THE EFFECTS OF LATE GESTATIONAL EXERCISE ON COLOSTRUM QUALITY AND NEONATAL IMMUNOGLOBULIN ABSORPTION

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Juan Simon Gavette

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

SUPERVISORY COMMITTEE:

Dr. Gerald Stokka
Chair

Dr. Eric Berg

Dr. Travis Hoffman

Dr. Glenn Dorsam

Approved:

June 28, 2021
Date

Dr. Marc Bauer
Department Chair

ABSTRACT

To investigate the effects that late gestational exercise has on ewe performance and colostrum IgG levels, and to evaluate how it impacts neonatal serum IgG absorption and future performance, thirty-two multiparous Dorset ewes were divided into two treatment groups and were housed in individual pens. The treatment group was walked for thirty minutes per day, every other day from the start of the third trimester to lambing. The results indicate that colostrum IgG levels were significantly greater in exercised ewes compared to ewes held in confinement. Increased locomotion impacted pregnant ewe feed intake, body weight, and average daily gain. Gestational exercise did not impact lamb birth weight or weaning weight. There were no significant differences in serum IgG levels amongst lambs after colostrum consumption but there were in serum protein. Further research is needed to better understand how and why gestational exercise increased colostrum IgG levels.

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LIST OF ABBREVIATIONS

ADCC	Antibody dependent cell mediated cytotoxicity
ADFI	Average daily feed intake
ADG	Average daily gain
AICC	Akaike Information Criterion C
ANPC	Animal Nutrition and Physiology Center
APC	Antigen presenting cell
ATP	Adenosine triphosphate
BCS	Body condition score
BIC	Bayesian Information Criterion
BW	Birth weight
Ca	Calcium
CP	Crude protein
DC	Dendritic cell
DM	Dry matter
E2	Estradiol
ETC	Electron transport chain
EX	Exercise
FA	Fatty acid
FcRn	Neonatal immunoglobulin receptor
FPT	Failure of passive transfer
GGT	Gamma-glutamyltransferase
HCL	Hydrochloric acid
Ig	Immunoglobulin
IgA	Immunoglobulin A

IgD	Immunoglobulin D
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IgM	Immunoglobulin M
NDSU	North Dakota State University
NO-EX	No exercise
P	Phosphorus
P4	Progesterone
PI	Passive immunity
RID	Radial immunodiffusion
Th	T helper cell
WW	Weaning weight

CHAPTER 1: LITERATURE REVIEW

Introduction

Colostrum quality is vital for neonatal ruminant species as a source of initial immune protection following parturition. The quality of colostrum in ruminant species is mainly dependent on immunoglobulin (Ig) or antibody levels that serve as the main defense mechanism outside of the sterile uterine environment. Additionally, colostrum is richly abundant in other key immune cells and proteins, fat content, lactose, enzymes, non-enzyme proteins, thermoregulatory cells and proteins, as well as other components that prime normal postnatal physiological functions. Research has shown that colostrum is essential for neonatal survival rates by providing needed nutrients and immunity to newborn farm animals. Newborn ruminants are dependent on the quality of colostrum to establish immune protection.

Both colostrum synthesis and its abrupt ending following parturition are impacted by many mechanisms and factors. Pregnant livestock, such as cattle, sheep, and goats, secrete colostrum in their mammary glands weeks before giving birth. The various mechanisms of synthesis include absorption and shuttling various components, especially water and Igs, from maternal blood sources into the mammary gland. The colostrum production process is dependent on hormonal feedback mechanisms and receptors. Various factors, including physiology, nutrition, fetal count, and environmental and management practices of the pregnant ruminant can influence the quality of colostrum.

The process and mechanisms of consuming and absorbing colostrum for initial immune protection is termed passive immunity (PI) and it is greatly impacted by several factors. The main factors that impact successful PI include the newborns’ ability to stand, find the udder, and properly nurse. Additionally, the timing of colostrum consumption, the quantity of colostrum Igs consumed, and neonatal stress (cortisol levels) may influence PI. After the newborn ruminant consumes colostrum, it passes through the esophageal groove directly into the abomasum. When colostrum bypasses the rumen and is sent directly to the gastric abomasum, the digestive physiology of the neonatal ruminant more resembles a monogastric digestive system for a short period of time. Colostrum Igs are protected from degradation through various mechanisms as they move to the small intestine. The small intestine of the neonate is unselectively permeable following birth which allows immune components and nutrients to be rapidly absorbed by intestinal cells and then shuttled into the newborn's blood stream.

Neonatal ruminants are born with a complex, fully developed but nonfunctioning immune system that must be primed to combat pathogenic microbes. This immune system is established during fetal development and is capable of mounting reactions as parturition nears. The immune system is critical to detect, combat, remove, and remember pathogenic microbes. Mammalian species are born with a complex immune system that has a wide range of cells available to combat immune challenges once they are introduced to pathogens after birth. The mammalian immune system involves 2 main branches called the innate and adaptive immune systems. The innate immune system is non-specific and responds very quickly. The adaptive immune system is much slower but has the ability to respond with extremely robust cellular and humoral responses to create memory to pathogens.

The function of colostrum in the establishment of PI is critical for humans and other mammals. Although this relationship has been widely studied, there are limitations in the literature relative to how exercise and movement patterns may impact colostrum and PI. In human models, maternal exercise has been studied for various reasons, especially for the control and prevention of excessive weight gain and gestational pains, as well as a means to minimize pain and discomfort before and after parturition. Numerous exercise routines, including aerobics, intense walking, muscle workouts or other types of endurance training have been explored in pregnant women, but few have attempted to make a correlation to how these routines could impact colostrum production, quality, postpartum absorption of colostrum Igs, and PI. Controlled research examining correlations between the impact of exercise and (or) movement patterns on colostrum and PI in livestock species are limited. In the few livestock studies evaluating gestational exercise, most have attempted to make correlations on the impact of exercise on maternal and fetal blood flow, fetal nutrient delivery, and gas exchanges. Currently, there appears to be a gap in the literature relative to the impact of gestational exercise on colostrum production, quality, and PI in both humans and animals.

This literature review will address both human, rodent, and domestic farm animal models, with a strong emphasis on sheep, to discuss the following: the production and components of colostrum, passive immunity, development and mechanisms of the neonatal immune system, and gestation and exercise.

The Production and Components of Colostrum

Colostrum is identified as the “first milk” secreted by mammals and differs from milk. The production of colostrum is controlled through hormone signaling and feedback triggered weeks before parturition. Proper mammary gland enlargement and secretion are vital for colostrum quality and to ensure that the quantity is sufficient for newborns. There are various factors that influence colostrum production, such as nutritional intake, breed type, age and parity, fetal count, and management practices. The components in colostrum differ or possess a different concentration than normal milk. The main element that differentiates colostrum from milk is the extremely high levels of Igs (especially the G isotype) which acts as the main defense mechanism before the neonate's own immune system is primed and functioning with the capability of creating its own Igs. Additionally, colostrum contains other various immune cells and proteins, fats, carbohydrates, vitamins, minerals, enzymes, hormones, and thermoregulatory proteins.

Production of Colostrum

Production of colostrum is limited and occurs under hormonal control and feedback. The period of colostrum production is defined as the time prior to parturition where Igs are secreted from the maternal blood into the mammary gland (Barrington et al., 2001). The cascade of colostrum production and the secretion of large quantities of Igs into the mammary gland is regulated by estradiol (E2) and progesterone (P4) (Castro et al., 2011). Signaling and feedback mechanisms generated by E2 and P4 result in a large cascade of immunoglobulin G (IgG) secreted from maternal blood into the mammary gland. As parturition nears, P4 levels, which have maintained pregnancy, begin to fall as E2 levels begin to rise (Delouis, 1978). These changing levels allow for mammary epithelial cells (and other local cells of the mammary gland) to begin colostrum synthesis. As P4 drastically falls, colostrum production becomes more rapid (Delouis, 1978). Neonatal immunoglobulin receptors (FcRn) are expressed in the mammary gland ductal and acinar cells (Tizard, 2018). During colostrum production, these FcRns mediate the transfer of IgGs from the maternal serum through the mammary gland epithelial cells. In the same mechanism that E2 and P4 control IgG secretion from maternal blood into the mammary, various water molecules and essential nutrients are bound by receptors and shuttled through mammary epithelial cells into colostrum (Patton and Jensen, 1975; Wheeler et al., 2007).

Scientists have evaluated changes in maternal serum IgG levels during the peripartum period as a means to quantify the initiation of colostrum production. Differential changes in IgG suggest that as parturition nears, the decline in maternal serum IgG is explained by colostrum production. After parturition, the maternal serum IgG levels increase because colostrum production has ended. According to Brandon et al. (1971), the production of colostrum is triggered weeks prior to parturition in ruminants. Ciupercescu (1977) reported blood IgG concentrations in sheep remained relatively constant in pregnant ewes until 15 days prior to parturition; after which they sharply decreased. Micusan and Borduas (1977) found pregnant goat serum IgG levels dropped drastically approximately 4 weeks prior to parturition. More specifically, Castro et al. (2006) found goat serum IgG levels decreased by more than 38% from the third month of gestation (assumed to be attributed to colostrum production) until parturition, then increased after delivery. Regardless of how much maternal serum IgG declined during colostrum production, the synthesis is only for a limited time. Brandon et al. (1971) reported that after delivery, ruminants abruptly stop secreting large quantities of IgGs into the mammary gland. This abrupt ending of large IgG concentrations is followed by synthesis of normal milk.

Factors that Impact Production of Colostrum

Nutritional factors during colostrum production are vital to ensure that good quantity and quality of colostrum is synthesized. Elevating the concentration of serum glucose and protein in the dam, especially during the final weeks of gestation, is important for ruminants to maintain energy, provide nutrients to the rapidly growing offspring, and synthesize appropriate levels of IgGs and nutrients in colostrum (Pecka-Kielb et al., 2018). Since many authors have concluded that colostrum production begins weeks prior to parturition, pregnant ruminants must be fed a proper quantity and quality of feed during the final weeks of gestation (Swanson et al., 2008). Banchero et al. (2015) found that increasing energy-rich rations fed to ewes prior to parturition greatly improves colostrum quantity and quality and decreases the viscosity of colostrum, allowing lambs to more effectively nurse. Ensuring that livestock have access to good quality feed (and preferably short-term supplementation of high energy grains) can help improve the Ig concentration of colostrum (Banchero et al., 2015). Production research has shown that supplementation and access to high energy feed has been linked to greater colostrum quantity and quality. Single bearing and twin bearing ewes that were supplemented with corn during the final week of gestation have been shown to have greater colostrum quantity with less viscosity than ewes not fed high energy supplements (Banchero et al., 2004). Additionally, Mellor and Murray (1985) concluded that underfeeding pregnant sheep prior to parturition decreased overall colostrum quantity, which may have negative effects on all nutritional components of colostrum.

It has been suggested that physiological age of the dam and parity number impact production of colostrum, but with varied conclusions. Age and parity number of Jersey cows influenced IgG levels in colostrum, with significant increases in IgG levels in the fourth parity and after (Silva-del-Río et al., 2017). Primiparous sows were found to have had greater levels of dry matter (DM), fat, lactose, and non-fat-solids, but parity number did not result in a significant concentration of Igs in colostrum (Segura et al., 2020). Research in sheep and goats have yielded similar conflicting results. In Awassi sheep, parity number did not influence energy content in colostrum but there were greater IgG concentrations in the colostrum of primiparous ewes (Higaki et al., 2013). Campion et al. (2019) reported that colostrum volume increased with age in a multi-breed study that evaluated volume and IgG levels in sheep. Their findings are similar to the conclusions made by Wohlt et al. (1981) who reported that colostrum volume increased as ewes age, especially from 1.5 to 4 years of age, and again from 4 to 8 years of age. The changes in colostrum production regarding age have not been fully explained. However, Campion et al. (2019) suggested that changes in colostrum production as age advances could be due to mammary gland development and the constant expansion, maturation, and survival of mammary gland epithelial cells.

Breed type has been suggested to have an impact both on the quantity and quality of colostrum produced in ruminants. According to Campion et al. (2019), variations in colostrum volume have been attributed to differences in breed type. Campion et al. (2019) reported that Leicester ewes averaged 383 mL of colostrum immediately following parturition, while Suffolk, Belclare, and terminal ewes yielded 624, 642, and 653 mL, respectively. Additionally, breed type has been suggested to influence IgG levels in colostrum. In certain breeds of sheep such as Rambouillet, Targhee, Columbia, and Finn crossbreed, colostrum IgG levels range from 60 to 70 mg/mL (Gilbert et al., 1988) compared to Polypay (79 mg/mL) (al-Sabbagh et al., 1995) and Karakul (125 mg/mL) (Hashemi et al., 2008). These studies agree with the findings of a large multi-breed study conducted by Campion et al. (2019) who concluded that sheep breed type impacts colostrum quantity and IgG concentration.

The number of fetuses in the uterus can impact colostrum synthesis. Nowak and Poindron (2006) reported that the amount of colostrum synthesized in the udder of ewes is dependent on the size of the litter. Twin bearing ewes tend to produce greater levels of colostrum than single bearing ewes. Alexander and Davies (1959) concluded that overall production and yield of colostrum is influenced more by the number of lambs that suckled than the number born. Hall et al. (1990) concluded that colostrum yield in sheep was greater in twin bearing ewes, but the colostrum produced per lamb was lower per lamb in twins and triplets versus singles. Work by McNeil et al. (1988) also provides evidence that twin bearing ewes may produce more colostrum, however, the quantity of colostrum per lamb was less than single born lambs finding that 30% of twin-bearing ewes had insufficient colostrum available for both lambs at birth.

Minimizing environmental and management stress factors can positively impact how pregnant livestock produce colostrum. Proper environmental conditions, including protection from harsh climate and weather, can reduce stress and increase comfort for gestating livestock. It has been suggested that proper management and handling can enhance ruminant colostrum production, especially when IgG is secreted into colostrum weeks prior to parturition (Vihan, 1988). Stress factors during gestation can impact secretion of Igs and nutrients into colostrum during synthesis (Yilmaz and Kasikci, 2013). Proper management of environmental factors that could impact hormone physiology is important because certain hormones play a major role in colostrum production prior to parturition (Castro et al., 2011). Managing the stress of cattle can reduce the release of the stress hormone cortisol. Cows possessing lower concentrations of serum cortisol generate a greater quantity and quality of colostrum that contains more IgG and total protein (Chernenko et al., 2017). Thus, the authors suggested that minimizing stress can have a positive impact on colostrum quality (Chernenko et al., 2017). Proper handling of livestock is important in all phases of production, especially pregnancy, to ensure the safety of the dam and fetus. Through mitigating environmental stress factors and improving animal handling practices, it is possible to have a positive impact on colostrum production.

Components of Colostrum

The main differentiation between colostrum and milk is the greater levels of IgG, as well as key physical and chemical differences (Khan and Ahmad, 1997). Although colostrum contains the same or similar components (Igs and other immune cells and proteins, enzymes, hormones, carbohydrates, fats, vitamins and minerals, and thermoregulatory elements) found in normal milk, the difference lies in the concentrations of these components in colostrum. That said, most of the differential components present in colostrum decrease as milk transitions sets in, with many of the colostrum components reaching zero or near zero percent in normal milk. Major physical and chemical differences exist between colostrum and milk. For example, the physical appearance of colostrum differs from milk. Milk is whiter and has a less viscous appearance and texture than the more yellow colostrum. The yellow color of colostrum is attributed to greater concentrations of carotenoids (McGrath et al., 2016). The greater density of colostrum versus milk is estimated to sharply decrease during the first 2 days postpartum (McGrath et al., 2016). Two of the main chemical differences in colostrum versus milk is overall fat and protein percent. In sheep and cattle colostrum, fat percent is around 9.94% and 6.66%, respectively, compared to milk at 6.39% and 3.26%, respectively (Hernández-Castellano et al., 2016). In sheep and cattle, colostrum protein percent is approximately 13.94% and 11.65%, respectively, compared to milk, which is around 6.60% and 3.71%, respectively (Hernández-Castellano et al., 2016).

Major Immunoglobulins

The IgG component in ruminant colostrum is the main antibody isotype and has 2 subtypes, IgG1 and IgG2 (Butler, 1969). In colostrum, IgG1 is the most abundant and most studied of the subclasses (Butler, 1969). Sheep colostrum has a much greater IgG content than milk, with levels estimated around 45.38 mg/mL compared to milk at approximately 0.91 mg/mL (Hernández-Castellano et al., 2016). Total IgG levels may differ across ruminant species. According to Tizard (2018), ewe colostrum contains around 4,000 to 6,000 mg/dL IgG, compared to 2,400 to 8,000 mg/dL IgG in cow colostrum.

Ruminant colostrum is richly abundant in IgG because neonatal farm animals cannot receive IgG unless they obtain and absorb colostrum. Unlike other mammals, such as humans and rodents, the ruminant placenta does not allow Igs to cross the maternal-fetal barrier and therefore must be obtained from colostrum (Wooding et al., 1986; Khan and Khan, 1991;

Argüello et al., 2004). The ruminant placenta is syndesmochorial (the chorionic epithelium is in direct contact with uterine tissues) and is made up of 6 complex maternal and fetal layers of tissue membranes that impede antibodies crossing to the fetus (Chucri et al., 2010; Tizard, 2018). While most authors have concluded that IgG does not cross the ruminant placenta, some investigators have reported that small levels of IgG may be detected in newborns. Sawyer et al. (1977) investigated PI in lambs and reported that some lambs were born with very minute traces of IgG in their serum prior to nursing colostrum. Additional studies by Waelchli et al. (1994) and Rabbani et al. (1990) reported that a very small trace of IgG was present in serum before newborn lambs and goat kids had nursed. The rare incidence of IgG placental crossing has not been explained, however, Khan and Ahmed (1997) believe that traces of Igs present in newborn lamb and goat kid serum is most likely due to in utero infections.

The IgGs that are secreted into the mammary gland of the dam are a representation of her exposure to various pathogens and have a limited half-life. When livestock species are exposed to antigens, they create Igs from B-lymphocytes that are dependent on T cell co-stimulation (Tizard, 2018; Abbas et al., 2020). These B-lymphocytes are specialized cells in the humoral immune system that initially create immunoglobulin M (IgM) and immunoglobulin D (IgD) and through different mutations, create IgG antibodies that circulate throughout the dam's blood stream and are very specific in their binding ability. Memory Igs are also created that continuously circulate in the animal's bloodstream looking for the specific pathogen that initiated their creation should that animal be exposed again (Tizard, 2018). The mixture of circulating Igs in the dam's bloodstream is secreted into colostrum and offers immune protection to the newborn that reflects the immune exposures of the dam. These IgGs are only able to offer immune protection for a short time. The half-life of these antibodies varies from 12 to 24 days in sheep and up to 29 days in cattle (Hedegaard and Heegaard, 2016).

When the immune system of ruminant species is elicited, B-lymphocytes create Igs that undergo affinity maturation and isotype switching to create a supply of various and specific antibodies that target specific microbes. Through various mechanisms, Igs undergo affinity maturation where portions of the Ig's variable regions are randomly mutated (Abbas et al., 2020). These variable regions of the mutated antibody increase the ability of secreted Igs to bind a specific antigen (Mishra and Mariuzza, 2018). Antibodies that have a high affinity for a respective antigen are proliferated, and Igs with a low affinity undergo apoptosis (Abbas et al., 2020). Following affinity maturation, Igs undergo isotype switching. The process of isotype switching involves point mutations in the Igs constant regions to transform Igs (usually IgM and possibly IgD) to IgG (Stavnezer and Amemiya, 2004; Abbas et al., 2020). Following affinity maturation and isotype switching, IgGs are ready to bind to specific antigens.

Antibodies have varying isotypes and structures, but all are composed of 2 light chains and 2 heavy chains (Butler, 1969). There are 5 distinct isotypes of Igs possessing variable structures, functions, carbohydrate content, and areas that they are located in (such as blood circulation versus mucosal regions) (Abbas et al., 2020). Specifically, for IgG, the structure and shape mimic a "Y" shape (Fig 1.1). The Fc fragment of IgG creates the stem of a "Y" shape with COOH terminus at its base and NH-2 terminal ends at the opposite ends (Butler, 1969). Two adjacent heavy chains make up the Fc fragment that contain 3 constant domains and 1 variable region (Butler, 1969). Two light chains are bonded to the heavy chains by disulfide bonds (Abbas et al., 2020). The light chains are composed of 1 constant domain and 1 variable region and are parallel to the upper portion of the heavy chains that create a "V" shape. These light chains account for the Fab fragments and contain COOH and NH2-termminal ends (Butler, 1969).

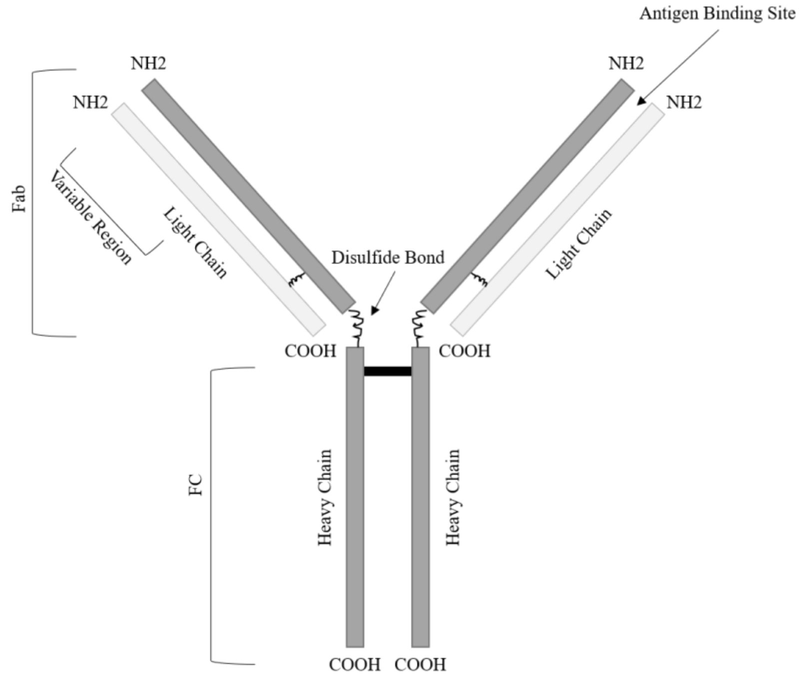

Figure 1.1. Structure of the Immunoglobulin G Isotype

Antibodies are created and structured in a way that allows for them to bind to specific microorganisms. The antigen binding site of IgG is composed of the “V” region on both the heavy and light chains (Abbas et al., 2020). Following affinity maturation and isotype switching, IgGs are ready to bind specific antigens. There are 2 antigen binding sites on IgG that are responsible for identifying targeted microbes (Butler, 1969). The binding of IgG to antigens occurs through a series of methods including reversible, noncovalent interactions, hydrogen bonding, hydrophobic interactions, and charge-based interactions (Abbas et al., 2020). Binding and targeting of IgG to antigens depends on epitopes or antigenic determinants that are made up of amino acids or monosaccharides present on the specific antigen (Butler, 1969; Abbas et al., 2020).

Minor Immunoglobulins

Colostrum contains varying levels of immunoglobulin A (IgA), IgD, immunoglobulin E (IgE), and IgM that account for around 15% or less of the total Igs present in ruminant colostrum (Khan and Ahmad, 1997; Smolenski et al., 2007; Cakebread et al., 2015; Tizard, 2018). The main effector function of IgA is to support anti-inflammatory reactions in mucosal immunity (Cakebread et al., 2015). Although very low in levels (around 5.36 g/L and 0.08 g/L in bovine colostrum and milk, respectively), IgA is an important mucosal protector and has strong stability against proteolytic degradation (Cakebread et al., 2015). The IgD isotype is not fully understood in ruminants and its mere presence is questioned in ruminants (Naessens, 1997). It has been disputed that unlike humans, rodents, and some primates, sheep and cattle may not have IgD present on the membranes of B-lymphocytes (Naessens, 1997). The IgE concentration in sheep colostrum is low, yet the antibody functions to mediate reactions against allergies. The concentration of IgE in sheep milk is assumed to be near zero and often is not detected (Hine et al., 2010). The antibody IgM functions in a very effective agglutinating (clumping microbes together) form against infectious microbes and is also suggested to assist in complement activation, which triggers different cascades of proteins that help kill foreign microorganisms (Butler, 1969). The levels of IgM present in ruminant colostrum (5.23 mg/mL in sheep) are greater than both IgA and IgE but are still extremely low in milk (0.38 mg/mL in sheep) (Cakebread et al., 2015; Hernández-Castellano et al., 2016).

Additional Immune Cells and Proteins

Colostrum provides other important immune cells, particularly innate immune cells and signaling mechanisms that aid in the initial protection for newborn ruminants. These immune cells are suggested to be in greater abundance in colostrum compared to milk (McGrath et al., 2016). Colostrum contains neutrophils, which aid in tissue destruction and inflammation, and dendric cells (DCs) and macrophages, which are essential for recognizing pathogens and communicating with the adaptive immune system (Stelwagen et al., 2009). Cytokines act as signaling molecules in mammalian immune systems and are important components of colostrum (Stelwagen et al., 2009). Cytokines such as interleukins, tumor necrosis factor, and interferons, stimulate and support various signaling mechanisms, immune reactions, and effector functions that promote the overall development of the neonatal immune system (McGrath et al., 2016; Tizard, 2018). Furthermore, colostrum contains complement proteins which are critical to innate immune reactions. Complement protein cascades either directly destroy foreign microorganisms or assist other immune system cells in destroying invading pathogens (Stelwagen et al., 2009).

Carbohydrates and Calories

Colostrum contains the initial energy components for neonatal ruminants. The main carbohydrate found in colostrum is lactose. As colostrum transitions to milk, lactose concentrations increase (Langer, 2009). According to Wohlt et al. (1981), lactose levels in Dorset sheep were 3.9% in colostrum and 4.6% at day 5, and 5.3% by day 28 of lactation. These levels agree with those of Fox (2009) and Nowak and Poindron (2006) that sheep lactose levels in colostrum were approximately 5%. Not only is lactose important for energy production, but Dai et al. (2000) also suggested that a proper level of lactose and other carbohydrates aids in initial immune development. Various other oligosaccharides are found in greater concentration in colostrum versus normal milk and act as sources of energy for the newborn (McGrath et al., 2016). Given that adequate amounts are consumed and absorbed properly, colostrum provides approximately 2 Kcal of energy per mL for neonatal sheep. This additional energy is important to promote newborn standing and vigor (McCance and Alexander, 1959; Eales and Small, 1981).

Wohlt (1981) reported that the energy present in the colostrum of Dorset sheep was approximately 1,683 cal/g and increased to an average of 1,722 cal/g by day 5, then stabilized at around 1,578 cal/g from days 28 to 56.

Fat

Fats are important nutrient components of colostrum. Not only do fats provide different nutritional elements, but they also aid in digestion and can offer specific health benefits. Contarini et al. (2014) suggested that certain fatty acids (FAs), bovine milk triglycerides, and membrane lipids may be effective in antimicrobial reactions (Contarini et al., 2014). Phospholipids are also important elements in colostrum that offer health benefits for protection against gastrointestinal infections (Contarini et al., 2014). According to Contarini (2014), colostrum is greater in many FAs, phospholipids, and cholesterol compared to milk. McGrath (2016) also concluded that cholesterol levels are greater in colostrum than milk; accounting for around 95% of the lipids found in cattle colostrum. Also, the type of various fats found in colostrum may increase or decrease as milk synthesis begins. Contarini (2014) suggested that short chain saturated FAs increased in normal milk, but long chain FAs decreased in milk compared to colostrum. Additionally, conjugated linoleic acid, which has been linked to controlling adipose tissue and serving as an antioxidant, has been found to greatly increase as lactation progresses and transitions from colostrum to milk (Contarini et al., 2014).

Vitamins

A greater concentration of fat-soluble vitamins is found in colostrum versus milk. Vitamin A can be found in many different forms in colostrum assisting in the maintenance of a strong and viable immune system and stable development and maintenance of mucus membranes, skin, and other tissues. Many authors have concluded that Vitamin A levels decrease as colostrum transitions to milk (McGrath et al., 2016). Vitamin E and its different compounds are also in colostrum at greater levels than in milk and are very important as an antioxidant to protect cells and tissues from free radical destruction (McGrath et al., 2016). Vitamin D and its different forms are present in colostrum to promote and regulate bone, skin, and hair development. It is suggested that vitamin D levels decline in postpartum milk production (McGrath et al., 2016). Different forms of Vitamin K are also present in colostrum and are critical in mediating coagulation and hemorrhagic disease. Like all other fat-soluble vitamins, Vitamin K is present in greater concentrations in colostrum than in milk (McGrath et al., 2016).

Water-soluble vitamins are also present in ruminant colostrum to help regulate internal organs and functions, and to support the overall development and health of the newborn. Vitamin C is a very important vitamin that supports formation of collagen, iron absorption, immune support, wound healing, and further maintenance of cartilage, bone, and teeth, along with various other functions. Neonatal ruminants cannot synthesis Vitamin C in their livers until about 3 weeks of age (McGrath et al., 2016). To compensate for the inability of newborns to synthesis Vitamin C, colostrum contains this vitamin and in slightly greater levels than in milk (McGrath et al., 2016). Additionally, various groups of Vitamin B (which support numerous physiological functions such as energy levels, cell metabolism, brain function, and immune function) are present in colostrum in greater concentrations than milk (McGrath et al., 2016).

Minerals

Various minerals are present in colostrum to further promote neonatal internal functions (such as bone and tissue development) and overall physiological health and maintenance. Colostrum is rich in minerals in different levels and forms. Calcium (Ca) and phosphorus (P), which are major mediators in bone and tissue growth and development, are important components of colostrum (McGrath et al., 2016). The amount of minerals present in colostrum is greater than in milk (Tsioulpas et al., 2007; McGrath et al., 2016). However, some data suggests that certain minerals such as Ca, P, and potassium are greater in milk than colostrum, but results have been mixed and do not offer definitive evidence (other than nutritional and environmental factors in the dam may influence the difference) (Kehoe et al., 2007; McGrath et al., 2016). Scientists have also reported that copper, iron, zinc, and manganese, which all support different physiological functions such as bone and tissue health, blood and hemoglobin concentrations, and immune functions, are present at varying concentrations in colostrum and milk (Lucey et al., 2009; McGrath et al., 2016).

Enzymes

There are unique enzymes found in colostrum that serve various functions for neonatal survival and priming of internal organs (Hernández-Castellano et al., 2016; McGrath et al., 2016). Gamma-glutamyltransferase (GGT) (present in greater concentrations in colostrum than milk) is an important enzyme that assists the transport of molecules across cellular membranes. It has also been suggested that high levels of GGT are positive markers for IgG levels in sheep and cattle colostrum, which ultimately may be used as markers for successful PI (Perino et al., 1993; McGrath et al., 2016). Antioxidant enzymes, such as lactoperoxidase and catalase are involved in catalytic transformation of reactive oxygen species and their by-products into stable, nontoxic molecules. An example of this action would be the stabilization of free radicals that could cause neonatal cell and tissue destruction (McGrath et al., 2016). It has been suggested that colostrum levels of antioxidant enzymes are superior to that of milk (McGrath et al., 2016). Additionally, proteinases richly present in colostrum compared to normal milk aid the initial breakdown of proteins into smaller peptides or single amino acids, aiding digestibility post-ingestion. The proteinase plasmin is one of the main proteinases found in milk but is about 10 times greater in colostrum (McGrath et al., 2016). Furthermore, lipases and esterases are greater in colostrum than milk and offer key benefits to the breakdown reactions of fatty acids.

Hormones

Colostrum contains numerous hormones that support nutrient and intestinal functions in neonatal ruminants, as well as general support for growth of internal tissues and organs. Thyroid hormones, such as triiodothyronine, are important to stimulate and mature the normal functions of the small intestine and assist in the regulation of metabolism. Additional functions of thyroid hormones in colostrum aid in body temperature, heart rate, vasodilation, and vasoconstriction. Thyroid hormones (in general) are suggested to be greater in ruminant colostrum compared to normal milk (Canapana and Baunarucker, 1995). Canapana and Baunarucker (1995) reported that the colostrum of ewes and other ruminants also contains insulin-like growth factor I and growth hormone, which are important hormones that mediate normal growth and development of tissues and bone. Furthermore, colostrum also contains the hormone insulin, which is vital for controlling and maintaining blood glucose levels. According to Aranda et al. (1991), insulin levels are greater in cattle colostrum at 327 ng/mL compared to 46 ng/mL in milk at day 7.

Thermoregulatory Proteins

Colostrum is a very important element in heat production in neonatal ruminants because of its thermoregulatory proteins and warm physical temperature, both of which provide heat to the newborn. According to Cannon and Nedergaard (1985) and Clarke and Symonds (1998), uncoupling proteins, primarily uncoupling protein 1, are an important element in colostrum. These uncoupling proteins will start a cascade of heat production by acting on the electron transport chain (ETC). Through uncoupling of the ETC, adenosine triphosphate (ATP) energy is created, resulting in heat production. Molecules of ATP created as a result of uncoupling proteins acting on the ETC may go on to further increase heat production through activation of brown adipose tissue (Cannon and Nedergaard, 1985). Brown adipose tissue is very important for thermoregulation in neonatal ruminants, especially those born in inhospitable outdoor environments. When ATP and guanosine diphosphate bind with thermogenin, a polypeptide found in brown adipose tissue, heat production occurs. Additionally, varying proteins and endocrine stimulatory factors can also activate brown adipose tissue, including thyroid hormones and catecholamines (Clarke and Symonds, 1998).

Passive Immunity

Colostrum is the source of maternal Igs that neonatal ruminants receive for initial immune protection by the mechanisms of PI. There are numerous factors that can impact how Igs are absorbed: the ability of neonates to stand and nurse, the timing of the first colostrum consumption, the quantity of colostrum IgG consumed, and stress levels of the newborn. Following nursing, colostrum is passed directly to the abomasum of the neonatal ruminant where it travels to the small intestine. Various defense mechanisms protect IgG from rapid degradation. However, these protection mechanisms are only available for a limited time in the neonatal digestive tract. In the small intestine, Igs are absorbed by unselective and highly permeable enterocyte cells. However, the enterocyte cells close after a limited time, after which, colostrum Igs cannot be absorbed. Morbidity and mortality rates in neonatal ruminants that consume a good amount of high-quality colostrum are much lower than in those that do not achieve proper PI.

Factors that Impact Transfer of Immunoglobulins

The ability of the newborn ruminant to properly obtain colostrum and achieve PI depends on its ability to stand, find the udder, nurse, and the dams' behavior and care. The parturition process stimulates behavior in the newborn and upon delivery, they are further encouraged to move and stand by being sniffed and licked by the dam (Nowak and Poindron, 2006). These processes also establish bonding between dam and offspring. Once they have stood, neonatal ruminants begin to explore for the udder and teat. Newborn lambs respond to the smell of amniotic fluids and inguinal wax in the rear of the ewe, which promotes exploration around the area where the udder is located (Ward and Vince, 1984). Further maternal care is needed to ensure that neonates are encouraged to continue to nurse and to ensure safety from predation and adverse weather through the seeking and identification of proper/appropriate shelter.

The timing of the first colostrum feeding has a major impact on achieving successful PI and avoiding failure of passive transfer (FPT). According to Alves et al. (2015), colostrum should be provided to newborn lambs in the shortest time possible after parturition. Specifically, authors agree that colostrum should be fed to small ruminants within 18 to 24 hours postpartum (Alves et al., 2015). Large ruminants are also encouraged to receive colostrum within hours of parturition (Shearer et al., 1992). In cattle, one of the major factors that influences successful PI is timing of colostrum consumption after birth (Shearer et al., 1992). While it is recommended that ruminants receive colostrum within 18 to 24 hours after birth, most authors believe that in order to avoid FPT, colostrum should be consumed within the first few hours. Increased morbidity and mortality rates have been observed in newborn ruminants who do not consume sufficient colostrum within the first hours of life (Kessler et al., 2019). Hungerford et al. (1999) suggested that delays in colostrum intake may leave neonatal calves at a greater risk of morbidity compared to calves that consume colostrum in a shorter time following birth. Outside of consuming colostrum immediately after birth, there is no fully defined time frame considered optimal for the achievement of optimal PI. However, Osaka et al. (2014) reported that FPT was reduced when Holstein calves consumed colostrum within 6 hours postpartum. Calves that consumed colostrum after 12 hours had a sharp decrease in IgG absorption rates, with even sharper declines after 18 hours (Osaka et al., 2014). Dwyer et al. (2016) suggested that if colostrum were not consumed by lambs within 6 hours postpartum, energy reserves may be exhausted which could lead to a sharp decrease in heat production and overall vigor of newborn lambs.

The quantity of colostrum IgG consumed is one of the most influential factors in achieving proper PI in neonatal ruminants. There is no set number that quantifies successful PI in newborns. However, many authors have made recommendations as to the amount of colostrum that should be consumed by newborns to have the greatest chance of successful PI. In sheep, Mellor and Murray (1986) recommended 180 to 210 mL of colostrum/kg of body weight during the first 18 hours after birth. Morrical et al. (1995) suggested that neonatal lambs and goat kids should consume colostrum at 10% of their body weight. The IgG levels in serum post-colostrum feeding are strong indicators of successful PI. Several authors have quantified a baseline of serum IgG concentrations to prevent FPT. O'Brien and Sherman (1993) define FPT in goat kids as < 12 mg/mL IgG in post-colostrum consumption serum. Other authors have suggested a 15 mg/mL baseline of serum IgG post-colostrum consumption in small ruminants (Hunter et al., 1977; Turquino et al., 2011; Alves et al., 2015). In newborn calves, Elsonhaby et al. (2019) defined FPT as < 10 g/L of serum IgG levels after colostrum consumption. Additionally, Perino et al. (1993) classified FPT in neonatal calves as < 8 mg/mL IgG in serum 24-hours after colostrum consumption. Furthermore, Wittum and Perino (1995) suggested that a good baseline for achieving proper PI in calves is 1,600 mg/dL IgG. Although there is no consensus for IgG concentrations postpartum and successful PI in ruminants, authors have reported threshold values that indicate FPT (Perino et al., 1993; Hungerford et al., 1999).

There are conflicting conclusions regarding neonatal stress and cortisol levels relative to whether cortisol enhances or depresses PI. According to Sangild (2003), cortisol stimulates, rather than inhibits macromolecule nutrient transport across the small intestine of the neonate. Elevated cortisol levels, especially in response to excessive physical and metabolic stress around the time of birth, could promote the development of the immature enterocytes and promote (rather than suppress) colostrum absorption in neonates (Sangild, 2003). Others have indicated that strong plasma cortisol levels are important for neonatal ruminants as a means to promote vigor, nursing, and ultimately intestinal absorption of IgG (Chniter et al., 2016). According to research by Chniter et al. (2016), plasma cortisol levels for lambs during the first 12-hours of life were above 200 ng/mL, decreased at 24 and 36-hours, and then remained stable until 48-hours in lambs from primiparous and multiparous ewes. In contrast, Sangild (2003) suggested that high cortisol levels may obstruct IgG absorption after colostrum consumption, potentially because of faster enterocyte cell maturity. Stress factors following parturition may cause a decrease in IgG consumption and absorption (Yilmaz and Kasikci, 2013). In both small and large ruminants, stress during parturition, especially dystocia, can have a major negative impact on cortisol levels (increasing them as the parturition process becomes more stressful to both dam and offspring). This may impact how and when colostrum is consumed by the stressed newborn (Chniter et al., 2016; Chernenko et al., 2017). Stott et al. (1976) reported that calves exposed to adverse environmental stressors had increased serum cortisol levels and decreased serum IgG. The authors suggested that increased cortisol levels in neonatal calves suppressed the permeability of the small intestine, impacting its ability to absorb IgG, ultimately impacting successful mechanisms of PI (Stott et al., 1976).

Mechanisms of Neonatal Intestinal IgG Absorption

Neonatal ruminant PI is dependent on colostrum being suckled and passing through the esophageal groove directly into the abomasum. For a brief period of time after parturition, newborn ruminants are born with a functioning esophageal groove that delivers colostrum directly from the esophagus to the abomasum of the 4-compartmentalized stomach (Titchen, 1976). The esophageal groove is developed during the fetal stage and is composed of muscular folds from the reticulorumen that are positioned when the newborn lifts its head to suckle. Nursing on the udder of the dam will allow the newborn's esophageal groove to close. When newborns nurse, the suckling reflex stimulates contraction of the esophageal groove from the cardia to the reticulo-omasal orifice, leading to a closure that does not allow colostrum to enter the reticulorumen (Dirr and Dirksen, 1989; Gaspers, 2015). Additionally, colostrum will come into contact with receptors located in the oropharyngeal area that further promote closure of the esophageal groove (Dirr and Dirksen, 1989). Failure to close the esophageal groove can lead to colostrum being passed directly into the rumen. Since the rumen is still immature and cannot process colostrum, the contents may remain in the rumen and cause bacterial fermentation, which can ultimately lead to ruminal acidosis and be fatal (Dirr and Dirksen, 1989).

Colostrum is passed from the esophagus to the abomasum where Igs, other immune cells, and proteins are protected from degradation before being passed onto the intestine. The secreted levels of hydrochloric acid (HCL) in the abomasum are low following parturition. This is attributed to low levels of parietal cells, which are responsible for the production and release of HCL (Hill, 1956; Guilloteau et al., 2009). These parietal cells increase considerably within the first 72 hours after birth, ultimately decreasing abomasum pH (Hill, 1956). The pH of the neonate's abomasum varies per specie and per individual, but is suggested to be around 5.8 at birth, then decrease to around 3.0 after 42-hours (Guilloteau et al., 1985). The elevated pH level in the neonate's abomasum serves many purposes, including decreased proteolytic activity of pepsin during the first 24-hours (Guilloteau et al., 1983). During this early postpartum period, the digestive enzyme pepsin is suppressed from digesting proteins into polypeptides. The suppression of these proteolytic and denaturing mechanisms will allow immune cells and proteins to pass through the neonatal abomasum with limited disruption.

Colostrum is passed from the abomasum to the small intestine where it is further protected from degradation to allow more efficient absorption. Colostrum leaves the abomasum through the pyloric valve to enter the duodenal portion of the small intestine. The small intestine reacts to the presence of colostrum by secreting pancreatic juices, which promote pancreatic proteases such as trypsin to be secreted (Zabielski and Naruse, 1999). Although Guilloteau et al. (2009) suggested that pancreatic secretions are minimal at birth, there may be sufficient secretions to threaten the viability of Igs. Immunoglobulins, especially IgG, are sensitive to trypsin degradation (Pineiro et al., 1978). Colostrum is rich in trypsin inhibitors (approximately 100-fold greater compared to normal milk) to better protect Igs from degradation (Sandholm and Honkanen-Buzalski, 1979). Trypsin inhibitors play a key role in protecting Igs in the small intestine.

The mechanism of intestinal lumen IgG absorption is through pinocytosis of enterocyte cells that are very permeable following parturition. The intestine of the newborn ruminant is highly permeable to absorb Igs and nutrients for a short period of time following parturition. According to Sawyer et al. (1977), the intestinal physiology of neonatal lambs is unselectively permeable, which allows all isotypes of Igs and other macromolecules to be rapidly absorbed. Upon arriving at the duodenum and ileum of the small intestine, colostrum IgGs are transported through the glycocalyx of the enterocytes by pinocytosis (Jochims et al., 1994; Weaver et al., 2000). The pinocytosis of IgG allows active transport across the enterocyte membrane to the basolateral membrane. Here IgG will undergo exocytosis into the lacteals and intestinal capillaries (Jochims et al., 1994; Mayer et al., 2002). Following exocytosis, IgGs will enter the circulation of the neonatal ruminant through the thoracic duct. The absorption of colostrum IgG into the blood ultimately defines the transfer of PI because the IgG now offers immune protection to the newborn. While most IgG is absorbed into the bloodstream, some IgG (selectively IgG1) is recycled back into the intestinal lumen to offer immune protection to the suckling neonate's gastrointestinal tract (Cervenak and Kacskovics, 2009).

Intestinal Closure

The cessation of unselective intestinal permeability to absorb IgGs and other macromolecules is termed closure and varies in timing. According to Weaver et al. (2000), closure varies per species and the mechanisms are not completely understood. In newborn lambs, Khan and Ahmed (1997) suggested that unselective permeability and absorption remains highest for the first 6 hours after parturition. Yilmaz and Kasciki (2013) found that small intestinal closure in neonatal lambs resulted in a loss of ability to absorb maternal Igs and other large macromolecules within 24 to 48 hours of birth. Cattle are very similar to sheep in regard to intestinal closure. Weaver et al. (2000) stated that enterocyte pinocytosis and the high permeability lasts approximately 24 to 36 hours. Additionally, Butler (1969) found that intestinal absorption of Igs and macromolecules occurred for 1 to 2 days following parturition in calves. As suggested by Weaver et al. (2000), the exact mechanisms for closure are not fully understood.

However, the authors suggested that closure occurs from an exhaustion of pinocytosis by intestinal cells and the rapid turnover of highly permeable enterocytes, which are replaced by more mature enterocyte cells (Weaver et al., 2000). Additionally, Jochims et al. (1994) reported that lysosomes begin to appear in the more mature enterocyte cells around 24-hours after parturition. Lysosomes contain degrative enzymes capable of degrading the immune cells they come into contact with inside the enterocyte (Jochims et al., 1994). The rise of lysosomal enzymes is suggested to be a major reason that the intestine undergoes closure.

Neonatal Survival Rates from Colostrum Consumption

Colostrum is richly abundant in many immunological and thermoregulatory components that aid in the survival rate of newborns. Consumption of colostrum is vital because newborn ruminants are born hypo-gammaglobulinemic (born without gamma globulins or sufficient ability to create them, with Igs being the most significant gamma globulins) (Khan and Ahmad, 1997). Their survival is dependent on obtaining proper Igs from their dam because of the lag period between primary antigen exposure and secretion of Igs (Gokce et al., 2014; Hedegaard and Heegaard, 2016). Controlled experiments have found the intake of colostrum to be positively correlated to survival rates in newborn ruminants. Vihan (1988) conducted several experiments to evaluate the impact of colostrum consumption on morbidity and mortality rates in sheep and goats. In one experiment, Vihan (1988) reported lambs that died had an average of 0.33 g/100 mL gamma globulin in their serum; however, lambs that survived had 1.75 g/100 mL gamma globulin in their serum. Additionally, Vihan (1988) reported that lambs who nursed colostrum immediately after being born had a 100% survival rate at 3 days of age and had greater gamma globulin levels from birth to 12-weeks of age. In contrast, lambs that were deprived of colostrum but fed milk instead had a 20% mortality rate in the first 3 days and had significantly less gamma globulin levels from birth to 12-weeks compared to colostrum-fed lambs (Vihan, 1988). Furthermore, Hodgson et al. (1992) reported even greater rates of morbidity and mortality among neonatal lambs who were deprived of adequate colostrum. Lambs that were deprived of colostrum had a morbidity and mortality rate of 80% and 67%, respectively (Hodgson et al., 1992). In comparison, lambs that were fed colostrum had a morbidity and mortality rate of 20% and 13%, respectively (Hodgson et al., 1992). Thermodynamic components are also present in colostrum that impact the newborn’s ability to thrive. Eales et al. (1980) reported that an adequate supply of colostrum received by newborn lambs prevented hypothermia in a more effective manner than shelter. Newborn animals are born wet and thus proper heat production is important. Colostrum is a key immunological and thermoregulatory element that has been shown to decrease morbidity and mortality rates in newborn animals.

The Development and Mechanisms of the Neonatal Immune System

Fetal ruminant animals develop a complex immune system throughout gestation that has the capability to respond to various antigens, although in various degrees in utero, as parturition nears. This naïve immune system is fully capable of identifying pathogens and mounting an initial immune response following birth; however, more advanced immunity is slow and not fully capable of creating its own Igs until weeks after parturition (Barrington and Parish, 2001). These neonates receive initial immune protection in the form of colostrum that is richly abundant in Igs, which carry out specialized effector functions to offer protection against pathogens. To create their own antibodies, the newborn ruminant must undergo an initial priming of their immune system to detect, combat, clear away, and remember specific microbes. This initial priming mechanism is done through the introduction of pathogens. Following initial pathogen exposure, the neonate’s immune system begins to operate its innate and adaptive immune systems. The innate immune system is the first line of defense that is rapid and acts in both a specific (ability to bind to pathogen associated molecular patterns) and non-specific (in the type of microbe) manner in sensing and combating microorganisms. The adaptive immune system is much slower, stronger, and possesses the ability to create memory cells that can rapidly identify and eradicate specific antigens upon secondary exposure.

The Development of the Fetal Immune System

The immune system of fetal ruminants develops during gestation and becomes fully established as parturition nears. Barrington and Parish (2001) found that the cells and proteins of the innate and adaptive immune systems originate from multipotent hemopoietic stem cells. Innate immune defenses, such as macrophages and DCs, along with complement proteins, are generated throughout gestation. The developing fetus also produces adaptive immune cells, such as B and T lymphocytes that are needed for humoral and cell mediated immunity (Fahey and Morris, 1978). Additionally, Barrington and Parish (2001) reported that nonimmune defense mechanisms, such as stomach acids, enzymes, and flora that colonizes mucosal surfaces upon birth, are also developing during gestation. Upon parturition, neonatal ruminants are born with a fully supplied, albeit naïve, adaptive immune system.

Fetal immune cells have the capability of responding to antigens inside the placenta and are therefore deemed ready for use after birth. Although the placenta acts as a strong barrier to prevent the crossing of pathogenic microbes, studies have suggested that the fetus is able to respond to antigenic stimulation. It has been suggested that an advanced immune reaction to antigens can be stimulated in fetal lambs (Fahey and Morris, 1978). In fact, fetal immune responses have been detected upon inoculation of various antigens. Fahey and Morris (1978) found that as fetal age increased, so did immune reactions and the capability of B-lymphocytes to create antibodies. Observations in fetal lambs have demonstrated that immune cells can mount a rejection reaction to skin allografts before birth (Schinckel and Ferguson, 1953). Additionally, Silverstein et al. (1964) reported that fetal lambs could reject skin allografts after 77 days of gestation, but not before 67 days. Others have suggested that fetal immune cells are capable of recognizing antigens and responding with varying immune reactions, including creation of antibodies specific for certain microorganisms (Silverstein et al., 1964; Fahey and Morris, 1978). The ability for fetal immune cells to respond to antigens is greater as gestation progresses, but not all antigens can be recognized and cleared (Fahey and Morris, 1978).

Immune Protection from Colostrum Immunoglobulins

Following the consumption and absorption of colostrum IgG, the maternal immune antibodies offer protection to the neonatal ruminant through specialized effector functions. Upon binding to specific antigens, IgG has 2 main effector functions which aid in the eventual destruction of foreign microorganisms in the newborn. The first main effector function of IgG is neutralization of microbes. Neutralization blocks foreign microbes from adhering to; or entering host cells. This action is accomplished when IgGs attach to the outer membrane of the invading microbe and disrupts their ability to bind or enter host cells. The second major effector function of IgG is to recruit effector cells and other molecules to kill the detected microbe through opsonization, complement activation, and antibody dependent cell mediated cytotoxicity (ADCC). Following opsonization of foreign microbes, phagocytic cells recognize and bind to the Fc receptors on IgG and engulf the coated microorganism. Pathogens coated with IgG can also activate the classical complement pathway (Butler, 1969). The complement system acts to kill foreign microbes through several mechanisms, including direct cytolysis, opsonization, and inflammation (Carroll, 1998). Destruction of foreign microbes by ADCC occurs when IgG binds to the foreign microbe, leading to a molecular shift that exposes the Fc region for recognition by leukocytes (Hashimoto et al., 1983). Cytotoxic cells such as natural killer cells or eosinophils have specific Fc receptors on their surfaces that bind with IgG. When IgG Fc receptors bind to cytotoxic Fc receptors, degranulation occurs, which signals the release of toxic molecules that cause lysis and destruction of targeted foreign microbes (Hashimoto et al., 1983; Abbas et al., 2020). Over time, the neonate will develop its own ability to produce antibodies, which will originate and function in the same manner as the IgGs that were originally received from the dam through PI.

Priming of the Neonatal Immune System

Following parturition, the immune system of neonatal ruminants is naïve and must be primed through pathogen exposure to begin normal functions outside of a sterile uterine environment. According to Tizard (2018), the immune cells of newborns must be stimulated to begin functioning in order to detect and bind to microbes, initiate cascades of effector cells that will combat and clear microorganisms, and create immune memory cells. Barrington and Parish (2001) suggested that the inability of the humoral and cell mediated immune systems to function is because the system has not been primed with innate immune reactions. Upon exposure to various microbes that act as priming mechanisms, innate immune cells will detect and present antigens to the adaptive immune system, which takes longer to respond. The primary response of neonates upon exposure to microbes involves a prolonged lag period where low levels of Igs are produced (Barrington and Parish, 2001). Thus, for initial protection, newborns depend on the immune components found in colostrum, such as Igs, cytokines, and other immune cells and proteins. Over time, the newborn's immune system will become more mature and be able to produce its own antibodies (Fahey and Morris, 1978). By around 2 weeks of age, the immune system of newborns is able to undergo cell-mediated and humoral immune reactions and signals (Barrington and Parish, 2001).

The Mechanisms of the Neonatal Immune System

The innate immune system is compiled of many fast-acting cells and proteins that function in both specific and non-specific mechanisms. It is the first line of defense in mammalian species for the detection of foreign microorganisms that follows with direct communication with the adaptive immune system. The invasion of microbes into animals can come in many forms, including through abrasions in external physical barriers, inhalation through the respiratory system, the eyes, and by consumption of contaminated food, water, or through contact with fomites. Epithelial layers, mucus membranes, and hair act as physical barriers to prevent or limit microbe invasion (Abbas et al., 2020). Foreign microbes that evade the physical barriers to enter an animal are engaged by the cells and proteins of the innate immune system within minutes to hours (Abbas et al., 2020; Byrne et al., 2020). Upon microbe invasion, antigen presenting cells (APCs), which include, macrophages and DCs, are the major detection cells that will be responsible for communicating with the adaptive immune system (Hamilos, 1989). Macrophages and DCs have pattern recognition receptors that specifically recognize pathogen-associated molecular patterns on invading microbes (Abbas et al., 2020). Macrophages and DCs phagocytize microbes and present small peptide fragments on their outer membranes (Hamilos, 1989). The activation of macrophages and DCs cells signal cytokines, neutrophil recruitment, eosinophils, basophils, and mast cells, as well as activate complement proteins (Barer et al., 2019; Abbas et al., 2020). Complement is a collection of many proteins that can aid in recruitment of phagocytes that directly infiltrate the membranes of foreign microbes, eventually killing them (Cooper and Nemerow, 1984). The 3 different pathways of the complement system can directly kill foreign cells or indirectly have them destroyed through phagocytosis or apoptosis (Cooper and Nemerow, 1984).

The adaptive system is the second branch of the immune system that acts slower but is much more robust and creates memory cells. The main reason that the adaptive immune system is slower is because it takes time for a specific antigen to activate lymphocytes; and proliferation of effector cells and antibodies requires a longer period of time than innate reactions. Following the detection and initial phagocytosis of invading microbes by the innate immune system, APCs will be the signaling mechanism between the innate and adaptive immune systems. In a chemotaxis manner, APCs are shuttled to the nearest lymph node where they meet with immature CD4 T cells; the first segment of the adaptive immune system. The APC binds to a CD4 T cell through 2 different signals (Abbas et al., 2020). Depending on the type of microbe, such as a bacteria, virus, or fungus, a different cascade of effector T cells will be activated from CD4 T cells (Abbas et al., 2020). Activated CD4 T cells create different T helper (Th) cells (Abbas et al., 2020). The activation of each respective Th cells (Th1, Th2, Th17, Tfh, and Treg) causes a clonal expansion of effector and memory T cells that detect, combat, and remember their respective microbe (Barer et al., 2019). In the case that intracellular microbes infect host cells, such as viruses, the immune system handles it in a similar fashion. Infected host cells are presented to CD8 T cells and, similar to CD4 T cells, trigger an array of effector functions (Abbas et al., 2020). Following CD4 or CD8 T cell activation, B cells are activated to secrete antibodies (Abbas et al., 2020). Activated B cells create antibodies that are specific to their respective microbe. When B lymphocytes are initially activated, IgM (and possibly IgD) are the membrane-bound isotypes initially present (Shi et al., 2019). These membrane-bound isotypes undergo affinity maturation and isotype switching to create stronger binding antibodies with stronger effector functions (Shi et al., 2019; Abbas et al., 2020). The production of antibodies and the processes of affinity maturation and isotype switching account for some of the longer period of time that it takes the adaptive immune system to respond compared to the innate immune system. Antibodies bind and coat microbes leading to deactivation, as both are engulfed and destroyed by macrophages (Barer et al., 2019). Additionally, antibodies play an important role in activating complement after initial microbe detection, leading to an even more vigorous immune response (Cooper and Nemerow, 1984). Similar to T cells, B cells will clonally expand and create memory cells that will survive in the host to respond faster and stronger upon a second exposure (Barer et al., 2019).

Gestation and Exercise

In humans and ruminant farm animals, gestation is accompanied by many changes to the mother and fetus that can be sensitive to external factors. Several factors can impact the overall quality of gestation, such as nutrition, health, stress, and exercise/movement patterns. Of the influential factors evaluated in human and animal models, published information on gestational exercise remains limited. Gestational exercise may play a critical role in the quality of gestation for dam and fetus and could influence colostrum quality and immune function in humans and animals. For years, physicians have focused on gestational quality of the mother and the role that exercise can play on overall comfort as the body undergoes major changes. However, few have explored the benefits of gestational exercise on colostrum production and composition or the newborn’s ability to utilize colostrum. In livestock, gestational exercise studies have focused on umbilical and placental blood flow, as well as management of maternal body weight and composition and the birth weight of offspring. Gestation continues to be widely studied in both humans and animals but with limitations in regard to the possible benefits of exercise on colostrum.

Factors Impacting Pregnancy in Humans and Animals

In humans and ruminants, the initiation of pregnancy, mechanisms of pregnancy, and means of pregnancy control are similar. Pregnancy is initiated by fertilization of a mature oocyte in females by male sperm. Fertilized eggs will eventually adhere to the endometrium layer of the uterus where the growth and development of the offspring will begin (Spencer and Bazer, 2004; Spencer et al., 2016). Similar to the mechanisms of gestation in humans, farm animals carry their fetus for a period of time in an enclosed placenta (Leiser et al., 1997). The placenta acts as a major element for the protection of the fetus and as a means for delivering key nutrients and oxygen to aid in the growth and development of the fetus(es) (Leiser et al., 1997). Pregnancy is maintained and controlled by both the placenta and corpus luteum through a major hormonal feedback mechanism (Spencer and Bazer, 2004). During the peripartum period, hormones initiate colostrum production and parturition (Macy, 1949; Castro et al., 2011).

One of the most influential factors impacting pregnancy in humans and animals is nutrition. Pregnancy requires essential nutrients to support the growth and development of the fetus, while simultaneously offering both maintenance and energy requirements for the dam. Similar to humans, ruminants require certain nutrients that elevate circulating glucose throughout gestation and provide nutrients for the development of colostrum. Gestating women are advised to follow a healthy diet and observe proper food hygiene to control and limit pathogenic microbial intake (Williamson, 2006). Along with obtaining proper nutrients, medical physicians strongly advise against the intake of drugs, alcohol, or using tobacco products due to the possible injuries or long-term impacts it may have on the fetus (Williamson, 2006). Likewise, livestock must receive proper quantity and quality of nutrients that are free of toxins and other pathogenic microbes. Improper nutrition can have major negative impacts on fetal growth and development, as well as possible long-term effects on the lifetime growth and development of humans and animals (Williamson, 2006; Symonds et al., 2010). Women who receive inadequate nutrient intake during gestation pose a risk to the developing fetus, with potential negative impacts following birth and/or accompanied with long-term effects such as growth restriction and metabolic disorders (Morrison and Regnault, 2016). In livestock, fetal nutrient restriction may negatively impact survival rates after parturition, reduce birth weight (BW) and weaning weight (WW), and decrease performance (Dunlap et al., 2015). Additionally, fetal nutrient restriction in livestock may lead to numerous physiological and metabolic syndromes as the newborn develops, including obesity, hyperglycemia, insulin resistance, and hypertension (Dunlap et al., 2015). Nutrition is one of the most important elements during gestation for both humans and animals because of the need to supply nutrients to the fetus while maintaining normal functions and energy of the mother.

Stress during gestation may have negative impacts on both gestational quality and lactation in humans and animals, as well as possible offspring health and performance in the future. Coussons-Read (2013) suggested that stress factors in human pregnancy can have a wide range of negative impacts both on the mother and child. Physiological stress may leave the gestating female's immune system more susceptible to acute or chronic infections and may predispose the baby to future health risks (Woods et al., 2010). Similar to animals, stress alters endocrine and inflammatory responses (which are already under abrasive changes from pregnancy) in the gestating woman. Greater and repeated exposures to stress elevate glucocorticoids (especially cortisol), which can predispose infants to altered stress reactions and can lead to long-term behavior and physiological problems (Coussons-Read, 2013). In livestock, stress from poor nutrition, transportation, restraint, handling, integration, or removal from the flock/herd can have a negative impact on pregnancy and lactation because of fluctuating levels of stress hormones (Borysenko and Borysenko, 1982; Chernenko et al., 2017). The quality of gestation and the capabilities to produce colostrum are also dependent on management and environmental stress to livestock. Pregnant animals that are exposed to prolonged and/or repeated handling or environmental stress, have lower quality gestation, and may face more health issues. Furthermore, their offspring could be at risk for having decreased vigor at birth, be smaller in size, have reduced performance, weigh less than contemporaries, and have decreased survival rates (Borysenko and Borysenko, 1982; Nowak and Poindron, 2006; Chernenko et al., 2017).

Gestational Exercise in Humans and Animals

Gestational exercise in women has been widely recommended for management of body weight gain, improved self-esteem, and for potential long-term benefits to the fetus. As pregnancy in women advances, weight gain of 12.50 ± 2.50 kg is excepted to account for the growing fetus, placenta, amniotic fluid, and increased blood volume (Lewis, 2014). While gestational weight gain is expected, excessive weight gain can lead to development of maternal diabetes, hypertension, and reduced overall health (Kominiarek and Peaceman, 2017). To help control and limit excessive gestational weight gain, many authors have suggested that moderate and safe exercise routines offer substantial benefits. Effective exercise routines like aerobics or endurance trainings are interventions suggested to control excessive weight gain. Albright (2016) suggested that women were more likely to participate in exercise routines during pregnancy if their physician recommended it. Wang et al. (2019) reported that women who participated in some type of exercise during pregnancy had decreased gestational weight gain compared to women that did not. Mentally, an increase in weight during pregnancy can have a negative impact on women. While women should expect their weight to increase as pregnancy advances, the added weight may leave them feeling more self-conscious. Lewis (2014) reported that the main reasons women exercise while pregnant is to control excessive gestational weight gain which has the psychological benefit of improved self-esteem. In addition to the maternal physiological and mental benefits of gestational exercise, controlling excessive gestational weight gain is linked to lower incidences of parturition issues. Moreover, it has been suggested that gestational exercise may be beneficial to the developing fetus by lowering its chances of developing long-term health problems such as metabolic disorders, obesity (especially leptin resistance), and diabetes (Kominiarek and Peaceman, 2017).

Modern society has begun to embrace the idea of a “fit pregnancy” (Garshasbi and Zadeh, 2005; Barakat et al., 2014). Women who partake in gestational exercise routines better maintain or increase overall fitness and toning of muscles (Lewis, 2014). According to Garshasbi and Zadeh (2005), one of the goals of gestational exercise may be to improve overall muscle function, biomechanics, posture, and strength of the lower back and pelvis. It has been reported that women in the second and third trimesters who adopt an aerobic and strengthening exercise routine have less lower back pain and increased spinal flexibility (Garshasbi and Zadeh, 2005). Additionally, exercise has been reported to improve overall functionality (ability to move freely in a more comfortable manner) and decrease lumbar and pelvic girdle pains in pregnant women (Kluge et al., 2011). These improvements in posture, muscle tone, and pain management as a result of gestational exercise may contribute to a more successful labor (Nascimento et al., 2012).

The immunological and colostrum consequences of gestational exercise have not been widely studied. Exercise during pregnancy has been implied to modulate the immune inflammatory response (Acosta-Manzano et al., 2020). Physical activity during pregnancy has also been linked to improved interleukin-6 and interleukin-8 immune function but has been negatively associated with interleukin-1β function and may suppress tumor necrosis factor α (Acosta-Manzano et al., 2020). As concluded by Acosta et al. (2020), the level of intensity of gestational exercise has produced mixed results regarding influence on key cytokines and immunometabolism markers. However, Acosta et al. (2020) cautiously suggested that some increase in physical activity may impact the cytokine profile without disrupting pregnancy. Physical activity may be used as a complementary therapeutic target for controlling immunometabolism responses (Acosta-Manzano et al., 2020). Additionally, Acosta-Mazano et al. (2019) concluded in a separate study that women who exercised during pregnancy had lower tumor necrosis factor compared to women that did not exercise. However, the results were not significant after adjusting for appropriate confounding factors, such as adjustments made amongst treatment groups for parity status and length of gestation at delivery (Acosta-Manzano et al., 2019). To date, Acosta-Manzano et al. (2020) have concluded that the scientific literature does not provide strong correlations between gestational exercise and the possible maternal or fetal immune benefits or major detriments.

Gestational exercise routines are recommended to be kept in moderation in type, duration, and intensity. It is suggested that pregnant women participate in varied exercise routines. Straight line or stationary exercise routines are deemed safe for pregnant women (Lewis, 2014). Exercise routines such as walking, jogging, swimming, and cycling are suggested as safe routines as long as they are kept in moderation (Lewis, 2014). Additionally, strength and endurance trainings are suggested to better improve overall muscle tone and posture (Nascimento et al., 2012). A combination of both walking and strength/endurance trainings has been linked to having many benefits for pregnancy pains and overall muscle toning and posture (Garshasbi and Zadeh, 2005; Nascimento et al., 2012). The duration of gestational exercise is varied. Exercising for 60 minutes, 3 times per week have yielded positive results in gestating women, such as controlled gestational weight gain and muscle toning (Garshasbi and Zadeh, 2005). Additionally, a summary of the literature by Berghella (2017) reported that scientists and physicians recommended 30 to 60 minutes of exercise, at least 3 to 4 days per week. Albright (2016) suggested that up to 40 minutes of moderate exercise was well tolerated by low-risk pregnant women. Furthermore, Lewis (2014) suggested that moderate gestational exercise 3 to 4 days per week may be beneficial to the mother's weight gain and overall mobility. The intensity of gestational exercise is strongly suggested to be kept in moderation for protection of the mother and fetus. Previously sedentary women are strongly advised to avoid intense workouts, while women who were previously active before pregnancy may be allowed to participate in more intense exercise routines (Nascimento et al., 2012). Workouts that become too intense become a physiological distress (as opposed to positive eustress) on the body and may negatively impact maternal heartbeat, increase incidences of hypoxia, and negatively impact the fetus. Regardless of the type, duration, and intensity of maternal exercise routines, it is strongly suggested that women partake in moderate and safe routines to protect themselves and the fetus.

In livestock species, the literature regarding maternal exercise is very limited and offers few benefits. Harris et al. (2013) reported that 30-minutes of gestational exercise in swine increased umbilical blood flow compared to gilts that were held in confinement from day 40 of gestation until parturition. Following weaning and rebreeding, gilts were entered into a second experiment following the same exercise routine. Once again, sows that were exercised during the second parity had increased umbilical flood flow compared to sows that were held in confinement during the final phase of gestation (Harris et al., 2013). The authors concluded that the exercise routine throughout gestation helped promote blood flow, leading to improved delivery of nutrients to the fetuses and placenta (Harris et al., 2013). In sheep, Curet et al. (1976) reported that gestational exercise promoted more evenly distributed blood flow to the placenta, which may have potential nutrient and gas exchange benefits for the fetus.

There is currently minimal evidence to suggest that moderate and safe gestational exercise poses a risk to the mother and/or fetus in both humans and animals. According to Nascimento et al. (2012), there is minimal evidence suggesting that maternal exercise in women is harmful to the fetus. Likewise, Lewis (2014) found no evidence suggesting risk to the fetus from gestational exercise. That said, exercise routines should be conducted safely. Exercise activities that can lead to injury and trauma to the fetus should be avoided. Activity sports, horse riding, and activities that lead to overheating, which may cause decreased fetal nourishment, should be avoided (Nascimento et al., 2012; Lewis, 2014).

In livestock species, scientific studies remain more conflicted. According to Chandler et al. (1985), 45-mintues of moderate exercise on a treadmill in late gestation decreased umbilical blood flow in sheep. However, it was also concluded that uterine and umbilical oxygen uptakes were not impacted as a result of gestational exercise (Chandler et al., 1985). Additionally, Emmanouilides et al. (1972) reported that gestational exercise in sheep compromised umbilical and uterine blood flow. However, the authors believed that the fetal lamb tolerated the maternal exercise well (Emmanouilides et al., 1972). Lotgering et al. (1983) suggested that maternal exercise in ewes may be stressful to the fetus by a reduction in uterine blood flow. However, despite increased body temperatures by both the ewe and fetus and decreased uterine blood flow, the authors concluded that uterine oxygen uptake remained stable posing minimal hypoxic and cardiovascular risk to the fetus from gestational exercise (Lotgering et al., 1983). If performed safely and in moderation, gestational exercise in humans and livestock has not been linked to serious risk factors to mother or fetus.

Research examining the impact of gestational exercise on the health and wellbeing of sheep is conflicting and sparse. There are no studies examining the relationship between maternal exercise during pregnancy on production and composition of colostrum and the subsequent benefit (or detriment) to the transfer of PI from the dam to the newborn lambs. Therefore, the hypothesis for this research is that exercising ewes in the final trimester does not have a significant impact on IgG levels in colostrum nor does it have a significant impact on neonatal Ig absorption. The hypotheses will be rejected only if there is a significant difference in treatment and control group IgG levels and neonatal IgG absorption at a P-value < 0.05.

The objectives of this research study are to evaluate several maternal and offspring characteristics that may or may not be influenced by gestational exercise performed at around the third trimester and lasting until lambing starts. Maternal traits evaluated are: step counts (for all days and only days when no sheep are exercised and confined in their pens), body weights, average daily feed intake (ADFI) and supplement intake, and colostrum IgG concentrations and quality of colostrum, which includes crude protein (CP), crude fat, and DM percentages. Traits measured in the lamb are: BW, WW, serum protein and cortisol levels prior to nursing, and serum IgG, protein, and cortisol after the consumption of their own dam’s colostrum.

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CHAPTER 2: THE INFLUENCE OF LATE GESTATIONAL EXERCISE ON DORSET EWE PERFORMANCE, FEED INTAKE, COLOSTRUM IgG, AND NEONATAL PASSIVE IMMUNITY AND PERFORMANCE

Abstract

The objectives of this research were to determine the impact of exercise during the final trimester of gestation on colostrum IgG levels and quality, ewe performance and feed intake, lamb serum IgG and protein levels after colostrum consumption, and growth through weaning. Multiparous, pregnant Dorset ewes (n = 32) were selected based on breed type, age/parity, weight, body condition score, and health status. Ewes were blocked through pairing ewes of similar age and weight in the opposite treatment group. Ewes were housed individually in pens of equal size. Sixteen ewes assigned to the non-exercise (NO-EX) group remained in their respective pens until after lambing. The 16 ewes assigned to the exercise (EX) group were exercised at their own pace by walking 30 minutes per day, every other day until lambing. Feed and supplement intakes were measured for all ewes and each ewe was weighed weekly until lambing. All lambs (n = 48) were weighed at birth and weaning. Blood samples were collected on lambs at birth and after which, each lamb was administered 8 ± 2% of colostrum per kg of birthweight. A second lamb blood sample was obtained 28 ± 4 hours after colostrum consumption. Maternal characteristics evaluated included: average steps per day during exercise and/or during confinement, feed intake, body weight gain over time, and colostrum IgG, crude protein, crude fat, and dry matter percentages. Offspring characteristics included: birth weight, weaning weight, postpartum performance, serum protein and cortisol at birth and after colostrum feeding, and serum IgG post-colostrum consumption. Data were analyzed using the mixed procedure of SAS. Exercised ewes averaged a greater number of steps per day throughout all of gestation ($P < 0.001$) and took more steps during days when they remained penned ($P = 0.0297$) compared to NO-EX. Ewes that remained penned throughout gestation (NO-EX) had greater average daily feed intake ($P = 0.0318$) and supplement intake ($P = 0.0165$). Ewes in NO-EX gained more weight over time ($P = 0.0230$) and had superior average daily gain ($P = 0.0457$). There were no differences seen for lamb birth weight, weaning weight, or average daily gain when separated by dam treatment. No differences were observed in serum cortisol concentration at parturition for lambs born to EX or NO-EX. Lamb serum protein concentration did not differ between treatments at birth, but EX serum possessed 0.66 g/dL greater protein content ($P = 0.039$) following colostrum consumption. Ewes from EX had a greater concentration (mg/dL) of IgG ($P = 0.0366$) in colostrum than NO-EX. Colostrum crude protein, crude fat, and dry matter percentages did not differ between treatments. Concentrations of serum IgG and cortisol in lambs at post-suckling did not differ across treatments.

Introduction

Human research has revealed the many benefits associated with gestational exercise. Gestational exercise routines such as aerobics, endurance training, muscle toning/stretching, or other posture exercises can play a role in a healthy pregnancy through management of excessive weight gain and gestational diabetes (Lewis, 2014; Albright, 2016). Given the large body of scientific literature extolling the benefits of exercise for human mother and developing baby, can the same benefits of exercise be applied in animal agriculture? Modern intensive management of livestock has evolved to be more confinement oriented; reducing the opportunity animals have to naturally “exercise.” This is especially true in sheep and goat operations where it may be more feasible to house animals in closer quarters due to their size and especially during harsh winter climates. This concentration of animal production and the subsequent restriction of animal movement during pregnancy has not been widely evaluated for many gestational components. Few studies have attempted to draw correlations between gestational exercise and maternal, fetal, and neonatal health, growth, and development. The research that has been conducted has focused primarily on understanding how gestational movement influences gas exchanges and blood flow/distribution from the dam to the developing offspring and how such exercise routines may influence feed intake in rodents (Chandler et al., 1985; Novak, 2012). Similar to how housing space is an important element to livestock production, colostrum is also an absolutely vital component in livestock survivability and future health of flocks and herds. Not only does colostrum provide essential immune cells and proteins that protect newborns, but it is also richly abundant in other key nutritional and thermoregulatory components (Khan and Ahmad, 1997). Numerous scientific studies have pointed to the importance of colostrum in neonatal ruminants and the many critical factors that can influence the quality of colostrum synthesized (Khan and Ahmad, 1997; Banchero et al., 2004; Campion et al., 2019).

Of the many factors that may influence colostrum quality, gestational exercise is one that has not been explored. Although a few studies have evaluated how gestational exercise in sheep and swine impacts placental blood flow and gas exchanges, a gap in literature exists to explain how gestational exercise influences colostrum IgG levels (Chandler et al., 1985; Harris et al., 2013). The literature also does not make implications into whether or not walking routines and patterns of the dam impacts her offspring's ability to absorb IgG. These limitations also exist in human gestational science. Although many scientists and physicians continue to promote physical exercise and activity for maternal benefits and possible long-term benefits to the baby, they do not provide implications to how it may influence immune components of colostrum and PI in the newborn.

If livestock have the ability to move with adequate space, versus being held in tighter packed facilities during gestation, this may impact their eating behaviors and weight gain. These changes may have impacts on the BW and performance of their offspring. Additionally, if livestock are provided with more adequate housing space to move freely during pregnancy, there may be beneficial impacts to their colostrum quality, especially IgG concentration.

The main objectives of this respective study were to analyze how late gestational exercise, performed from approximately the start of the third trimester to lambing, impacts the ewe and her newborn(s). The traits of interest in the ewe were how an exercise routine or how being held in confinement impacts maternal movement activity (movement behaviors both on days exercise occurs and days that no exercise occurs), feed intake, body weight gain, and colostrum IgG and quality. The traits of interest in the lamb were how their dam's exercise routine or confinement impacted their birthweight, weaning weight, average daily gain, serum IgG after colostrum consumption, and serum protein and cortisol both at birth and after colostrum feeding.

Materials and Methods

All live animal procedures were reviewed and approved by the North Dakota State University Institutional Animal Care and Use Committee (Protocol #A20081). All animals were sourced from the North Dakota State University Sheep Unit (Fargo, ND) and all control and treatment procedures were performed at the North Dakota State University Animal Nutrition and Physiology Center (Fargo, ND).

Selection of Research Animals

Ewes for inclusion in the research were selected from the flock of multiparous Dorset ewes (n = 69) available from the North Dakota State University (NDSU) Sheep Unit. Selection criteria for inclusion were: ewes must be pregnant, all ewes must be multiparous (had at least one successful parturition and raised a lamb to weaning), and must be around 45 to 50 days pregnant as determined by ultrasonography. The initial ultrasound was used to confirm pregnancy and to obtain a prediction of the number of fetuses present. Age, body weight, and body condition score (BCS) was recorded at the time of the first ultrasound. Ewes were blocked by treatment with a pair of similar age, BCS, weight, and predicted fetal count. Ewes that could not be matched closely were not used in the project. The final sample group included 16 pairs (n = 32) with 14 pairs of identical age and 2 pairs were 1 year apart. The average weight difference between pairs was 3.25 ± 3.25 kg. Ewes within a pair were randomly assigned to control (no exercise; NO-EX) or treatment (exercise; EX). A second ultrasound was performed between 75 to 80 days of gestation to confirm continued pregnancy and recount fetuses. Following the confirmation of pregnancy, all candidates were placed in a large pen and evaluated for extreme stress response such as excessive running and/or escape attempts, waste excretion, or vocalization in response to human movement and vocalization. No ewes exhibited extreme stress behavior. The final 32 pregnant Dorset ewes were transported to the NDSU Animal Nutrition and Physiology Center (ANPC).

Acclimation Period

Ewes were weighed upon arrival to ANPC and assigned to individual pens. Each pen (1.524 × 1.220 meters) was fitted with plastic, slatted flooring material, and included a feed and water trough. All pens were on the same side of the ANPC Sheep Wing, in 2 rows, directly in series to one another, with exit/entry gates on opposing sides. After entry processing, ewes were placed in their individual pens and provided *ad libitum* access to hay for the first week.

All ewes were given 3 days (including the day they arrived) to rest and recover from transportation and become familiarized with their new environment. On day 4, the 16 EX ewes were taken from their pens and walked, as a group, outside of ANPC. A circular exercise pen was assembled on the north lawn of the ANPC. The circular pen was composed of 2 portions, an outer and inner ring (Fig 2.1). Sheep were gently encouraged to exercise within the confines of the panels to maintain a constant forward circular motion. Day 1 and 2 of acclimation included walking ewes at their own pace and as a group for 10 minutes in the morning prior to feeding. On days 3, 4, and 5, the previous routine was extended to 20-minute exercise bouts. By day 6 and 7, the exercise bouts were increased to 30-mintues. Acclimation exercise bouts occurred at the same time each day by consistent personnel. Following exercise, ewes were returned to their pens and fed. All EX-sheep were given 1 full day of rest following the 7-day exercise acclimation period.

Figure 2.1. Exercise pen composed of an inner and outer circular section that facilitated movement of sheep in a continuous circular motion as a group for 30 minutes per day, every other day, from the start of the third trimester until lambing.

Exercise Period

The exercise protocol and data collection began after 1 week of the ewes becoming acclimated to handlers, exercise, and the exercise pen. The exercise protocol was administered by consistent technicians familiar to the sheep and occurred at approximately 800 hours on each exercise day. The EX sheep were moved from their home pens to the exercise pen (Fig 2.1) where they completed a constant exercise bout for 30 minutes per day, every other day, until the first EX ewe gave birth. To discourage grass eating in the exercise pen, the grass was mowed short, and sheep were prompted to move forward at their own pace while a technician walked behind them. To quantify steps, Fit Bit Flex step counters were placed on the front leg above the knee of the 4 lightest and 4 heaviest EX ewes and their respective NO-EX pairs also received them. Step counting began immediately upon placement on the first day of the exercise routine.

Ewe Health, Body Weights, and Feed and Supplement Intake

Approximately 3 weeks prior to the start of lambing, all ewes were administered a *Clostridium perfringens* C, D, and tetanus vaccination. Body weights were recorded every Wednesday morning at approximately 700 hours, even when days of weight recordings were also on days of exercise. Final body weights were collected 3 days before the first ewe lambed. At the start of the research study, feed allotment was provided for each ewe based on a percentage bodyweight basis whereby ewes were provided feed at 2.50% per kg of live weight. Average daily feed intake (ADFI) was recorded. For the first 4 weeks, feed provided per day was adjusted for each sheep based the consumption patterns of the previous week. On week 5, feed rations were increased by 10% from the previous week’s allocation (that amount was continued until the conclusion of the project) and were administered a temporary corn supplement prior to the start of a soybean meal pellet supplement. Three weeks prior to the start of lambing, ewes were provided a soybean pellet supplement, which was calculated for each individual ewe. The supplement calculation took into consideration how much hay each respective ewe was consuming and the proximate analysis of the hay to best provide sufficient nutrients. Supplement was always fully consumed and was increased whenever hay consumption was increased. All supplement was provided to the ewe at their morning feeding and lasted until they were sent back to the NDSU Sheep Unit. All feed was submitted to the NDSU Nutritional Laboratory for proximate analysis and was used in feeding calculations and supplement requirements (Table 2.1).

Table 2.1. Proximate analysis of hay, corn supplement, and soybean pellet meal supplement fed to ewes during the third trimester of gestation.

Nutrient	Hay	Corn Supplement	Soybean Meal Supplement
Dry Matter, %	95.75	88.21	88.68
Ash, %	8.87	1.32	5.53
Crude Protein, %	10.00	7.98	16.16
Nitrogen, %	1.5992	1.2760	2.5848
Fat, %	1.17	-	-
Starch, %	3.21	-	-
Non-Detergent Fiber, %	66.27	18.46	19.89
Acid Detergent Fiber, %	41.65	3.21	10.74
Acid Detergent Lectin, %	6.69	0.27	0.60
Calcium, %	0.5468	0.0072	0.8685
Phosphorus, %	0.2220	0.2666	0.3622

Lamb Birth Weight and Blood Samples

Following the birth of the first lamb, all exercise routines were stopped, and ewes were closely monitored 24-hours per day until full-term, noninduced lambing ended across both groups. The lambing procedures were the same across both groups. In the case of dystocia or other complications, assistance was offered.

Lambs that did not require assistance at birth remained with the ewe to receive maternal attention (licking and maternal imprinting). Once lambs were strong enough to stand, they were weighed and afterwards, had 10 mL of blood withdrawn from their jugular vein using a 22-gauge needle with a 12-inch catheter. The blood was immediately placed into a red top serum tube (BD Vacutainer Serum, Franklin Lakes, NJ) and properly secured and labeled.

Colostrum Collection and Feeding

The ewe was milked evenly from both teats using a hand milker that attached to the ewe's teat. To ensure clean colostrum, the ewe's teats were primed by hand to allow the first of the colostrum to be drained out prior to obtaining colostrum from the milker. The amount collected was based on the BW of the lamb(s). From the collection, 14 mL was used as a colostrum sample and was placed into a polystyrene conical tube. Each lamb received 8 ± 2% of its BW of colostrum. Colostrum was fed using a small tuber that fed directly into the newborn's stomach. Following colostrum feeding, lambs were returned to their dams.

Serum Separation and Equipment Sanitation

Blood and colostrum samples were set at room temperature for 30 minutes after collection. Blood was then placed in a centrifuge and spun (4^{O}C, 1,550 XG) for 20 minutes. After centrifuge, serum was separated with a pipet. Serum was placed in 2-mL plastic serum tubes. All serum samples were properly labeled and frozen immediately at -20^{O}C until further analysis. Colostrum samples were placed in the same freezer as the serum samples at a temperature of -20^{O}C until further analysis. Any supplies used to obtain, or feed colostrum, were washed following use and air dried. Supplies were rinsed with hot water and washed using Nolvasan (chlorhexidine diacetate) mixed appropriately with water. One mega omh water was used for the final rinsing of supplies.

Lamb Blood Sample Post-Colostrum Consumption and Weaning

A second blood sample, using the same procedures as the first one, was obtained from all lambs 28 ± 4 hours following colostrum consumption. Following the second blood sample, ewes and lambs remained at the ANPC until the NDSU Sheep Unit deemed them strong enough to be transported back. Lambs were weaned from their dams at an age of 68 ± 13 days to record and calculate an adjusted WW (adjustment factors specific to Dorset sheep), adjusted average daily gain (ADG), and birth to weaning performance.

Colostrum IgG and Serum IgG Analysis

Ewe colostrum and second lamb serum samples (obtained after colostrum consumption) were tested for IgG levels on a volume basis. Saline [0.85% (wt/vol) sodium chloride] was used as a dilution. All serum and colostrum samples were thawed at room temperature. For each colostrum sample preparation, 875 µL of saline and 125 µL of colostrum was placed into individual, 1.5 mL-microcentrifuge tubes. For each serum sample preparation, 600 µL of saline and 200 µL of serum were placed into individual, 1.5 mL-microcentrifuge tubes and vortexed. After vortex, 5 µL per colostrum and serum samples were loaded onto radial immunodiffusion plates (RID) for ovine (Triple J Farms, Bellingham, WA). Following loading of all plates, they were sealed in their respective bags and placed in styrofoam containers for 48 hours at room temperature. After 48 hours, each plate well was measured using a caliper. Three ring diameter measurements were taken to obtain a final average ring diameter that was recorded. The average coefficients of variance for colostrum and serum IgG were 4.82% and 6.28%, respectively.

Colostrum Crude Protein, Crude Fat, and Dry Matter Analysis

Colostrum samples were analyzed for CP and crude fat (Eurofins Nutrition Analysis Center, Des Moines, IA). The CP was analyzed using a combustion method to extract a protein percentage (AOAC 992.15; AOAC 990.03; AOCS Ba 4e-93). Crude fat was analyzed using acid hydrolysis to extract a fat percentage (AOAC 954.02).

The DM percentages were analyzed by heating 1 mL of colostrum in aluminum pans (5.5 cm) as recommended by the NDSU Nutritional Analysis Laboratory (Fargo, ND). All samples were heated at a temperature of 135^{O}C for 2 hours using Isotemp Drying Oven (Thermo Fisher Scientific, Waltham, MA).

Serum Protein and Serum Cortisol Analysis

All lamb serum protein was analyzed by Biuret method using the Total Protein Reagent Set T7528-1L (Pointe Scientific, Township, MI) with Bovine Serum Albumin P5369-10m (Sigma Aldrich, St. Louis, MO) as the standard and Data-Trol Normal Control Serum TR40001 (Thermo Fisher Scientific, Waltham, MA) as the control. Following loading, each microtiter plate was ran using a Synergy H1 Microplate Reader (Biotek, Winooski, VT) for 12 minutes with results recorded following the end of the test.

All lamb serum samples collected prior to and post-colostrum consumption were analyzed for cortisol using a solid-phase, competitive chemiluminescent enzyme immunoassay kit according to the instructions provided by the manufacturer (IMMULITE 1000; Siemens Healthineers, Los Angeles, CA). All samples were analyzed on the same day with an average intra-assay coefficient of variance of 4.3025%.

Statistical Analysis

Data were analyzed using the mixed procedure of SAS, version 9.4 (SAS Institute, Cary, NC). Ewe pairs served as a blocking factor. Depending on the model used and if it was critical for biological reasons, ewe pair served as a covariate, fixed effect (n = 16), or was not included in the analysis. Model fit based on class effects and covariates were assessed across models using standard Akaike Information Criterion C (AICC) and Bayesian Information Criterion (BIC) statistics from SAS output.

Results

The final model analyzed for traits of ewe steps, feed and supplement intake, weight, and performance, and lamb weight and performance are provided in Table 2.2. The final model analyzed for traits of ewe colostrum components and lamb serum traits are provided in Table 2.3. Additionally, for each trait of both tables, ewe pair is identified in the analysis as either not included, analyzed as a fixed effect, or analyzed as a covariate. The determination of how to assess ewe pair was based on comparing each output (excluding ewe pair, analyzing ewe pair as a fixed effect, or analyzing it as a covariate) with one another and identifying the lowest AICC, which accounted for sample size parameter estimates, and BIC, which accounted for fit of model, and residual variance for error of model. Results were considered significant at a level of $P \leq 0.05$ and a tendency at $P \leq 0.10$.

Table 2.2. Variables used in final statistical models to assess ewe and lamb trait differences attributed to ewes subjected to late gestational exercise versus ewes who did not exercise and whether ewe pair was not used, used as a fixed effect, or used as a covariate for each trait.

	Ewe Traits							**Lamb Traits**			
	Fit-Bit All Days[5, a]	Fit-Bit Non-Exercise Days[6, a]	Average Daily Feed Intake[a]	Supplement Intake[a]	Weight[c]	Feed to Gain[a]	Average Daily Gain[b]	Birth Weight[c]	Average Daily Gain[b]	Weaning Weight[b]	Weight Gain[7, b]
Fixed											
Pair[1]	-	-	-	-	-	-	-	-	*	*	*
Age	-	-	-	-	-	-	-	-	-	-	-
Sex	-	-	-	-	-	-	-	*	-	-	-
Birth Type[2]	-	-	*	*	*	-	-	*	-	-	-
Treatment[3]	*	*	*	*	*	*	*	*	*	*	*
Week	-	-	*	*	*	-	-	-	-	-	-
Weight	-	-	-	-	*	*	-	-	-	-	-
Treatment*Week[4]	-	-	-	-	*	-	-	-	-	-	-
Covariate											
Pair	-	-	-	-	*	-	*	*	-	-	-
Random											
Dam	-	*	*	*	-	*	*	-	*	-	-
Maternal Grand Sire	-	-	-	-	-	-	*	-	*	-	-
Maternal Grand Dam	-	-	-	-	*	-	-	-	-	-	-
Sire	-	-	-	-	-	-	-	*	*	-	-
Paternal Grand Sire	-	-	-	-	-	-	-	-	*	*	*

* = Used in the statistical model

\- = Not used in the statistical model

a = Trait analyzed excluding ewe pair as a blocking factor due to AICC, BIC, and residual variance values

b = Trait analyzed using ewe pair as a fixed effect due to AICC, BIC, and residual variance values

c = Trait analyzed using ewe pair as a covariate due to AICC, BIC, and residual variance values

[1] Pair = Ewe pair (blocks), 1 ewe from the control group and 1 from the treatment group paired together based on similar age and weight

[2] Birth Type = Number of lambs born, either single or twin

[3] Treatment = Treatment group, either NO-EX (control, ewes that were not exercised through the third trimester of gestation) or EX (treatment, ewes that were exercised through the third trimester of gestation)

[4] Treatment*Week = Treatment by week interaction

[5] Fit-Bit All Days = Average ewe steps recorded by a Fit-Bit Flex on all days

[6] Fit-Bit Non-Exercise Days = Average ewe steps recorded by a Fit-Bit Flex on days that no sheep were exercised

[7] Weight Gain = Total lamb weight gained from birth until weaning

Table 2.3. Variables used in final statistical models to assess ewe colostrum traits and lamb serum traits attributed to ewes subjected to late gestational exercise versus ewes who did not exercise and whether ewe pair was not used, used as a fixed effect, or used as a covariate for each trait.

	Ewe Colostrum Traits				**Lamb Serum Traits**					
	Immunoglobulin G[c]	Crude Protein[a]	Crude Fat[c]	Dry Matter[c]	Immunoglobulin G[b]	Protein at Birth[a]	Protein at 24 to 32 Hours[6, a]	Protein Difference[7, b]	Cortisol at Birth[a]	Cortisol at 24 to 32 Hours[8, c]
Fixed										
Age	-	-	-	-	*	-	-	-	-	-
Bleed Age[1]	-	-	-	-	*	-	-	*	-	-
Pair[2]	-	-	-	-	*	-	-	-	-	-
Colostrum Intake[3]	-	-	-	-	*	-	-	-	-	-
Birth Type[4]	-	-	-	-	*	*	*	*	*	*
Sex	-	-	-	-	*	*	*	*	*	*
Treatment[5]	*	*	*	*	*	*	*	*	*	*
Covariate										
Pair	*	-	*	*	-	-	-	-	-	*
Random										
Dam	-	*	*	*	-	*	*	*	*	*
Maternal Grand Sire	-	-	-	-	-	*	*	*	-	-
Sire	-	-	*	*	-	*	*	*	-	*
Paternal Grand Sire	-	-	-	-	-	*	*	*	*	-
Paternal Grand Dam	-	-	-	-	-	*	*	*	-	-

* = Used in the statistical model
- = Not used in the statistical model
a = Trait analyzed excluding ewe pair as a blocking factor due to AICC, BIC, and residual variance values
b = Trait analyzed using ewe pair as a fixed effect due to AICC, BIC, and residual variance values
c = Trait analyzed using ewe pair as a covariate due to AICC, BIC, and residual variance values

[1] Bleed Age = Bleed age (hours) of lamb when blood sample was obtained

[2] Pair = Ewe pair (blocks), 1 ewe from the control group and 1 from the treatment group paired together based on similar age and weight

[3] Intake = Amount of colostrum consumed by lamb as a percentage of its birthweight

[4] Birth Type = Number of lambs born, either single or twin

[5] Treatment = Either NO-EX (control, ewes that were not exercised through the third trimester of gestation) or EX (treatment, ewes that were exercised through the third trimester of gestation

[6] Protein at 24 to 32 Hours = Lamb serum protein at age 28 ± 4 hours

[7] Protein – Difference = Difference of total lamb serum protein levels from birth to age 28 ± 4 hours

[8] Cortisol at 24 to 32 Hours = Lamb serum cortisol at age 28 ± 4 hours

Ewe Step Counts

Ewes subjected to exercise every other day from the third trimester to lambing averaged more steps per day (*P* < 0.001) than ewes who remained in individual pens without exercise (Table 2.4). The EX sheep also averaged more steps per day on the days that they remained confined to their individual pens than did the NO-EX sheep on the same day (Fig 2.2).

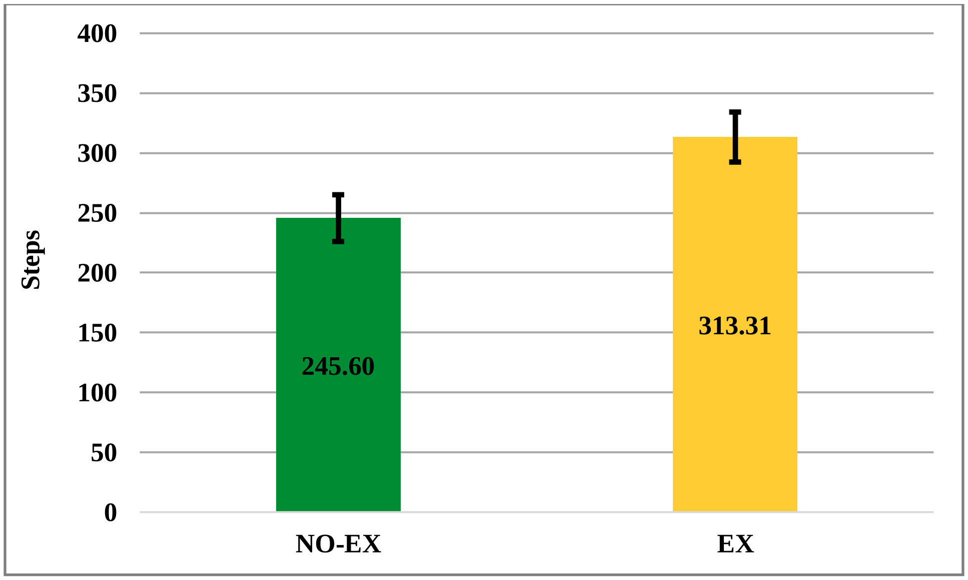

Figure 2.2. Least square means and standard errors for the average step counts per day when no sheep were exercised, separated by treatment group as exercised (EX) versus ewes not exercised (NO-EX) from the third trimester to lambing.

Ewe Body Weight and Average Daily Gain

There was a significant (*P* = 0.023) bodyweight over time interaction observed (Fig 2.3). The first week on test, EX and NO-EX did not differ in bodyweight. Differences were seen on week 2 as EX lost weight while NO-EX continued to gain. No differences were seen between treatments from weeks 3 to 7, however EX ewes remained lighter. The NO-EX ewes finished the research trial heavier than EX at the end of week 8 (Fig 2.3). Ewes that remained in pens without exercise had a greater ADG (*P* = 0.046; Fig 2.4) compared to EX but did not differ in feed efficiency expressed as feed to gain (*P* = 0.120; Table 2.4).

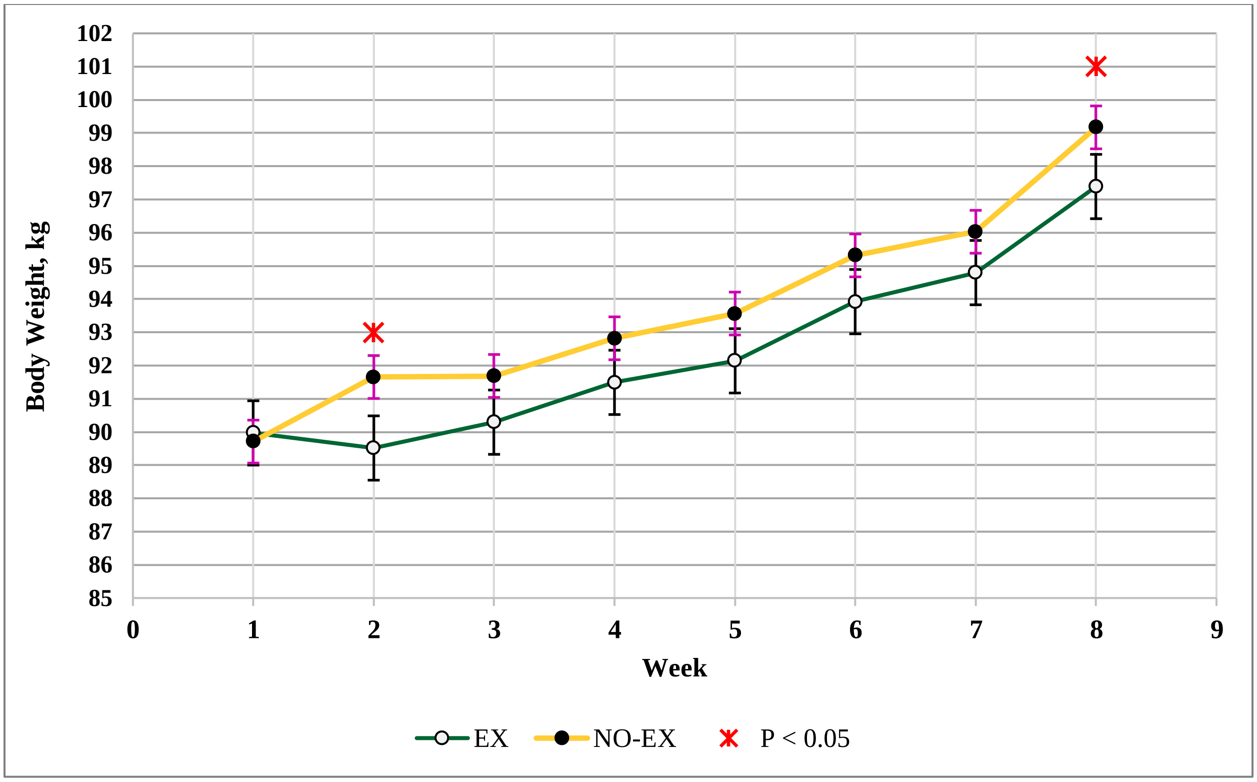

Figure 2.3. Least square means and standard errors for body weight (kg) over time for ewes that were exercised (EX) versus ewes not exercised (NO-EX) from the third trimester to lambing. *significance at $P < 0.05$.

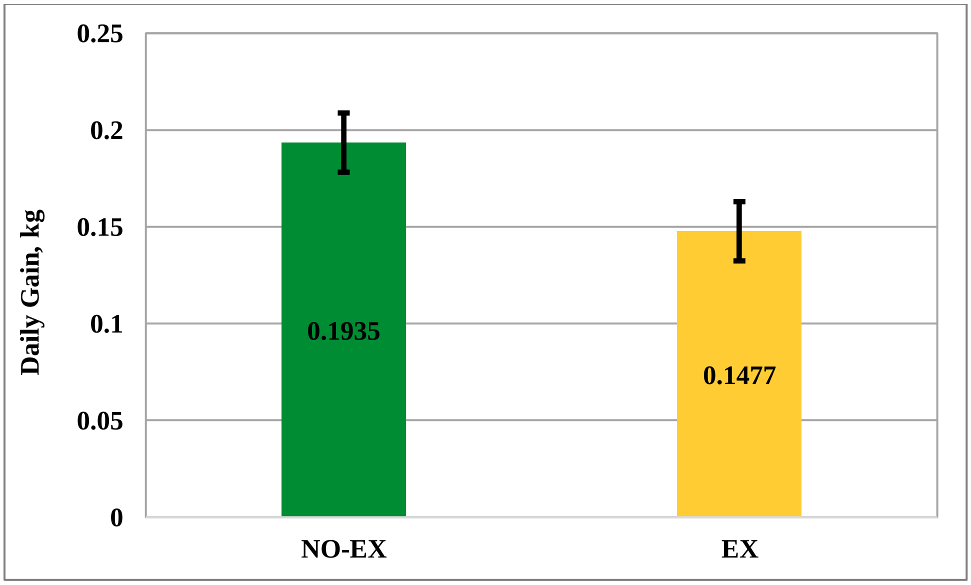

Figure 2.4. Least square means and standard errors for average daily gain (kg) for ewes that were exercised (EX) versus ewes not exercised (NO-EX) from the third trimester to lambing.

Ewe Feed and Supplement Intake

Ewes that were not subjected to exercise consumed more hay (*P* = 0.003; Fig 2.5) and soybean meal pellet supplement (*P* = 0.017) than EX ewes (Table 2.4)

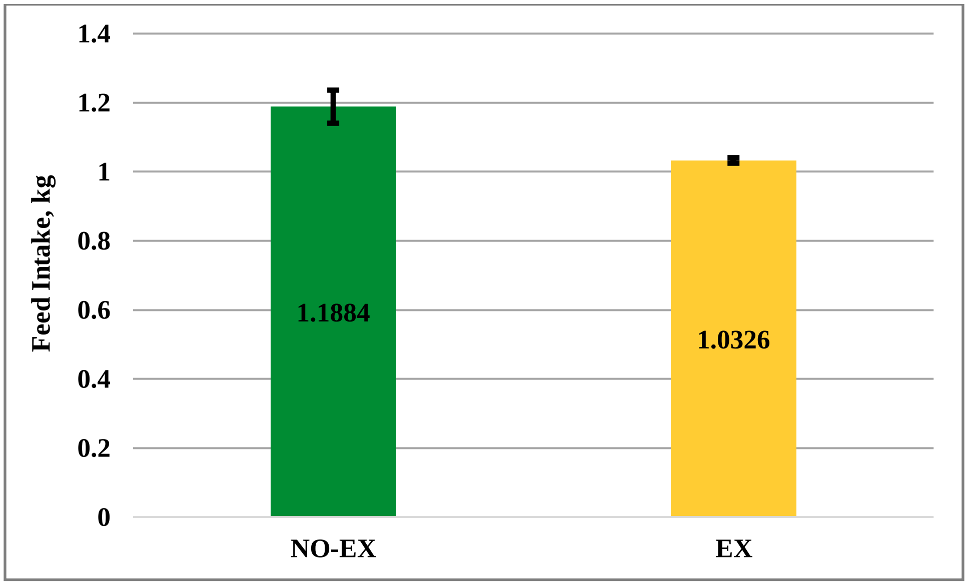

Figure 2.5. Least square means and standard errors for average daily feed intake (kg) for ewes that were exercised (EX) versus ewes not exercised (NO-EX) from the third trimester to lambing.

Table 2.4. Least square means and standard errors for total ewe steps, supplement intake, and feed efficiency separated by treatment group and lamb birth weight, weaning weight, and average daily gain separated by dam treatment group.

	Treatment		
	NO-EX[1]	**EX[2]**	***P*-Value**
Ewe Traits			
Step Counts - All Days	226.29 ± 92.68	1817.63 ± 104.68	< 0.0001
Soybean Meal Supplement Intake, kg	0.2096 ± 0.0077	0.1814 ± 0.0079	0.0165
Feed to Gain Efficiency, kg	0.9199 ± 0.3868	1.7982 ± 0.3868	0.1198
Lamb Traits			
Birth Weight, kg	4.8555 ± 0.2109	4.8336 ± 0.1915	0.9104
Average Daily Gain, kg	0.6105 ± 0.0354	0.5656 ± 0.0350	0.3811
Weaning Weight, kg	24.1298 ± 0.9588	23.4577 ± 0.9588	0.6299
Weight Gain, kg	19.5045 ± 0.9505	18.8126 ± 0.9505	0.6108

[1] NO-EX = Ewes that were not exercised through the third trimester of gestation

[2] EX = Ewes exercised through the third trimester of gestation

Lamb Birth Weight, Performance, and Weaning Weight

Lambs born to EX ewes did not differ (*P* = 0.9104) in BW than lambs born to NO-EX ewes (Table 2.4). There were no differences in lamb weaning weight (*P* = 0.6299), total weight gained from birth to weaning (*P* = 0.6108), or average daily gain (*P* = 0.3811) despite NO-EX lambs having a very slight advantage in growth (Table 2.4).

Traditional differences were seen with ram lambs born heavier than ewe lambs (*P* = 0.0197) and singlets weighing more than lambs born with a twin (*P* = 0.0168; data not shown). Furthermore, birth type (single or twin) had a significant impact (*P* = 0.001) on weaning and ADG whereby singles gained more daily compared to twins (data not shown). No treatment by sex or treatment by birth type interactions were observed.

Colostrum IgG, Crude Protein, Crude Fat, and Dry Matter

Ewes in EX had greater colostrum IgG (*P* = 0.0366) compared to ewes in confinement (Fig 2.6). Colostrum CP, crude fat, and DM percentages were not different by treatment (*P* = 0.2317, *P* = 0.5576, and *P* = 0.9333, respectively; Table 2.5).

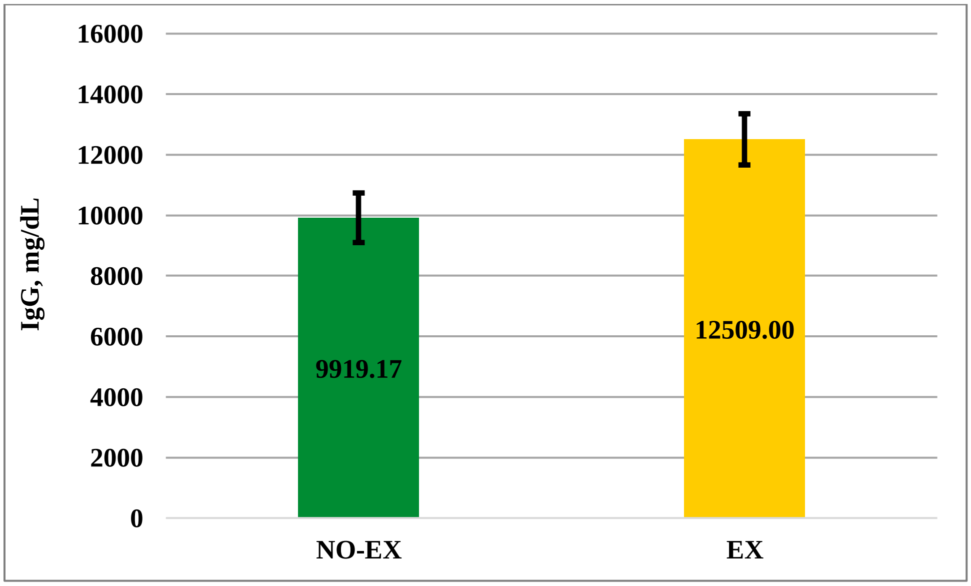

Figure 2.6. Least square means and standard errors for colostrum IgG (mg/dL) for ewes that were exercised (EX) versus ewes not exercised (NO-EX) from the third trimester to lambing.

Lamb Serum IgG, Serum Protein, and Serum Cortisol

Lamb serum IgG levels obtained after colostrum consumption were not different (*P* = 0.3402) by treatment (Table 2.5). There was no treatment effect (*P* = 0.6012) on neonatal serum protein levels prior to colostrum consumption (Table 2.5). There was a treatment effect (*P* = 0.0390) on lamb serum protein after colostrum consumption (Fig 2.7). Additionally, sex of lamb had a treatment effect (*P* = 0.0266) on serum protein post-colostrum feeding, with females having more (7.4899 ± 0.1773 g/dL) compared to males (6.9133 ± 0.2148 g/dL; data not shown). The total difference in serum protein levels from birth to after colostrum feeding was different for treatment (*P* = 0.0017; Table 2.5). Serum cortisol levels were not different for treatment at birth (*P* = 0.6012) or at the time of the second bleeding (*P* = 0.8801; Table 2.5).

Table 2.5. Least square means and standard errors for colostrum components, separated by treatment group, and lamb serum components, separated by dam treatment group.

	Treatment		
	NO-EX[1]	**EX**[2]	***P*-Value**
Ewe Colostrum Traits			
Colostrum Crude Protein, %	17.4784 ± 0.8190	18.9206 ± 0.8490	0.2317
Colostrum Crude Fat, %	10.2733 ± 2.1483	9.4225 ± 1.9922	0.5576
Colostrum Dry Matter, %	32.8820 ± 2.4709	33.1230 ± 2.4216	0.9333
Neonatal Lamb Serum Traits			
IgG at Age 28 ± 4 Hours, mg/dL	4654.03 ± 250.39	5013.44 ± 254.41	0.3402
Protein at Birth, g/dL	4.1729 ± 0.0895	4.1158 ± 0.0738	0.6012
Cortisol at Birth, µg/dL	4.5340 ± 0.4000	4.0430 ± 0.4088	0.3829
Cortisol at Age 28 ± 4 Hours, µg/dL	10.1515 ± 0.9571	9.9440 ± 0.9778	0.8801
Total Protein Difference, g/dL[3]	2.8925 ± 0.1408	3.4846 ± 0.1271	0.0017

[1] NO-EX = No exercise, ewes that were not exercised through the third trimester of gestation

[2] EX = Exercise, ewes that were exercised through the third trimester of gestation

[3] Total Protein Difference = Total difference in lamb serum protein (g/dL) between birth and age 28 ± 4 hours

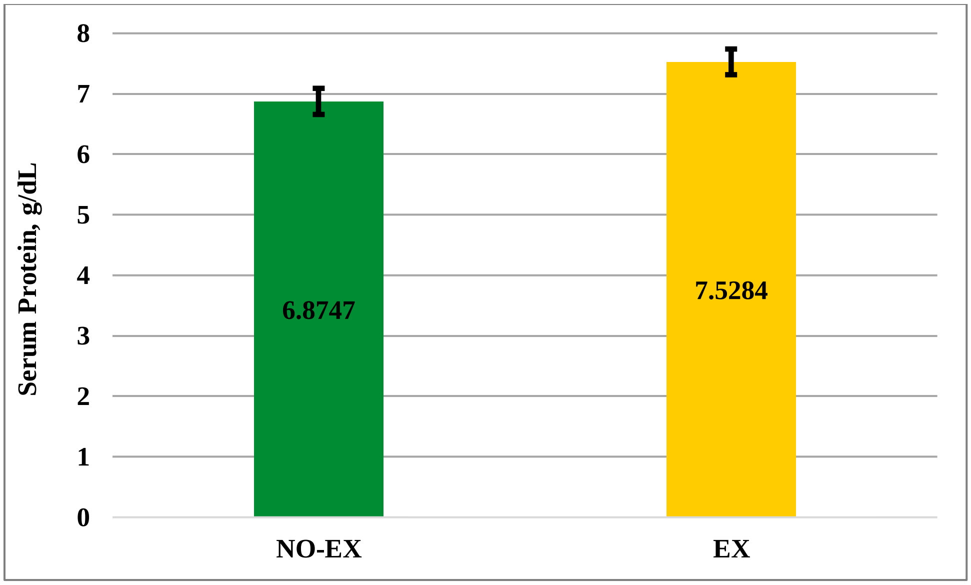

Figure 2.7. Least square means and standard errors for lamb serum protein (g/dL) separated by their dam treatment group as ewes that were exercised (EX) versus ewes not exercised (NO-EX) from the third trimester to lambing.

Discussion

Ewe Step Counts

It was expected that ewes in EX would take more steps than NO-EX ewes since they would be walked every other day for 30 minutes. Analysis of the non-exercise days also showed that EX ewes still took significantly more steps, even on the days they were confined to their individual pens like their NO-EX counterparts. This increase in steps during non-exercise days may be attributed to EX sheep having improved muscle movement/mobility or positive pregnancy adjustments compared to their counterparts. In humans, women are advised to follow an exercise routine for many physical and mental benefits (Wolfe and Davies, 2003). Women who are active before and during pregnancy have been shown to experience a greater sense of comfort as their body adjusted to advancements in pregnancy (Petrov Fieril et al., 2014). Gestational exercise has made women feel more confident and accomplished, improved back pains and posture as their bodies adjusted to the weight gain, experienced less headaches and fatigue, and many women have reported improved sleeping patterns (Petrov Fieril et al., 2014). It has also been reported that women who take a break from their exercise routine during pregnancy often revert back to feeling uncomfortable or restless and sleeping less, which has been suggested to be from deviating from their normal exercise routine (Petrov Fieril et al., 2014). Novak (2012) suggested that in rodents, exercise improved neuro activity that led to decreased perceptions of stress, improved mood, and the feeling of a sense of reward from exercise (which has also been suggested to reduce feed intake). Sheep share many similarities to humans in their gestational physiology (Morrison et al., 2018) and may have similar behaviors and patterns with regard to gestational activity. Ewes in EX may have experienced similar outcomes from taking days off from walking such as women have reported when they do not exercise during pregnancy. Although EX ewes only took 1 day off in between exercise days, the data indicate that they remained more active in their pens than their NO-EX pairs. These changes in steps and movement patterns during non-exercise days may indicate that EX ewes felt more restless on their days off or at least created a sense of desire or habit for them to be in motion as opposed to sedentary. Observational evaluation of EX ewes indicate that the exercise routine may have impacted their posture and overall body comfort. Ewes that were walked appeared to be fit, agile, and comfortable as they continued to put on weight due to the advancement of pregnancy. Their responses to the increased locomotion are similar to observations seen in humans and rodents who were more active during pregnancy. To better gauge how EX sheep felt or behaved on days off should be investigated further in the future to identify if there is a sense of restlessness or behavioral patterns in animals that have access to movement compared to animals in confinement.

Ewe Body Weight and Average Daily Gain

The results confirmed the expectation that gestational weight gain and ADG would be better controlled in EX compared to NO-EX ewes. In studies evaluating human pregnancies, one of the main outcomes of gestational exercise has been lower gestational weight gain. Lewis (2014) suggested that women are expected to gain around 12.50 ± 2.50 kg during pregnancy. To better control excessive gestational weight gain, numerous scientists have suggested that women should partake in gestational exercise routines such as aerobics, muscle training, and endurance training. Several authors have suggested that control of gestational weight gain via exercise can improve body comfort, locomotion, posture, and muscle tone (Lewis, 2014; Kominiarek and Peaceman, 2017; Wang et al., 2019). The reduced gestational weight gain of EX ewes may have been attributed to less ADFI and moving significantly more throughout the third trimester. Exercise requires energy. As pregnancy advanced and ewes gained more weight, more energy would be needed to support the exercise routine as well as deliver nutrients and energy to a rapidly growing fetus, maintain maternal energy, and initiate lactation. The energy cost for EX ewes would vary per individual and is dependent on several different factors (such as weight and ability to handle exercise), however, it has been shown that carbohydrate oxidation, lipid oxidation, and energy demands are greater in more strenuous locomotion compared to low levels of exercise or no movement (Yoshioka et al., 2001). Even after an exercise routine has ended, Yoshioka et al. (2001) suggested that post-exercise homeostasis and metabolism continues to favor greater oxygen consumption and lipid oxidation, which may be mediated by greater beta-adregenic stimulation. To better understand if gestational exercise played a role in EX's control of weight gain, future studies may wish to study metabolism patterns and levels in animals that have the ability to move freely compared to animals held in closer facilities.

Ewe Feed and Supplement Intake

We hypothesized that EX ewes would eat more feed to make up for energy lost during exercise. However, the results show that NO-EX sheep ate significantly more feed and since supplement amount was calculated based off how much each ewe was eating, NO-EX sheep also ate more supplement. The elevation of body temperature associated with exercise may be one reason for the reduced intake in EX ewes. The research project was conducted during the months of August and September where it was often hot and humid in the mornings. Although internal body temperatures were not taken, it would be expected that EX would have a greater internal body temperature during and shortly following exercise. Cannas et al. (2004) suggested that body heat in sheep may have a direct impact on maintenance requirements, therefore, it would be expected that EX would eat more. However, EX ewes still ate less while using more energy. Additionally, Silanikove (1992) suggested that a hot environment decreased voluntary food intake of animals while simultaneously increasing maintenance requirements. It is also conceivable that ruminants avoid eating (especially forage) when they are hot because of the heat of fermentation and the potential decrease in appetite. In cattle, Maurya et al. (2012) suggested that physical work or walking stress increases heat, which ultimately decreases gut motility and rate of passage that may result in reduced appetite and less feed intake. In rodents, the impacts of exercise on feed intake are mixed. Bi et al. (2005) concluded that giving rodents access to an exercise wheel increased their activity and decreased their feed intake. In contrast, Swallow (2001) reported that exercise activity through rodents running on a wheel increased feed intake. It remains unclear why some animals may eat more or less when subjected to exercise. A second reason for the reduced feed consumed by EX ewes may be because they were tired after exercise. Since they were fed immediately after exercising, the tired sensation may have altered their desire to eat in the morning. Fillon et al. (2020) suggested that exercise may have an impact on eating behaviors, especially in morning appetite following exercise. In humans, exercising prior to eating a meal has been shown to have greater benefits in controlling the amount of food consumed, resulting in better regulation of weight gain (Fillon et al., 2020). Behavioral observations during the exercise routine showed that ewes began to tire at approximately 20 minutes of walking. As gestation advanced, the exercise routine became more strenuous on the ewe, even after being subjected to the routine many times and being familiarized with it. The physical demand and energy lost during walking may have been enough to alter their morning appetite upon being returned to their individual pens.

Lamb Birth Weight, Performance, and Weaning Weight

The results confirmed the expectation that gestational exercise would not significantly impact BW. Since EX ewes ate less feed and expended more energy for exercise, it would have been conceivable that offspring of EX ewes weighed less at birth; yet no differences were seen across treatments. In women, studies evaluating the impact of gestational exercise on BW are mixed. According to Bisson et al. (2017), women who participated in more maternal exercise and activity during the second and third trimesters had babies that were lighter weight compared to women who were more sedentary. The authors suggested that further evaluation should be performed to better conclude what caused the decrease in BW and whether the decrease predisposes the baby to future health risks (Bisson et al., 2017). Other scientists have reported no correlation between exercise and offspring BW (Haakstad and Bo, 2011; Pathirathna et al., 2019). However, there have been observations where maternal exercise during pregnancy has increased BW. In mice, females that ran on running wheels prior to and during pregnancy birthed heavier offspring compared to mice that did not have access to a running wheel (Eclarinal et al., 2016). These conflicting results from other species show the need for similar projects in medium and large ruminants. It is conceivable that even though EX ewes gained less weight throughout the trial and ate significantly less feed, the fetus was spared growth retardation. At parturition, there were no differences in BW, suggesting that more controlled gestational weight gain and lower ADFI, along with increased locomotion during the third trimester, is safe for neonatal BW and future performance and WW.

Colostrum IgG

This research study may be a steppingstone into evaluating the impacts of exercise on colostrum quality. To the best knowledge of the research team, no known studies have evaluated how exercise activities and routines during the last trimester of mammalian species impacts their colostrum IgG levels, plus CP, crude fat, and DM. When gestational exercise is studied in humans, rodents, or livestock species, elements such as gestational weight gain, blood flow, placental gas exchanges, and placenta blood flow are the focus (Garshasbi and Zadeh, 2005; Harris et al., 2013; Wang et al., 2019). When ruminant mammary secretions are evaluated though as a result of access to walking, milk is evaluated, rather than colostrum, and often in dams that have been lactating for a period of time (Matthewman et al., 1993). In the limited research conducted in how exercise impacts immune components, studies are focused on inflammatory responses and variations in cytokine and chemokine profiles (Acosta-Manzano et al., 2019; Acosta-Manzano et al., 2020). Without a clear precedence or knowledge of how the exercise would impact the colostrum IgG levels of EX, it was safest to hypothesize that exercising ewes in the final trimester would not have a significant impact on colostrum IgG.

Possible explanations for the increase in EX colostrum IgG include the concept of immunoenhancements through exercise/acute stress and rate of blood flow. The first major explanation for greater colostrum IgG in EX sheep may be that acute variations of stress have been linked to immunoenhancement in humans and animals. According to Dhabhar (2009), humans and animals that experience short periods or acute phases of stress may have increased immune functions, especially in leukocyte circulation, macrophage and DC capabilities, and neutrophil circulation. It has been reported that acute stress increases immune functions because the body is mobilizing various immune components for defense and the possibility of damage (Dhabhar, 2009). Increases in heart rate and blood pressure as indicators of stress may alter the body's ability to handle stressful situations, and as a result, may suppress or improve the ability of circulating lymphocytes to be activated (Dhabhar, 2009). Additionally, Irwin (1990) and Dhabhar (2009) have suggested that stress hormones such as glucocorticoid are some of the main mediators in how leukocyte distribution is handled as a response to acute stress. While some animal studies have shown the opposite effect, human studies have shown that certain stress levels, often eustress (positive stress such as exercising) can increase leukocytes (Goldstein and McEwen, 2002; Dhabhar, 2009). Dhabhar (2009) directly stated that exercise may activate the sympathetic nervous system and induce greater levels of norepinephrine, which could increase leukocyte numbers. Regarding leukocytes, it is unclear as to what type of leukocytes authors are referring to when they claim that eustress or acute stress may increase those immune cells. However, if leukocyte circulation is increased in the blood, it may increase B-lymphocytes and ultimately the amount of IgG created. One of the limitations of this research study was that blood stress markers were not measured in EX. However, based off behavioral patterns of ewes, it did not appear that physically walking them for 30 minutes at a time made them feel more nervous or anxious to the degree where the exercise could be classified as a negative stress. It was observed that they were tired after walking, but no major stress responses were observed in ewes.

The sheep quickly became accustomed and comfortable to leaving their pens and walking outside. This strongly implies that the exercise routine acted more as a positive stress, and as suggested by various authors, this eustress routine could have mobilized various leukocytes to circulate in the blood at greater quantities, while at the same time making APCs and lymphocytes more effective and better undergo maturation.

If the concept of stress is evaluated as a possible explanation for increased leukocyte count, then a differentiation of positive and negative stress must be made. According to Dhabhar (2009), how the body reacts to stress is dependent on how the brain perceives the potentially stressful situation. When physical stress is present, these situations can pose a challenge to homeostasis and the safety of the animal or human (Dantzer and Mormede, 1983). If the situation is deemed to be more psychological, a stress reaction can also occur as the brain perceives and anticipates there to be a situation or event that will put them in danger or at the very least, alter homeostasis (Dhabhar, 2009). When there is a stressful situation that arises, the body is prepared to mount a reaction to this by various hormones that are triggered because of the activation of the sympathetic nervous system and the hypothalamic-pituitary adrenal axis (Dhabhar, 2009). When the stressful situation or event is chronic/prolonged, this can have serious consequences on the animal or human (Merlot et al., 2013). The constant surge of stress hormones and cytokines can damage host cells and tissues and suppress immune function. However, when these stressful situations do not pose great risk and are perceived by the brain as minimal and acute phases of stress, such as exercise that provides some stress but in a positive manner, the body may actually boost its immune capabilities (Dhabhar, 2009). The mechanisms and reasoning behind increased immune function because of acute/positive stress are not fully understood.

When EX sheep were exercised, there was minimal evidence to suggest that this routine was a negative stress factor. Based on behavioral observations, the ewes did not appear to be in any danger, and it was not believed that they perceived the exercise pen or routine as an event that put them in danger. During and after the acclimation period, ewes responded well and comfortable to the exercise routine and pen. The exercise pen was located and designed in such a way that sheep could clearly see their surroundings, stay together as a group, and walk on dirt. This allowed them to perceive the exercise routine and area as comfortable and to minimize the chances of their experience being classified as a negative stress.

If EX ewes did have increased immune cells, especially lymphocytes, because of their bodies responding to the acute stress of being removed from the building and walked outside, they could have also had increases in blood flow which could have caused IgG to be better synthesized into colostrum. This concept might go hand and hand with the elevated immune cells that have been suggested to accompany exercise. Colostrum is synthesized in sheep and other livestock weeks prior to parturition (Brandon et al., 1971). The production process is controlled by hormones and FcRn receptors that shuttle Igs and various nutrients across mammary epithelial cells (Patton and Jensen, 1975). The source of Igs and nutrients that contribute to the components of colostrum mainly originate from maternal blood sources (Tizard, 2018). There may be a link between maternal blood flow rates and absorption of IgG into the mammary gland. Although blood flow was not measured during the research trial, the act of removing ewes from their pens, walking them outside of the housing unit and to the exercise pen, and walking them for 30 minutes and then returning them, should have increased blood flow. Chandler et al. (1985) has concluded that exercising pregnant sheep promotes blood flow. If EX ewes did have increased blood flow from the time they were removed from their pens to the time they returned (and shortly after), it would be expected that maternal protein, lipid, hormone, and various other substances in the blood would be circulating throughout the body at faster rates compared to ewes held in confinement. If blood flow rates were greater in EX ewes, maternal serum IgG may have been circulating at faster rates. The increase in blood flow may have brought IgG proteins into proximity where FcRn receptors are located, or at the very least, allowed for more IgGs to be in the presence of FcRn receptors more times than when ewes did not have elevated blood flow. Since IgG is shuttled into the mammary gland through the mediation of FcRn receptors (Tizard, 2018), an increased level of IgGs encountering FcRn receptors could explain why EX ewes shuttled more IgG into their colostrum. With such limitations into the knowledge of how exercise impacts IgG absorption into the mammary gland for colostrum production, further research is essential to obtain a clearer understanding of the mechanisms that may be contributing to the variations of IgG levels in colostrum.

Colostrum Crude Protein, Crude Fat, and Dry Matter

It was not expected that the CP percentage of colostrum would be impacted by late gestational exercise. While EX ewes did have slightly greater CP levels in their colostrum, it was not significant. One reason the greater levels may have been present was that EX had greater IgG levels in their colostrum compared to NO-EX. Short periods of physical exercise, especially aerobic trainings, have been shown to impact protein and fat concentrations in milk of women, along with water levels (Mirzajani and Salehzadeh, 2015). Following the completion of a short exercise routine, greater levels of protein have been observed in the milk of lactating women (Mirzajani and Salehzadeh, 2015). However, these greater protein concentrations became more normal as exercise routines became longer, eventually to the point where they were no longer significantly different to women who were not exercising (Mirzajani and Salehzadeh, 2015). The findings of Dewey et al. (1994) are consistent with those from previous studies that showed that exercising during lactation did increase protein levels in milk; however, those levels gradually stabilized after a certain time. Matthewman et al. (1993) concluded that in dairy cross cattle, cows that were allowed to walk did not have any significant differences in milk protein compared to cows held in confinement. In rodent milk, there also has not been a significant difference in protein levels observed between exercised and sedentary females (Treadway and Lederman, 1986). Many scientists have concluded that exercise decreases milk protein and one major reason for that may be that protein synthesis in the muscle mass begins and then disperses after the fat mass is hydrolyzed in the blood (Peterson et al., 1990; Shirai et al., 1999; Hanyu et al., 2004). Since EX ewes were physically walked right up until lambing, the act of exercising may have allowed for more mobilization of proteins to be secreted into colostrum, but not enough to greatly alter CP in colostrum and cause them to be significantly different compared to NO-EX.

Like CP, the crude fat of colostrum levels after gestational exercise have not been studied in depth. It was not expected that there would be a significant difference in colostrum fat between treatment groups. Although Dewey et al. (1994) suggested that exercise may better synthesize fat in human milk, the results of their study do not show any significant difference in fat levels in the milk of women who exercised compared to less active women. However, it is still suggested that exercise may still increase fat in milk due to mobilization of fatty acids and the transferring of these lipids into mammary glands (Mirzajani and Salehzadeh, 2015). In contrast, milk evaluated from lactating dairy cross cattle have shown that milk fat during and shortly after walking increase, but gradually level out post-exercise and do not have significant differences compared to cows that are not walked (Matthewman et al., 1993).

When evaluating DM percentages, it was not expected that there would be a difference in the colostrum of EX versus NO-EX. Even though EX ewes were subjected to continuous exercise throughout the period when colostrum would have been synthesized, there is no evidence to suggest that their colostrum had any more or less water content in it compared to NO-EX ewes. In human milk, exercise has not shown to significantly change water percentage, although it may be expected since exercise usually causes bodily water loss (Mirzajani and Salehzadeh, 2015). The major limitations of previous human and rodent studies on DM, as well as CP and crude fat, have been that experimental units were evaluated during the lactation of milk rather than colostrum, often with studies beginning weeks to months postpartum.

Lamb Serum IgG, Lamb Serum Protein, and Lamb Serum Cortisol

It was not expected that EX lamb serum IgG levels would be greater than NO-EX lambs. Lamb serum at birth was not analyzed due to the sensitivity limitations of the RID test kits that were used to measure serum IgG and because numerous authors have suggested that neonatal ruminants are born with essentially zero IgGs in their serum (although possible traces may be found, suggested to be present due to in utero infections) (Sawyer et al., 1977; Khan and Ahmad, 1997; Chucri et al., 2010; Tizard, 2018). Since PI is mainly dependent on the timing of the first colostrum and the quantity of IgGs consumed (Morrical et al., 1995; Alves et al., 2015), it was not expected that PI difference would be observed amongst newborns. There is currently no evidence that suggests that exercise would have a major impact on intestinal epithelial permeability in newborns, which is why it was not expected that lamb PI would be significantly impacted. It was expected though after analyzing colostrum IgG levels that EX lambs would have greater serum IgG levels compared to NO-EX lambs. Lambs from EX ewes did have numerically greater serum IgG after colostrum consumption. However, these results were not significant. This suggests that although EX ewes provided more IgG to their lambs by way of colostrum, their lambs were only able to absorb slightly more compared to NO-EX lambs. The post-colostrum serum IgG concentrations may suggest that although newborns receive more IgG through colostrum, their intestines are only capable of absorbing a certain amount.

There was no significant difference in lamb serum protein levels at birth. This result was expected since fetal protein levels were not expected to be increased or decreased based on the act of walking EX ewes. In humans, exercise has been shown to cause variations in blood protein levels due to the mobilization of adipose and free fatty acids, along with changing the profiles of various hormones such as insulin (Wang et al., 2016). The serum protein levels of ewes were not measured during the study, so it is not clear whether they had differences in serum protein levels prior, during, or post-exercise compared to NO-EX sheep. Even if there would have been differences in ewe serum protein concentrations, there is no evidence found that would suggest that these differences would somehow impact the fetal blood protein levels of EX ewes.

There was a significant difference in lamb serum protein levels after colostrum consumption that is most likely attributed to colostrum antibodies. Total lamb serum protein would include the major proteins of albumin and gamma globulins (the globulin type of Igs). The most reasonable explanation for EX lambs having greater serum protein after colostrum feeding is most likely because they consumed colostrum that had a greater concentration of IgG. Given that IgG antibodies are proteins, it is conceivable that they would have more total protein after they consumed colostrum. According to Barrington et al. (2001), antibodies account for around 90% of the total protein present in colostrum. These levels are extremely high compared to milk, especially since Barrington et al. (2001) also suggested that colostrum IgG levels are 5 to 10 times greater in colostrum than serum.

Serum cortisol levels were obtained both at birth and after colostrum feeding to assess if there would be any differences that may impact how neonatal lambs absorb Igs. The results indicate that EX lambs did not have any serum cortisol differences at birth or after colostrum feeding. It was expected that at birth, lambs would have greater cortisol levels due to the stress of parturition and learning to walk and nurse. The findings from this experiment agree with previous authors that have suggested that cortisol levels are high at birth and gradually decrease and stabilize as newborns adapt to their new environment (Hoyer et al., 1990). It has been well studied and documented that dams and/or newborn ruminants that are subjected to severe stress have elevated glucocorticoid levels, especially in the case of dystocia (Vannucchi et al., 2015). In severe cases of dystocia or high stress during the peripartum period, elevated cortisol levels in the dam can cross the placenta and elevate serum cortisol levels of the fetus (Vannucchi et al., 2015). In this research study, most ewes gave birth without assistance. In the lambs that did require assistance, the cortisol levels were mixed, indicating that some lambs had greater cortisol, and some had lower cortisol compared to the average levels. Another reason for analyzing cortisol shortly after birth and after colostrum consumption was to determine if cortisol does gradually decrease with time; and if there were differences in EX cortisol levels, would it have impacted intestinal absorption. Previous scientific studies have yielded conflicting results in how and if cortisol influences the permeability of intestinal epithelial cells that aids in the eventual PI of newborn ruminants (Sangild, 2003; Chniter et al., 2016). Although EX lambs had slightly lower cortisol levels both at birth and after colostrum consumption, it was not significant, and it is apparent that it did not improve or suppress their ability to absorb IgG.

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CHAPTER 3: GENERAL CONCLUSIONS AND FUTURE DIRECTIONS

This research study may be one of the first that has evaluated how exercise/increased locomotion in the third trimester of ruminant pregnancy impacts the dam and offspring. As highlighted in both previous chapters, there are limitations in research that have explored this area of animal science. One of the major reasons for these limitations may have to do with how livestock species are managed. How relevant or important is understanding locomotion during pregnancy? Based on the evidence that has been collected on the ewe, especially her colostrum IgG levels, and serum protein results of the lamb, this may be of great relevance in the future.

One of the key observations from this experiment was that EX ewes had lower ADFI and gained less weight as pregnancy advanced. These findings reflect results seen in previous human and rodent studies that evaluated physical activity versus remaining more sedentary in pregnant females. While it was expected that EX would eat more since they were more active and expended more energy throughout the third trimester, ADFI and weight gain were significantly lower compared to ewes held in confinement. This control in gestational weight gain may be explained by the act of walking significantly more and eating less feed compared to NO-EX. These results could be further studied in the future to better understand to what degree exercise impacts the weight of ewes. The NO-EX ewes were held in individual pens that greatly limited their ability to move. Future experiments may wish to adjust pen sizes and housing to see as to how extreme pen sizes must be for confined females to begin to eat more and gain more compared to dams that are given more space to move. At some degree, pen sizes/housing spaces may be altered in such a way that it makes an impact on eating behaviors and weight gain.

The overarching objective of this research study ultimately was to evaluate if physically walking ewes compared to remaining in complete isolation impacts colostrum IgG concentration in colostrum and if there is a subsequent increase or decrease in offspring serum IgG. To the best knowledge of the research team, there are no studies that have evaluated how increased locomotion in ruminant species impacts IgG concentration in colostrum or absorption of IgG. With these limitations, it becomes challenging to make conclusions about how and why EX ewes synthesized greater IgG in their colostrum compared to NO-EX. Since all ewes were treated the same, fed the same rations and supplement, and were closely related both genetically and physiologically (by pairing ewes), the exercise routine is the strongest explanation for the variations in colostrum IgG. The main question from this explanation then is how exercise better shuttled IgG from the blood of the dam into her colostrum. As suggested in Chapter 2, if EX ewes underwent a form of eustress (positive stress) from being walked for 30 minutes per day, this form of acute stress may have enhanced certain immune functions. If various immune cells such as leukocytes and APCs are suggested to be improved in quantity and function from eustress, EX ewes may have had increased function of these cells, especially leukocytes. The reason for improved leukocyte mobilization is not fully understood by authors who make this suggestion. It is also not clearly understood that of those increased leukocytes how many of those are B-lymphocytes. Given these possible explanations for increased colostrum IgG, research studies in the future could take more consideration into the serum immune components of the ewe. This was a limitation through this study since ewe serum was analyzed. The serum of ewes could provide results for 2 major components. The first one being the dam serum IgG levels to evaluate to what degree and when IgG levels were fluctuating amongst NO-EX and EX. This could help make a stronger case for how the exercise routine impacted colostrum IgG. The second major reason to obtain ewe blood samples during the exercise period is to analyze leukocyte count and other immune cells of interest. If future studies obtained serum before and after exercise routines, perhaps the theory that acute phases of stress increases leukocytes can be better built upon. Furthermore, lymphocyte count and maturation could be heavily investigated to see if there is a correlation between them and eventual colostrum IgG levels.

The serum IgG of newborn lambs was of big interest in this research study and while numerically lambs from EX did have greater serum IgG, it was not significant from a statistical standpoint. Once colostrum IgG was analyzed, it was expected that lambs from EX would have much greater serum IgG since they consumed colostrum with more IgG. The results suggest that there is a baseline of colostrum IgG that newborns can absorb. Research studies in the future that evaluate this concept should take intestinal physiology and colostrum intake into account. Future studies should investigate the actual intestines to see if there are any variations between lambs born from dams of EX or NO-EX. Additionally, it is suggested that future studies remove lambs from their dams after birth to prevent additional nursing after initial colostrum feeding. This would help make serum IgG results more accurate since in this study, the ability to remove lambs from their dams was limited.

The behavioral observations of sheep in this experiment were limited and could be further evaluated to better understand why EX ewes moved more inside their pens on days off from exercise and to better evaluate if the exercise routine acted as a positive stress factor. As addressed in Chapter 2, there are suggestions that increased locomotion in human and rodent pregnancy tends to create both physical and mental benefits. Additionally, it has been suggested that deviating from an exercise routine or taking a break from it may leave females feeling more agitated and restless. To better gauge this concept, future studies may wish to evaluate EX sheep more closely on days held in confinement. These observations could include movement patterns, sleep patterns, and other observational patterns that would evaluate if EX ewes feel restless or agitated inside their pens. How and why do EX sheep respond differently in confinement compared to ewes normally held in confinement? Additionally, EX ewes could be further observed for signs of positive stress. Based on the behavior of ewes before, during, and after exercise, it is not believed that they were under negative stress from being walked. To better make the case that this walking routine acted as eustress, closer observations may be made on sheep. These observations could include rate of movement, response to stimuli in and around the exercise pen, vocalization, waste excretion, temperament, and possibly cortisol levels.

Experimental studies in the future that evaluate pregnancy and the impacts that movement activities have on the colostrum IgG levels of the dam should be aimed at making evaluations that pertain to real industry settings. While it is not assumed that most sheep and goat operations would house animals in the type of environment that they were in for these research purposes, there are still implications that can be built upon in livestock operations. Sheep were housed in the manner that they were for research purposes and intensely to see if that magnitude of confinement versus walking every other day yields useful results. If they do produce results, such as in this research study, then future studies may wish to build upon that in a manner that more mimics a real operation.

The conclusions from this experiment suggest that the ability for animals to move and walk make an impact on their ADFI, weight gain, and their colostrum IgG levels. These conclusions have the capability to further advance other fields of gestational science. Given that sheep share many similarities to humans and rodents in gestational physiology and health, these results and implications may be of some use to better understanding the modern culture of a “fit pregnancy” and the continual fascination with physical activity during human pregnancy and to help explain many of the benefits that have emerged in the field of gestational exercise.

With the constant shifts and means to evolve in the livestock industry come new opportunities to advance livestock production. The data and conclusions from this experiment offer implications into applicable ruminant production. Livestock operations may wish to evaluate how pregnant animals are housed and held when in confinement, especially if animals are in very close quarters during the gestation phase. Housing size and the ability for animals to move and walk during gestation may have great animal welfare and physiological benefits that should be further explored.