trimester	5.71	4.64	0.28	0.009
BCS[3], end of third trimester	5.40	5.08	0.26	0.78

Data adapted from Gardner, 2017.

[1] Maintenance treatment consisted of cows (n = 15) that did not have a nutritional insult during the second trimester while cows (n = 17) from the restricted treatment had a nutritional restriction.

[2]Initial values were taken at the beginning of the second trimester and end values at the end of the second trimester

[3]Body condition score, BCS

Table 6. Nutrient analysis and yields of cow pasture

Item	Maintenance Pasture[1]		Restricted Pasture[2]	
	As-fed Basis	Dry Matter Basis	As-fed Basis	Dry Matter Basis
Moisture, %	43.09	---	39.72	---
Dry matter, %	56.91	100.00	60.28	100.00
Crude protein, %	6.21	10.91	8.70	14.43
Acid detergent fiber, %	23.77	41.76	18.55	30.78
Neutral detergent fiber, %	36.30	63.80	29.25	48.52
Total digestible nutrients, %	31.52	55.38	40.36	66.96
Pasture yield (kg/ha)	4057.66	2309.04	2757.24	1662.08

Data adapted from Gardner, 2017

[1]A 54 acre irrigated pasture grazed by the maintenance cows in the study

[2]A 6.4 acre non-irrigated pasture grazed by the restricted cows in the study

Table 7. Nutrient analysis of calves' feedlot grower ration

	Grower ration[1]	
Item	Wet matter basis	Dry matter basis
Moisture, %	43.22	0.00
Dry matter, %	56.78	100.00
Crude protein, %	7.38	13.00
Acid detergent fiber, %	10.74	18.92
Neutral detergent fiber, %	21.81	38.41
Total digestible nutrients, %	42.04	74.04
Calcium, %	0.32	0.56
Phosphorus, %	0.18	0.32
Potassium, %	0.78	1.38
Magnesium, %	0.10	0.17

Data adapted from Gardner, 2017

[1]Grower ration was fed to calves for an 84 day "grower" period and consisted of approximately 43% corn silage, 27% barley concentrate, 27% alfalfa hay, and 3% vitamin and mineral premix on dry matter basis.

Table 8. Sequences for MHC isoform analysis with RT-qPCR[1]

MHC-IIa	AB059398.1	FP	ATTGCTGAATCCCAGGTCAACA
		TP	CAGTGAAGAGTGATCGTGTCCTGATGCT
		RP	TTGTGCCTCTCTTCAGTCATCC
MHC-IIx	AB012850.1	FP	GCTCCTTACCTCCGAAAGTC
		TP	CATTGAGGCCCAGAATAAGCCT
		RP	CTCTGCACAGTTGCTTTCAC
MHC-slow	AB059400.1	FP	CTCTTCTGCGTCACCATCAAC
		TP	TACAATGCCGAGGTAGTAGCCG
		RP	CCTCACTCCTCTTCTTGCCC

[1]Forward primer (FP), reverse primer (RP), and TaqMan probe (TP) sequences indices along with GenBank accession number for the genes analyzed using the TaqMan primer and probe system of real-time PCR.

Table 9. Sequences for mRNA analysis on RT-qPCR[1]

Ezh2	XM_015470758.2	FP	TTTACTGTTGGCACCGTCTGAT
		TP	TTCATCTCGGAATACTGTGGAGAG
		RP	ACACTTTCCCTCTTCTGTCTGC
MamL1	XM_024994729.1	FP	CCCTGGACACACTTCAGTTTCT
		TP	TCTCTTCCCTCAAACTCAGGC
		RP	CCATCTGGGTTATGCTGGAAGT
Cdc25A	NM_001101100.2	FP	TTCCACTGCGAGTTCTCTTCTG
		TP	GATACGTGAGAGAGAGGGATCG
		RP	CTTCAGGACATACAGCTCTGGG
Pax3	XM_871932.4	FP	CCCAGAGGGCAAAGCTTACA
		TP	AGGCCCGAGTACAGG
		RP	ACGGCGGTTGCTAAACCA
Pax7	XM_015460690.2	FP	AGGACGGCGAGAAGAAAGC
		TP	AAGCACAGCATCGAC
		RP	CCCTTTGTCGCCCAGGAT
IGF-1R	XM_606794.3	FP	TTCGCACCAACGCATCAG
		TP	TCCTTCCATCCCCC
		RP	GTTTGAGGCCGAGAGGACATC

[1]Forward primer (FP), reverse primer (RP), and TaqMan probe (TP) sequences indices along with GenBank accession number for the genes analyzed using the TaqMan primer and probe system of real-time PCR.

Table 10. Relative expression of MHC isoforms in skeletal muscle.

	Treatment[1]			
	Maintenance[2]	Restricted[2]	Fold Change[3]	P-value
Beginning of the Feedlot				
MHC-IIa	0.5185 ± 0.075	0.4865 ± 0.055	0.94	0.73
MHC-IIx	5.1915 ± 0.641	5.555 ± 0.542	1.07	0.67
MHC-I	2.326 ± 0.287	2.8137 ± 0.243	1.21	0.21
End of the Feedlot				
MHC-IIa	0.5488 ± 0.118	0.5787 ± 0.111	1.05	0.76
MHC-IIx	5.2485 ± 0.350	4.525 ± 0.322	0.86	0.14
MHC-I	2.6633 ± 0.440	2.7139 ± 0.399	1.02	0.92

[1]Maintenance treatment consisted of calves (n=16) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves (n=18) that were born from cows that did have a nutritional restriction during the second trimester

[2]Values are calculated as $2^{-\Delta CT}$

[3]Fold change value represent relative change in expression of the restricted calves when compared to the maintenance calves

Table 11. mRNA expression in skeletal muscle of offspring at the beginning and end of the feedlot phase

	Treatment[1]			
	Maintenance[2]	Restricted[2]	Fold Change[3]	P-value
Beginning of Feedlot[4]				
Pax3	36.3 ± 27.2	52.8 ± 23.1	1.46	0.64
Pax7	129.7 ± 17.5	123.4 ± 15.7	0.95	0.69
Cdc25A	3.6 ± 0.8	3.4 ± 0.7	0.95	0.83
MamL1	27.3 ± 3.9	25.5 ± 3.8	0.94	0.58
Ezh2	54.6 ± 8.8	53.4 ± 7.6	0.98	0.89
IGF-1R	0.67 ± 0.18	0.55 ± 0.16	0.84	0.27
End of Feedlot[4]				
Pax3	145.9 ± 576.3	1163.6 ± 510.8	7.98	0.20
Pax7	165.1 ± 18.0	128.9 ± 17.4	0.78	0.13
Cdc25A	9.3 ± 2.2	7.4 ± 2.0	0.8	0.37
MamL1	29.7 ± 4.0	35.5 ± 3.6	1.19	0.30
Ezh2	55.4 ± 6.1	59.9 ± 5.6	1.08	0.59
IGF-1R	52.2 ± 7.1	49.5 ± 7.1	0.95	0.78

[1]Maintenence treatment consisted of calves (n=16) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves (n=18) that were born from cows that did have a nutritional restriction during the second trimester

[2]Values are calculated as $2^{-\Delta\Delta CT}$ and represent the least squares mean ± SEM

[3]Fold change value represent relative change in expression of the restricted calves when compared to the maintenance calves

[4]paired box transcription factor 3 (*Pax3*), paired box transcription factor 7 (*Pax7*), insulin-like growth factor-1 receptor (*IGF-1R*), mastermind like transcriptional coactivator 1 (*MamL1*), cell division cycle 25 A (*Cdc25A*), enhancer of zeste homolog 2 (*Ezh2*)

[5]Samples were collected from the *biceps femoris* muscle at this time point

[6]Samples were collected from the *longissimus lumborum* muscle at this time point

Table 12. miRNA expression in *longissimus lumborum* of offspring at weaning.

	Treatment[1]			
	Maintenance[2]	Restricted[2]	Fold Change[3]	P-value
miR-1	37.20 ± 27.16	63.85 ± 24.15	1.72	0.34
miR-27b	8.13 ± 1.50	2.64 ± 1.33	0.32	**0.005**
miR-133a	4.59 ± 3.21	13.53 ± 2.41	2.95	**0.04**
miR-133b	3.61 ± 9.20	42.52 ± 7.12	11.78	**0.003**
miR-181d	1.68 ± 5.04	16.75 ± 4.31	9.97	**0.03**
miR-206	63.14 ± 23.02	41.67 ± 20.58	0.66	0.46
miR-214	1.75 ± 3.13	10.72 ± 2.62	6.13	**0.04**
miR-424	1.85 ± 0.98	5.41 ± 0.88	2.92	**0.01**
miR-486	1.60 ± 1.10	5.88 ± 0.94	3.675	**0.007**

[1] Maintenance treatment consisted of cows (n = 16) that did not have a nutritional insult during the second trimester while cows (n = 18) from the restricted treatment had a nutritional restriction.
[2]Values are calculated as $2^{-\Delta CT}$ and represent the least squares mean ± SEM
[3]Fold change value represent relative change in expression of the restricted calves when compared to the maintenance calves

Table 13. miRNA expression in the *biceps femoris* of offspring at the beginning of the feedlot phase.

	Treatment[1]			
	Maintenance[2]	**Restricted**	**Fold Change[3]**	**P-value**
miR-1	215.8 ± 195.1	455.6 ± 186.3	2.11	0.30
miR-27b	0.94 ± 0.59	2.18 ± 0.55	2.32	0.12
miR-133a	26.7 ± 28.8	109.7 ± 26.7	4.11	0.05
miR-133b	8.3 ± 60.9	398.8 ± 56.3	48.05	0.001
miR-181d	0.24 ± 0.62	1.61 ± 0.58	6.71	0.12
miR-206	29.5 ± 85.9	263.0 ± 76.2	8.92	0.05
miR-214	0.33 ± 0.20	0.83 ± 0.20	2.48	0.01
miR-424	0.11 ± 0.12	0.45 ± 0.12	4.09	0.03
miR-486	6.95 ± 3.92	22.68 ± 3.63	3.26	0.007

[1]Maintenance treatment consisted of calves (n=16) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves (n=18) that were born from cows that did have a nutritional restriction during the second trimester

[2]Values are calculated as $2^{-\Delta CT}$

[3]Fold change value represent relative change in expression of the restricted calves when compared to the maintenance calves

Table 14. miRNA expression in the *longissimus lumborum* of offspring at the end of the feedlot phase.

	Treatment			
	Maintenance[2]	**Restricted[2]**	**Fold Change[3]**	**P-value**
miR-1	312.5 ± 31.9	285.9 ± 29.1	0.91	0.55
miR-27b	1.33 ± 0.19	1.18 ± 0.18	0.89	0.06
miR-133a	39.0 ± 13.3	77.7 ± 10.1	1.99	0.03
miR-181d	0.63 ± 0.16	0.49 ± 0.14	0.78	0.52
miR-206	192.1 ± 172.4	307.9 ± 155.9	1.60	0.60
miR-214	0.67 ± 0.18	0.55 ± 0.16	0.82	0.62
miR-424	0.08 ± 0.03	0.06 ± 0.04	0.75	0.44
miR-486	5.85 ± 0.92	2.97 ± 0.92	0.51	0.04

[1]Maintenance treatment consisted of calves (n=16) that were born from cows that did not receive a nutritional insult during the second trimester. Restricted treatment consisted of calves (n=18) that were born from cows that did have a nutritional restriction during the second trimester

[2]Values are calculated as $2^{-\Delta CT}$

[3]Fold change value represent relative change in expression of the restricted calves when compared to the maintenance calves

Table 15. Correlations between miRNA and mRNA at the beginning of the feedlot phase

	Pax3[1]	Pax7[1]	Ezh2[1]	MamL1[1]	Cdc25A[1]	IGF-1R[1]
miR-27b	0.04	-0.07	-0.14	-0.10	-0.07	-0.14
miR-133a	-0.02	-0.08	-0.09	0.01	-0.02	-0.12
miR-181d	-0.06	-0.31	-0.13	-0.24	-0.14	-0.09
miR-214	-0.06	0.06	0.01	-0.13	0.22	0.15
miR-424	-0.12	-0.07	-0.17	-0.10	-0.07	-0.10
miR-1	-0.09	-0.06	-0.16	-0.12	-0.07	-0.12
miR-133b	0.47*	0.02	-0.25	-0.22	-0.29	-0.23
miR-206	0.47*	-0.23	-0.25	-0.13	-0.37†	-0.28
miR-486	-0.04	-0.16	-0.15	-0.16	-0.15	-0.08

[1]Values in column represent R value between corresponding mRNA and miRNA.
*Significant correlations ($P \leq 0.05$)
†Tendency ($P \leq 0.1$)

Table 16. Correlations between miRNA and mRNA at the end of the feedlot phase

	Pax3[1]	Pax7[1]	Ezh2[1]	MamL1[1]	Cdc25A[1]	IGF-1R[1]
miR-27b	0.04	-0.58*	-0.48*	-0.53*	-0.36	-0.60*
miR-133a	0.13	-0.30	-0.05	-0.03	-0.14	-0.15
miR-181d	-0.17	0.02	-0.12	-0.30	-0.47*	-0.40†
miR-214	-0.12	0.36	-0.17	-0.27	-0.06	0.02
miR-424	-0.02	-0.05	-0.04	-0.05	0.001	0.33
miR-1	0.08	-0.01	0.11	-0.25	-0.26	-0.19
miR-133b	0.03	-0.04	0.02	-0.11	-0.24	-0.22
miR-206	-0.20	-0.14	-0.31	-0.05	-0.45†	-0.24
miR-486	-0.06	-0.51*	-0.32	0.99*	-0.27	0.15

[1]Values in column represent R value between corresponding mRNA and miRNA.
*Significant correlations ($P \leq 0.05$)
†Tendency ($P \leq 0.1$)