EFFECTS OF NUTRIENT RESTRICTION, REALIMENTATION, AND PARITY ON UMBILICAL HEMODYNAMICS IN THE PREGNANT EWE

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ABSTRACT

Sheep are normally managed within grazing systems; forage availability and quality are dependent upon seasonal conditions such as drought and humidity. It is therefore important for producers to know when during gestation it is critical to supplement animals with additional feed. Previous research has shown that nutrient restriction during mid-gestation causes a decrease in umbilical blood flow with a possible consequent decrease in fetal body weight and size. Our findings indicate that a decrease in umbilical blood flow upon nutrient restriction during mid-gestation is not solely a consequence of the restriction itself or an additional effect of parity. Moreover, it appears very probable that such a decrease is also an effect of maternal age during pregnancy. In order to address this question further investigation is needed.

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CHAPTER 1. LITERATURE REVIEW

Introduction

Sheep are normally managed within grazing systems; forage availability and quality are dependent upon seasonal conditions such as drought and humidity. Forages with lower quality not only have less nutrient components but also have lower digestibility (Milchunas et al, 2004). This can impact animal nutrient availability, particularly during pregnancy.

Various environmental factors affect fetal and placental development. Nutrition is the most important among these factors (Wu et al., 2004). During times of drought, ewes may experience deficient total energy requirements. Furthermore, during different stages of conceptus development, ewes may need additional nutritional supplements. Therefore it is important for producers to know when during gestation it is critical to supplement animals with additional feed.

Similarly, in farm animals, parity has an important effect in reproduction. Typically birth weight and number of offspring increases as parity increases (Jacquot and Vessey, 1998; Lafi et al., 2009). Studies that investigate the combined effects of nutrient restriction and parity are scarce.

The majority of placental growth happens in the first two thirds of pregnancy, with the placenta reaching its maximum weight by d 90 in sheep (Redmer et al., 2004). Fetal umbilical blood flow (UBF) increases proportionally with fetal weight until birth (Rudolph and Heymann, 1967). Placental weight however does not increase after d 90 of gestation (Rudolph and Heymann, 1967; Stegeman, 1974). Therefore the increase in UBF after d 90 of gestation is due to a decrease in placental vascular resistance (Rudolph and Heymann, 1967; Vonnahme, 2012), and an increase in the number of vessels in the placenta (Rudolph and Heymann, 1967). Impairing placental growth or uteroplacental blood supply, with insults such as nutrient restriction, can affect fetal growth trajectory (Redmer el al., 2004).

Summarizing the importance of the above mentioned, this literature review describes the blood volume increase in pregnant animals, fetal circulation in the sheep, intrauterine growth restriction, nutrient restriction in pregnant sheep, realimentation in pregnant sheep and the parity effects in sheep.

Blood volume increase in pregnant animals

In every physiological state an adequate blood volume is necessary for normal nutrient and oxygen delivery to the tissues. Similarly blood is necessary for the collection and elimination of metabolic waste products and CO_2 from the body. In mammals an inadequate blood volume during pregnancy can have detrimental physiological consequences in the mother and the fetus.

The average blood volume (blood volume) in humans is about 71 mL/kg in men and 70 mL/kg in women (Dien and Lentner, 1970). This means that an 170 lbs. man will have approximately 5486 mL of blood and a same weight woman around 5408 mL of blood. Heavier individuals will therefore have increasing amounts of blood.

Universities and research institutions have developed values of average blood volumes of laboratory and farm/research animals. Sheep have a blood volume of around 60 mL/kg according to the NDSU IACUC (NDSU, 2013). These values vary as a consequence of species and body weight. It is specified in some of these tables that cattle up to 400 kg and horses up to 500 kg have an average blood volume of 60 mL/kg and 72 mL/kg respectively (NDSU, 2013). This specification of body weight is explained by the variation of blood volume present in different body tissues. A kg of muscle will have a higher blood volume than a kg of bone (Everett et al., 1956).

When the values of blood volume published by the universities and research institutions are compared with early blood volume studies similarities and differences can be found. Little

difference is seen when we compare values of blood volume in dogs, 85 mL/kg according to NDSU IACUC (NDSU, 2013), and 79 mL/kg according to Courtice (Courtice, 1943). On the other hand, blood volume in rabbits has an important difference between NDSU average values (56 mL/kg; NDSU, 2013) and the cited study (70 mL/kg; Courtice, 1943). This difference in values could be a result of blood volume measuring methods. Early studies investigating blood volume used radioactive isotopes and exsanguination to find volumes of laboratory animals (Courtice, 1943; Goodlin et al., 1981), whereas new investigations rely on dyes and non-radioactive isotopes (Silver et al., 1998; Rumball et al., 2008). The accuracy of each method has been debated.

Blood volume during pregnancy

Blood volume in rabbits increases 62% by the final period of pregnancy (Nuwayhid, 1979). The vast majority of studies in humans show an increase of blood volume during pregnancy (Pritchard, 1965; Longo, 1983; Silver et al., 1998; Torgersen and Curran, 2006). In human studies, blood volume increase has ranged between 20% and nearly 100% (Pritchard, 1965), with the increase being proportionally higher to the number of offspring carried by the mother (Pritchard, 1965). Others have shown an increase between 25% and 50% (Torgersen and Curran, 2006). The average increase in blood volume during pregnancy in humans appears to be around 45% (Pritchard, 1965; Longo, 1983; Torgersen and Curran, 2006).

In women, blood volume increases during pregnancy in a moderate rate in the first trimester, it increases rapidly during the second trimester with the last third experiencing a slight increase in blood volume (Pritchard, 1965). The increase in hematocrit (Ht) is usually the opposite, catching up with blood volume prior to parturition (Pritchard, 1965). Plasma volume increases at high rates during the first two trimesters and stabilizes on the third, being the principal reason of blood volume increase during the first two thirds of pregnancy (Longo, 1983). These variations in

the increase of the main components of blood during pregnancy are the explanation of the physiologically normal "pregnancy anemia" observed in women during the end of the second trimester and the beginning of the third trimester (Pritchard, 1965).

In sheep, blood volume expansion during pregnancy has been debated. Some studies show blood volume expansion during pregnancy (Barcroft et al., 1939; Caton et al., 1975; Daniel et al., 1989). Others show that non-pregnant ewes and pregnant ewes exhibit small or no differences in blood volume (Metcalfe and Parer, 1966; Rumball et al., 2008).

Theoretical mechanisms of blood volume increase during pregnancy

There are two main reasons for the importance of blood volume increase during pregnancy in females. The mother needs to compensate for the new metabolic demands of the enlarged uterus (Pritchard, 1965; Torgersen and Curran, 2006) and counteract the blood loss of parturition (Pritchard, 1965; Torgersen and Curran, 2006). An adequate blood volume increase is also necessary in order to protect mother and fetus from the deleterious effects of a reduced venous blood return and cardiac output (Pritchard, 1965; Torgersen and Curran, 2006). Pregnant women can handle more blood loss that non-pregnant women. They can lose up to 35% of their blood volume before showing signs of hypovolemia (Pritchard, 1965; Torgersen and Curran, 2006).

While it is well established why maternal blood volume increase would need to occur, there is still debate on how blood volume increases. There are currently two theories that attempt to explain blood volume expansion: the decreased vascular resistance theory and the endocrine theory.

The decreased vascular resistance theory describes a mechanism by which blood volume could increase during pregnancy (Schrier and Briner, 1991; Duvekot et al., 1993). When the female becomes pregnant a new vascular system is added to the main vascular system (Schrier and Briner,

1991; Duvekot et al., 1993). This new addition decreases the total vascular resistance of the cardiovascular system of the mother (Schrier and Briner, 1991; Duvekot et al., 1993). This in turn increases the heart rate in the mother, which activates the plasma volume regulating mechanisms in the liver, kidneys, and adrenal glands (Schrier and Briner, 1991; Duvekot et al., 1993). As plasma volume increases, blood volume increases as well (Schrier and Briner, 1991; Duvekot et al., 1993).

The endocrine control theory (Longo, 1983) suggests a fetal influence on blood volume in the pregnant female. As gestation advances, the fetus, and its adrenal glands increase in size (Longo, 1983). As adrenal gland size increases there is an increasing production of dehydroepiandrosterone, a hormone that stimulates estradiol production in the mother (Longo, 1983). Estradiol then stimulates the renin-angiotensin system, which ultimately increases plasma volume (Longo, 1983). This theory also suggests a mechanism through which erythrocytes increase during pregnancy. During gestation, placental size increases and as placental tissue grows there is an increasing production of somatomammotropin (i.e. placental lactogen) and progesterone (Longo, 1983). These two hormones stimulate the production of erythropoietin in the mother, which finally stimulates the production of erythrocytes (Longo, 1983).

Consequences of an inadequate blood volume increase during pregnancy

In women, failure to increase blood volume during pregnancy has been related to pregnancy-induced toxemia (preeclampsia), fetal growth retardation, and premature labor (Goodlin et al., 1981). Similarly, risks of blood loss during parturition are greater with women losing up to 1 L of blood during normal labor and 1.5 L or more during a cesarean section (Pritchard et al., 1965). Failure to increase blood volume during pregnancy could be the cause or the consequence of many feto-maternal illnesses. An inadequate function of the mechanisms

necessary to increase blood volume in a state of decreased vascular resistance could consequently increase heart rate and produce vasoconstriction (Lund and Donovan, 1967; Goodling et al., 1981). This could increase blood pressure and therefore be one of the causes of preeclampsia (Lund and Donovan, 1967; Goodling et al., 1981). Another way of understanding an inadequate blood volume increase could be by the existence of a reduced vasodilatory capacity of the cardiovascular system of the mother previous to pregnancy (Assali and Vaughn, 1978; Campbell, 1983). This would prevent blood volume increase and favor preeclampsia due to a reduced vessel compliance (Assali and Vaughn, 1978; Campbell, 1983). Other feto-maternal illnesses such as fetal growth retardation could be a consequence of this state (Assali and Vaughn, 1978; Campbell, 1983).

In accordance with the idea of inadequate blood volume increase as the cause of pregnancy related illnesses, some studies have shown that fetal growth retardation can happen independent of preeclamptic states but with failure to increase blood volume (Lund and Donovan, 1967; Grunberger et al., 1979). Similarly, pregnant women with hypovolemia can have all ranges of blood pressure with hypertension probably expressing cardiac compensation and hypotension representing malnutrition (Lund and Donovan, 1967).

A sheep study done in 2008 showed that periconceptional undernutrition does not affect blood volume on days 65 and 120 of gestation (Rumball et al., 2008). However this study did not measure blood volume during the period of nutrient restriction and did not measure blood volume in adequately fed pregnant ewes.

As mentioned before, when females become pregnant a new vascular system is added to the main maternal vascular system (Schrier and Briner, 1991; Duvekot et al., 1993). This new vascular system is comprised of the fetal and placental vessels that have specific anatomical and physiological characteristics.

The fetal circulation in the sheep

Fetal circulation has been widely studied in sheep and humans (Kiserud, 2006). There are some differences between sheep and humans that are important to take into account, (e.g. the sheep fetus has smaller brain relative to body weight, greater growth rate and two umbilical veins; Kiserud, 2006). However the main concepts are similar; thus, if there are important differences, they will be mentioned within the text. Fetal circulation is different from the adult circulation as the lungs are not functional yet so gas exchange occurs in the placenta; therefore, less blood is distributed towards the lungs (Murphy, 2005). The fetus also has three special structures that contribute to the different paths through which blood is distributed, this does not happen in the adult (Murphy, 2005). These structures are the ductus venosus, the ductus arteriosus and the foramen ovale (Kiserud, 2006). These three structures act as shunts and are essential distributional arrangements that make the fetal circulation a flexible and adaptive system for intrauterine life (Kiserud, 2006).

The ductus venosus connects the intra-abdominal umbilical vein to the inferior vena cava at its inlet to the heart (Kiserud, 2006). The blood flow with the highest oxygenation, coming from the ductus venosus, also has the highest kinetic energy and presses open the foramen ovale valve, entering the left atrium (Kiserud, 2006). In lambs, approximately 50% of the umbilical vein blood is shunted through the ductus venosus (Edelstone and Rudolph, 1979). The physiological role of the ductus venosus is not well understood (Kiserud, 2006). Although agenesis of the ductus has been linked to abnormalities and fetal demise in humans, it is also found in fetuses that exhibit normal growth (Kiserud et al., 2000). The shunting seems more prominent during mid-gestation rather than late gestation (Kiserud et al., 2000). In primates, normally, around 80% of the umbilical blood perfuses the liver; however, during hypoxia more blood goes through the ductus, ensuring

more oxygen to the heart and brain (Behrman et al., 1970; Kiserud, 2006). Similarly, the amount of blood flow going through this ductus increases as umbilical blood flow increases (Rudolph and Heymann, 1967). It is normally present until birth in the lamb and the human (Rudolph and Heymann, 1967).

The ductus arteriosus constitutes a wide muscular vessel, connecting the pulmonary arterial trunk to the descending aorta (Kiserud, 2006). In humans, normally the shunt closes two days after birth (Huta et al., 1984). A patent ductus arteriosus is a common clinical problem (Kiserud, 2006). Failure of the ductus arteriosus to close results in a left to right shunt, the opposite direction to that in the fetus (Murphy, 2005). This ultimately results in an increased volume and workload to the left atrium and left ventricle, and eventually left heart failure (Murphy, 2005).

The foramen ovale is an oval opening between the right and left atria. The inferior venous inlet to the heart should be viewed as a column of blood that ascends between the two atria from below (Lind and Wegelius, 1949; Kiserud, 2006). This column hits the interatrial ridge, the crista dividens, and is divided into a left and right arm (Kiserud, 2006). The left arm fills the "windsock", formed between the foramen ovale valve and the atrial septum, to enter the left atrium (Kiserud, 2006). The right arm is directed towards the tricuspid valve and joins the flow from the superior vena cava towards the right ventricle (Kiserud, 2006). The foramen ovale usually closes shortly after birth, however 25% of the human population has a patent foramen ovale (Mann et al., 2014). A persistent foramen ovale generally does not cause clinical problems; however, it has been linked with a higher risk of clot related brain strokes (Lechat et al., 1988).

Fetal blood circulation

In sheep, fetal blood volume is around 110 – 115 ml/kg (Brace, 1983). Fetuses are capable of a faster restoration of blood volume due to high diffusion rates between fetal compartments

(Kiserud, 2006). Deoxygenated blood arrives at the placenta via the umbilical arteries and is returned to the fetus through the umbilical veins (Murphy, 2005; Kiserud, 2006). The placenta must therefore receive the deoxygenated blood from the fetal systemic organs and return oxygen rich blood to the umbilical venous system, continuing towards the fetal arterial system (Murphy, 2005). The fetal cardiovascular system is designed in a way that the most oxygenated blood is delivered to the myocardium and brain (Murphy, 2005). Between 50 and 60% of the blood in the umbilical vein bypasses the ductus venosus to enter the inferior vena cava (Murphy, 2005). At the junction of the inferior vena cava and the right atrium is a tissue flap known as the Eustachian valve (Murphy, 2005). This flap tends to direct, guided by chemoreceptors, the more oxygenated blood towards the foramen ovale and into the left atrium (Murphy, 2005; Kiserud, 2006). The less oxygenated blood that is picked up by the inferior vena cava from the lower portions of the body is directed towards the tricuspid valve and into the right ventricle (Kiserud, 2006). The better-oxygenated blood is directed from the left atrium to the left ventricle and then is ejected towards the ascending aorta (Murphy, 2005). This way the highest oxygenated blood is delivered to the brain and coronary circulations (Murphy, 2005). The deoxygenated blood from the inferior vena cava, superior vena cava and the coronary sinus is directed across the tricuspid valve into the right ventricle (Murphy, 2005). This blood is then ejected to the pulmonary artery (Murphy, 2005). Only a small portion of this blood goes to the lungs, due to the high pulmonary vascular resistance; meanwhile, the major portion bypasses the lungs through the ductus arteriosus and towards the descending aorta (Murphy, 2005). This way, the left ventricle is predominantly dedicated to the coronary circulation and upper body, while the right ventricle is the main distributor to the lower part of the body, the placenta and lungs (Kiserud, 2006). The lowest oxygen saturation is measured in the abdominal vena cava, and the highest in the umbilical vein (Kiserud, 2006).

The myocardium grows by cell division until birth; growth beyond birth is due to cell enlargement (Kiserud, 2006). Increasing heart rate may be the single most prominent means of increasing cardiac output in the fetus (Kiserud, 2006). The two ventricles pump in parallel and the pressures in both of the ventricles are mostly the same (Kiserud, 2006).

It seems that during late gestation, fetal right and left ventricular outputs are similar (Rudolph and Heymann, 1967). In earlier gestation, outputs have not been measured. However, in human fetuses, right and left ventricular masses are similar towards the end of the pregnancies but the left ventricle is proportionally heavier in early gestation (Rudolph and Heymann, 1967). Similarly, in humans the right ventricle receives about 65% of the venous return and the left ventricle about 35% (Murphy, 2005). This suggests that ventricular outputs in sheep could vary during gestation (Rudolph and Heymann, 1967). Total fetal-lamb cardiac output measured in various stages of gestation ranges from 198 ml/kg/min to 361 ml/kg/ml (Rudolph and Heymann, 1967). These variations could be due to the gestational age of the measurement (Rudolph and Heymann, 1967). The percentage distribution of cardiac output is summarized in Table 1.1.

Table 1.1. Percentage distribution of cardiac output towards the organs of lamb fetuses (Gestational ages 95 to 140 days)

Organs	**Percentage**
Brain	4.9
Lungs	4.6
Gastrointestinal tract	5.4
Kidneys	2.2
Spleen	1.6
Liver (From hepatic artery only)	1.6
Placenta	41.2
Upper carcass	14.9
Lower carcass	19.9

Adapted from: Rudolph and Heymann, 1967.

Before d 90 of gestation, 20 to 25% of the fetal cardiac output goes to the placenta; however, at the end of pregnancy the amount increases to 60% (Rudolph and Heymann, 1967).

This increase is mainly accounted by the increase in UBF (Rudolph and Heymann, 1967). At d 130 of gestation, the mean UBF in sheep is around 57% of the total fetal cardiac output (Dawes et al., 1954). Placental blood flow has been found to be fairly stable and chiefly determined by arterial blood pressure (Kiserud, 2006). Fetal UBF increases proportionally with fetal weight until birth (Rudolph and Heymann, 1967). Placental weight however does not increase after d 90 of gestation (Rudolph and Heymann, 1967; Stegeman, 1974). Therefore, the increase in UBF after d 90 of gestation is due to a decrease in placental vascular resistance (Rudolph and Heymann, 1967; Vonnahme, 2012) and an increase in the number of vessels in the placenta (Rudolph and Heymann, 1967).

As previously stated, before birth, cardiac output to the lungs is only around 4.6% (Rudolph and Heymann, 1967). However in the newborn, blood flow to the lungs increases dramatically because of the stimulus of oxygen and the mechanical stimulus of expansion (Rudolph and Heymann, 1967). It has been estimated that the fetal lamb has an alveolar and bronchial fluid volume of around 50 mL at term (Rudolph and Heymann, 1967). This is similar to alveolar gas volumes in infants of similar weight (Rudolph and Heymann, 1967). In the fetus, sustained hypoxia forces an adaptational shift to less oxygen demand, reduced DNA synthesis and growth with a gradual return towards normal concentrations of blood gases and endocrine status (Kiserud, 2006). Subtle differences in the development of autocrine, paracrine, endocrine and metabolic functions induced by nutritional or circulatory variations during pregnancy could have lasting effects with increased risks of cardiovascular and endocrine diseases in adult life (Kiserud, 2006).

Intrauterine growth restriction

Intrauterine growth restriction (IUGR) is the state in which an infant has not achieved its optimal growth in utero (Anthony et al., 2003) and it is a concerning problem in human

pregnancies. Effects of IUGR on the cardiovascular and metabolic systems of the newborn later in life have been analyzed in humans and animals (Ford et al., 2007; BjarnegÅrd et al., 2013).

The influence of the maternal environment before fertilization and during gestation on the future offspring has been proven many times. The study of the effects of IUGR in animal models is not only important for its implications in human health, but also because of its significance for animal production. The sheep has been used as a model for studying IUGR because it has some resemblances with human reproduction which include: 1) a relatively long gestation length (i.e. ~150 days); 2) generally carries singleton or twin fetuses; and 3) the villous tree of the sheep cotyledon is structurally similar to the human placenta (Reynolds et al., 2005; Barry and Anthony, 2008; Ireland et al., 2008). Additionally, the ewe model allows repeated manipulations for maternal and fetal sampling/data collection to determine how both maternal and fetal tissues respond to physiological stimuli. This makes it possible to evaluate the fetal and maternal effects of IUGR (Reynolds et al., 2005).

Several models of IUGR have been applied in different species. Heat stress, corticosteroids, high altitude exposure (hypoxia), and nutritional causes of IUGR have been analyzed in humans and have been used as sheep models for IUGR (Anthony et al., 2003; Redmer et al., 2004; Reynolds et al., 2005). Intrauterine growth restriction can also be genetic (Robinson et al., 1995); however, in this review I will focus on the animal models mentioned above that are used to mimic environmental conditions in livestock production and that are used for research.

Regardless of the experimental approach employed to study IUGR, utero-placental blood flow is reduced (Reynolds et al., 2005; Vonnahme, 2012). Adequate utero-placental blood flow is critical for normal fetal growth, experimental conditions designed to investigate fetal growth retardation and placental insufficiency commonly share reduced uterine and umbilical blood flows

(Vonnahme, 2012). An understanding of the factors that impact utero-placental blood flow permits the comprehension of the placental function and thus placental growth (Vonnahme, 2012). Doppler ultrasonography can be used to measure UBF. Other methods of determining blood flow are very invasive and require increased number of animals to determine blood flow at different time points during pregnancy (Mellor and Matheson, 1979; Vonnahme and Lemley, 2012).

Heat stress

In mature cows, heat stress during mid-gestation causes fetal weights to be 82% of those fetuses from control cows (Reynolds et al., 1985). Similarly, liver, kidney, heart and spleen weights are reduced in the fetuses from the heat stressed animals (Reynolds et al., 1985). However, fetal lung weight increases (Reynolds et al., 1985). Oxygen, glucose and lactate metabolites taken from umbilical vein blood samples are not affected in heat stressed animals (Reynolds et al., 1985). On the contrary, they are lower in the uterine artery of the dams (Reynolds et al., 1985). Uterine and umbilical blood flow values of heat stressed animals is 70% of those not stressed (Reynolds et al., 1985).

In sheep studies performed during mid-gestation, heat stressed adult ewes showed decreased fetal and placental weights by the end of pregnancy (Bell et al., 1987; Galan et al., 1998) and at lambing (Galan et al., 1999). In contrast, another study showed that fetal weight is not affected near term (Bell et al., 1989). Fetal liver weights are decreased in heat stressed animals (Bell et al., 1987; Bell et al., 1989); however, brain weight is not affected (Bell et al., 1987). Interestingly, heart weight is greater in heat stressed animals (Bell et al., 1989), probably because of the fetal hypoxemia and the consequent compensatory increase in heart work that heat stressed fetuses exhibit (Bell et al., 1989). Decreased cotyledonary weights are responsible for the decrease in placental weight (Bell et al., 1987; Bell et al., 1989). However, cotyledonary number between

heat stressed and non-heat stressed animals does not change (Bell et al., 1987). Oxygen saturation, partial pressure of oxygen (PO2), and pH in maternal arterial and uterine venous blood are not affected by heat stress (Bell et al., 1987). On the other hand, oxygen saturation alone is decreased in the umbilical vein. Oxygen saturation and PO2 are decreased in the umbilical artery in heat stressed fetuses (Bell et al., 1987). Similarly, heat stressed fetuses (Bell et al., 1987), but not dams (Bell et al., 1989), exhibit lesser blood glucose concentrations than thermoneutral control animals. Progesterone levels are decreased in heat stressed ewes (Bell et al., 1989) and estrogen levels do not vary (Bell et al., 1989). Uterine and umbilical blood flows are lesser (Bell et al., 1987), and pulsatility index (PI) is greater in the umbilical artery of heat stressed fetuses (Galan et al., 1998). Resistance index (RI), however, is not affected (Galan et al., 1998).

The duration of heat stress and the gestational stage of gestation contributes to the severity of the fetal effects observed (Galan et al., 1999). During mid-gestation, when ewes are stressed for 80 d (d 35 – 115), fetal and placental weights are lesser than when animals are stressed for 55 d (d 35 – 90; Galan et al., 1999). Similarly, when ewes are stressed for a longer period of time, biparietal distance, abdominal girth, and femur and tibia measurements are lesser in heat stressed fetuses (Galan et al., 1999). However, when the stress is reduced to 55 days, only abdominal width is decreased in these animals (Galan et al., 1999).

Corticosteroids

In sheep, the impacts of glucocorticoids, namely betamethasone and dexamethasone, have been tested to determine their effects on fetal and placental growth, and umbilical and uterine blood hemodynamics (Jobe et al., 1998; Jellyman et al., 2004; Schwab et al., 2006).

Protocols of glucocorticoid injections have included single (0.5 mg/kg of betamethasone, IM, Jobe et al., 1998), double (170 μl/kg of betamethasone, IM, Schwab et al., 2006; 12 mg of

dexamethasone per animal, IM, Jellyman et al., 2004) or triple doses (0.5 mg/kg of betamethasone, IM Jobe et al., 1998). One study reported fetal weight decreases between 11% with one injection of betamethasone up to 25% with three injections of betamethasone when compared to controls (Jobe et al., 1998). Similarly, a triple dose of betamethasone decreased weights of lungs, brain, kidneys, liver, and placenta (Jobe et al., 1998). On the other hand, another study reported no differences in fetal weights when ewes were injected twice with betamethasone (Schwab et al., 2006). Biparietal distance seems to decrease with increased number of glucocorticoid injections (Jobe et al., 1998). Maternal and fetal oxygen saturation and PO2 are not affected by glucocorticoids (Jellyman et al., 2004; Schwab et al., 2006). In the fetus, hematocrit decreased 21% upon an acute injection of betamethasone (Jobe et al., 1998) compared to controls. However hematocrit values recovered by lambing (Jobe et al., 1998). Moreover, glucocorticoid injection does not affect fetal or maternal hemoglobin concentration (Jobe et al., 1998; Schwab et al., 2006).

Maternal blood pressure and uterine artery blood flow are not affected by glucocorticoids injected in the last third of pregnancy (Jellyman et al., 2004; Schwab et al., 2006); however, fetal blood pressure is increased (Jellyman et al., 2004; Schwab et al., 2006). In one study, UBF decreased with the injection of betamethasone, and this decrease was maintained for 23 h (Schwab et al., 2006). However, another study reported that UBF increased after a double injection of dexamethasone (Jellyman et al., 2004). The same study also reported that PI and RI values were not correlated with UBF measurements and did not change with glucocorticoid treatment (Jellyman et al., 2004).

High altitude

High altitude causes hypoxemic conditions. In women, the placenta shows significant adaptations in high altitude (Robinson et al., 1995). Larger intervillous space, reduced volume of

chorionic villi, placentas with more trophoblast and more villous stroma have all been found in women with short and long adaptation periods to high altitudes (Robinson et al., 1995). Oxygen transfer is facilitated by movement of the fetal capillaries to the periphery of the chorionic villi (Robinson et al., 1995). Similarly, in humans, pregnancies developed in high altitude cause a decrease in birth weight (Robinson et al., 1995; Galan et al., 2000; Aksoy et al., 2015). However, placental weight is either unchanged or increased (Robinson et al, 1995; Aksoy et al., 2015). Ultrasound velocimetry measurements (PI, RI and S/D ratios) in the fetal mid-cerebral and umbilical arteries are not affected (Galan et al., 2000; Aksoy et al., 2015). On the other hand, PI and RI values are lower in both uterine arteries when compared to sea level pregnancies in women (Aksoy et al., 2015).

Similar results are found in sheep maintained during the full length of pregnancy at high altitude (Parraguez et al., 2005; Parraguez et al., 2015). Lamb birth weight is decreased at high altitude (Parraguez et al., 2005; Parraguez et al., 2015). Fetal ultrasonography measurements (biparietal distance, thorax and abdominal widths) are also decreased in these animals (Parraguez et al., 2005; Parraguez et al., 2015). Similar to humans, placental weights in sheep are not affected by altitude (Parraguez et al., 2015).

Blood PO2 and saturation of hemoglobin are lower (Parraguez et al., 2015), and hemoglobin concentration is higher in animals at high altitude (Parraguez et al., 2015). During the first 60 days of pregnancy, ewe estrogen and progesterone levels are decreased in high altitude pregnancies when compared to sea level pregnancies (Parraguez et al., 2015).

Nutritional causes of intrauterine growth restriction

Two principal models of nutritional influence on IUGR have been studied. Overnourishing adolescent sheep and nutrient restriction at different stages of the pregnancy (Anthony et al., 2003;

Redmer et al., 2004; Reynolds et al., 2005). Before analyzing the effects of nutrient restriction in pregnant ewes, which will be studied in the following section of this literature review as well as in the next chapters of this thesis, it is important to briefly synthetize the effects of overfeeding adolescent pregnant ewes on fetal growth.

Overfed adolescent sheep

Overfeeding pregnant adult sheep generally results in an increased lamb birth weight (Muñoz et al., 2009; Symonds et al., 2010). On the contrary, when pregnant adolescent sheep are overfed, paradoxical effects on the fetus can be seen (Wallace et al., 1999). Overfeeding adolescent sheep during the full length of the pregnancy results in decreased birth weights, placental weights, cotyledon weights and cotyledon numbers relative to moderately fed adolescent ewes (Wallace et al., 1999; Wallace et al., 2001). Similarly all major organ weights, with the exception of the adrenal glands, are decreased in the fetus (Wallace et al., 1999; Wallace et al., 2001). Uterine arteries in the dam and umbilical vein and artery blood flow in the fetus are decreased when adolescent ewes are overfed (Wallace et al., 2001). Oxygen and glucose levels are also decreased in both ewe and fetal blood (Wallace et al., 2001). Hematocrit, however, is increased in over-nourished animals (Wallace et al., 2001).

Abruptly decreasing the amount of feed to previously overfed young sheep, after the first third of pregnancy, neutralizes the detrimental effects in fetal and placental weights (Wallace et al., 1999). On the other hand, if adolescent ewes are overfed after the first third of the pregnancy, lamb birth and placental weights are affected (Wallace et al., 1999).

Nutrient restriction in pregnant sheep

Various environmental factors affect fetal and placental development. Nutrition is the most important among these factors (Wu et al., 2004). Diet during pregnancy is an important modifiable

element that can have a substantial influence on the viability and body composition of the newborn (Symonds et al., 2010). Changes in the amount or composition of feed consumed by the mother from the time of ovulation through to lactation have the potential to significantly reset the growth trajectory of the majority of fetal organs and tissues (Symonds et al., 2010). Maternal nutritional status can program nutrient partitioning and ultimately growth, development, and function of the major fetal organ systems (Vonnahme et al., 2014). However, the differences in the impacts of nutrient restriction may depend on the duration and intensity of the restriction (Reynolds et al., 2005; Vonnahme, 2012). In contrast to what is seen in the pregnant adolescent ewe, and despite the fact that in the adult ewes severe under nutrition in all stages (particularly late pregnancy) can influence fetal growth, the notion is that during pregnancy nutrient partitioning favors the conceptus at the expense of the dam (Luther et al., 2005).

In sheep, the fetal placenta attaches to discrete sites in the uterine wall called caruncles (Vonnahme, 2012). These caruncles are aglandular and appear as knobs along the uterine luminal surface of non-pregnant animals (Vonnahme, 2012). The placental membranes attach to these sites via chorionic villi in areas termed cotyledons (Vonnahme, 2012). The caruncular-cotyledonary unit is called a placentome and it is the primary functional area of physiological exchange between mother and fetus (Vonnahme, 2012). At different stages of gestation the relative growth rates of the placenta and fetus vary greatly (Robinson et al., 1995). Normal placental growth is a requirement for normal fetal growth (Robinson et al., 1995).

Offspring born at an above average weight have an increased chance of survival compared to those born at a below average weight in all domestic livestock species (Vonnahme, 2012). Utero-placental blood flow increases dramatically to support the nutritional demands of the rapidly growing fetus (Vonnahme at al., 2014). In the undernourished model, nutrient ability in maternal

plasma is reduced, therefore uptake by the gravid uterus is limited (Vonnahme, 2012). In adult ewes an increased placental blood flow, combined with an increase in the extraction of glucose and oxygen helps to maintain the fetal supply of these essential substrates (Robinson et al., 1995). In addition, placental consumption of glucose and oxygen is reduced (Robinson et al., 1995). In normal pregnancies, resistance of the utero-placental arteries decrease as gestation advances (Vonnahme, 2012). In women and sheep increased umbilical artery resistance (PI and RI) is correlated with reduced birth weights (Robinson et al., 1995; Vonnahme, 2012). Along with these findings greater umbilical and uterine artery blood flows have been repeatedly associated with heavier fetuses and lambs (Galan et al., 1997; Rigano et al., 2001; Ferrazi et al., 2002; Reynolds et al., 2005; Lemley at al., 2012). In adolescent sheep, UBF decreases in mid and late gestation nutrient restriction (Lemley et al., 2012). Similarly, the percentage of increase in PI is higher in restricted animals compared to control (Lekatz et al., 2013). However, mean PI and RI are not affected by the restriction (Lemley et al., 2012; Lekatz et al., 2013).

The placenta modulates fetal growth and maternal metabolism through the synthesis and secretion of steroid and peptide hormones, growth factors and cytokines (Robinson et al., 1995). In the same way, it has been suggested that E2 and P4 can modulate the expression of some growth factors (Robinson et al., 1995). Estrogen produces vasodilation via NO (White et al., 2002). Studies have shown that arteries from reproductive tissues exhibit greater sensitivity to E2 than vessels from non-reproductive tissues (Royal et al., 2012). Progesterone maintains pregnancy through the promotion of uterine growth and the suppression of myometrial contractility (Graham and Clarke, 1997). Both hormones are key for the development of a successful pregnancy and can vary in animals undergoing nutrient restriction. While the importance of these hormones is indisputable, a thorough review of their importance is out of the purview of this literature review.

Nutrient restriction during early gestation

Before implantation the local maternal environment can determine the size of the future placenta, hence the fetus (Robinson et al., 1995). Manipulation of the embryos *in vitro* has highlighted the importance of early influences on the future growth of the fetus and placenta (Robinson et al., 1995). Maternal nutrition, partly through the regulation of ovarian activity, modifies the maternal endocrine environment required for maintenance of early pregnancy (Robinson et al., 1995). In sheep, an enhancement of placental growth can be achieved by administering P4 to the mother during the first six days of pregnancy (Robinson et al., 1995). Similarly, before pregnancy, maternal nutrition influences the impact of variation of nutrient exchange with the conceptus in early pregnancy, and subsequently, is thought to alter the growth rate of the placenta (Robinson et al., 1995). The majority of placental growth happens in the first two-thirds of pregnancy, with the placenta reaching its maximum weight by d 90 in sheep (Redmer et al., 2004). Table 1.2 displays a review of studies showing the effects of early-gestation nutrient restriction on fetal, placental and blood measurements.

Table 1.2. Ovine nutrient restriction on early and early mid-gestation

Study	Days of restriction	Amount of restriction	Time of measurement	Fetal/lamb weight	Fetal organ weights	Placental weight	Velocimetry measurements	Maternal E2/P4	Glucose/ O2/PO2
Kotsampasi et al., 2009	0 - 30	50% of requirements	Birth	ND	____	____	____	____	____
Burrage et al., 2009	1 - 31	50% of requirements	D 127	ND	ND	____	Fetus: ND in HR, arterial pressure or femoral and carotid blood flows.	____	ND in fetal or maternal blood
Burrage et al., 2009	1 - 31	40% of requirements	D 127	ND	ND	____	Fetus: ↑ HR, ND in arterial pressure or femoral and carotid blood flows.	____	ND in fetal or maternal blood.
Rhodes et al., 2009	1 - 65	70% of requirements	Birth	ND	____	____	____	____	____
Andrade et al., 2013	0 - 110	50% of requirements	D 110	↓	____	____	____	____	____

ND = No difference; ↓= decreased; ↑= increased; ___ = Not measured; HR = heart rate; D = day

Nutrient restriction during mid-gestation

In humans, small placental volumes at mid gestation are associated with small-for-gestational-age babies at term (Robinson et al., 1995). Placental growth retardation precedes fetal growth retardation (Robinson et al., 1995). In the ewe, the growth of the cotyledonary mass is exponential during the first 70 or 80 d of pregnancy, slowing during the last third of pregnancy (Vonnahme, 2012). Similarly, at mid-gestation, the fetal metabolic rate and visceral organ growth are at their greatest (McMullen et al., 2005). From mid to late gestation, the capillaries in the caruncle increase in area and number, this is modest compared to the cotyledon (Vonnahme, 2012). The increase in cotyledonary vasculature is critical for the rapid fetal growth during the second half of gestation (Ma et al., 2011). The large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends on the dramatic growth of the utero-placental vascular beds during the first half of pregnancy (Vonnahme, 2012). Also at mid-gestation, the fetal metabolic rate and visceral organ growth are at their highest (McMullen et al., 2005). Table 1.3 displays a review of studies showing the effects of mid-gestation nutrient restriction on fetal, placental, and blood measurements.

Table 1.3. Ovine nutrient restriction on early-mid and mid gestation

Study	Days of restriction	Amount of restriction	Time of measurement	Fetal/lamb weight	Fetal organ weights	Placental weight	Velocimetry measurements	Maternal E2/P4	Glucose/ O2/PO2
Kotsampasi et al., 2009	31 - 100	50% of requirements	Birth	ND	____	____	____	____	____
Ma et al., 2011	28 – 78	50% of requirements	D 78	↓	↓ brain	↓	____	____	Fetal and maternal: ↓ glucose
Zhou et al., 2008	28 - 78	50% of requirements	D 78	↓	↓ Liver, lungs, longissimus muscle	____	____	____	____
Andrade et al., 2013	0-110	50% of requirements	D 110	↓	____	____	____	____	____
Lekatz et al., 2014	50 - 90	60% of requirements	D 132	ND	____	ND in placentome, cotyledon or caruncular wt.	____	↑ P4 in during restriction	____
Sharkey et al., 2009	30 – 80	50% of requirements	Birth	ND	____	____	____	____	____
Sebert et al., 2008	30 - 80	50% of requirements	Birth	ND	____	____	____	____	____
Gilbert et al., 2005	28 - 78	50% of requirements	Birth	ND	____	____	____	____	____
Murdoch et al., 2003	28 - 78	50% of requirements	D 78	↓	____	____	____	ND	____
Satterfield et al., 2011	35 - 125	50% of requirements	D 125	↓	↓ stomach, brown adipose tissue	ND in placentome No. or wt	____	↑ P4	Maternal glucose: ND

Table 1.3. Ovine nutrient restriction on early-mid and mid gestation (continued)

Study	Days of restriction	Amount of restriction	Time of measurement	Fetal/lamb weight	Fetal organ weights	Placental weight	Velocimetry measurements	Maternal E2/P4	Glucose/ O2/PO2
Wallace et al., 2010 ¥	4 to birth	75% of requirements	Birth	ND	____	ND in total placental wt; ↓cotyledon wt.	____	____	Maternal: ↓ glucose from D 112 to 140
Fahey et al., 2005	30 - 70	50% of requirements	Birth	ND	Various muscles not different	ND in wt; ↑ No. of placentomes per placenta.	____	____	____
Fahey et al., 2005	55 - 95	50% of requirements	Birth	ND	Various muscles not different	ND	____	____	____
Mellor and Murray, 1982	From D 35 to 50 until ewes lost 5 kg. (Max. D 142).	60 to 80% of requirements	D 142	At D 90 fetuses were shorter, at D 142 fetuses were lighter and smaller, ↓ growth rate	↓ Crown-rump length	↓ at day 142	____	____	Maternal: ↓ glucose at D 105

ND = No difference; ↓= decreased; ↑= increased; ___ = not measured; HR = heart rate; D = day; ¥ = ewe lambs

Nutrient restriction during late gestation

Exponential fetal growth occurs during the last third of pregnancy (Redmer et al., 2004). The fractional distribution of cardiac output to the uterus increases from 0.5% in the non-pregnant ewe to over 16% in the late pregnant ewe (reviewed in Vonnahme et al., 2014). Of the overall increase in blood flow to the gravid uterus by late gestation, more than 85% is directed towards the caruncular vascular beds (reviewed in Vonnahme et al., 2014). Therefore, a decreased birth weight is a common result of late-gestation nutrient restriction with several studies reporting IUGR (Ferrazzi et al., 2002; Redmer et al., 2004; Lemley et al., 2012; Table 1.4). There is evidence in sheep that the reduction in birth weight in late pregnancy is highest when maternal protein intakes are low (Luther et al., 2005). Sometimes there is a lack of difference in birth weights, yet there are reported differences in postnatal performance (Vonnahme et al., 2014). Early postnatal health may be better predicted by birth weight, however many of the phenotypes that are economically important to the producers (reproductive function, milking ability or carcass quality) may not be predicted by birth weight alone (Vonnahme et al., 2014). It is hypothesized that trajectory growth, including prenatal growth that is dependent on placental function, is a better predictor of postnatal performance in livestock (Vonnahme et al., 2014). Reduction of fetal growth during the last period of pregnancy sets three days after maternal under nutrition begins (Mellor and Matheson, 1979). Table 1.4 displays a review of studies showing the effects of late-gestation nutrient restriction on fetal, placental and blood measurements.

Table 1.4. Ovine nutrient restriction on mid-late and late gestation

Study	Days of restriction	Amount of restriction	Time of measurement	Fetal/lamb weight	Fetal organ weights	Placental weight	Velocimetry measurements	Maternal E2/P4	Glucose/ O2/PO2
Scheafer et al., 2004	50 - 130	60% of requirements	D 130	↓	____	____	____	____	____
Burrage et al., 2009	104 - 127	50% of requirements	D 127	ND	ND	____	Fetus: ↑ gut flow	____	Fetal and matern.: ND
Rhodes et al., 2009	65 - 128	70% of requirements	Birth	ND	____	____	____	____	____
Meza-Herera et al., 2015 §	100 to birth	Wheat straw, non-supplemented	Birth	↓	____	↓Cotyledon number and diameter	____	____	____
Yakubu et al., 2007	110 to birth	50% of requirements	____	ND	↓ Lungs, kidneys	____	____	____	____
Tygesen et al., 2007	Approx. 105 to birth	Approx. 50% of a.l. silage + supplement	Birth	↓	____	____	____	____	Matern. glucose: ND
Hou et al., 2013 ¥	Approx. 105 to birth	50% of requirements	Birth	↓	____	____	____	____	____
Feng et al., 2007	90 to birth	0.33 MJME*kgw$^{-0.75}$*d^{-1} vs a.l.	Birth	↓	____	____	____	____	↓ matern. Glucose
Feng et al., 2007	90 to birth	0.175 MJME*kgw$^{-0.75}$*d^{-1} vs a.l.	Birth	↓	____	____	____	____	↓ matern. Glucose
Lekatz et al., 2010	90 - 130	60% of requirements	D 132	↓	____	ND in placentome, cotyledon or caruncular weights	____	↑ P4 during restriction (NS)	____

Table 1.4. Ovine nutrient restriction on mid-late and late gestation (continued)

Study	Days of restriction	Amount of restriction	Time of measurement	Fetal/lamb weight	Fetal organ weights	Placental weight	Velocimetry measurements	Maternal E2/P4	Glucose/ O2/PO2
Lekatz et al., 2010	50 - 130	60% of requirements	D 132	↓	____	ND in placentome, cotyledon or caruncular weights	____	↑ P4 during restriction (NS)	____
Shukla et al., 2014	50 - 130	60% of requirements	D 130	ND	↑ Right ventricle base thickness	____	____	____	____
Hyatt et al., 2008	115 to birth	50% of a.l. + supplement	Birth	↓ ∩ and ND £	↓ liver	____	____	____	Fetus: ND in hepatic glycogen
Vonnahme et al., 2013 ¥	50 to birth	60% of requirements	Birth	____	____	____	____	↑ P4; ↑ E2 in mid gestation	____
Lekatz et al., 2013	50 - 130	60% of requirements	Until D 108	____	ND on US biparietal, abdominal widths and HR (during pregnancy)	ND on US placentome area (during pregnancy)	ND in PI and RI through restriction period; ↑ % PI change.	____	____
Reed and Ward et al., 2014	64 - 135	60% of requirements	D 135	↓	↓ Heart girth Ø, GI tract, stomach and liver	____	____	____	____

Table 1.4. Ovine nutrient restriction on mid-late and late gestation (continued)

Study	Days of restriction	Amount of restriction	Time of measurement	Fetal/lamb weight	Fetal organ weights	Placental weight	Velocimetry measurements	Maternal E2/P4	Glucose/ O2/PO2
Fahey et al., 2005	85 - 115	50% of requirements	Birth	↓	↓Semitend. and vastus lateralis muscle	ND	____	____	____
Lemley et al., 2012 ¥	50 - 130	60% of requirements	D 130	↓	D 110: ↓ US abdominal width; D 130: ↓ carcass weight, biparietal diameter, spleen, liver, lungs, and large intestine; ND in brain, heart and kidneys	ND in total placenta or placentome weight and number	↓ UBF on D 80 – 110; ND in PI and RI; ↓ uterine artery blood flow on d 130.	____	____

ND = No difference; ↓= decreased; ↑= increased; ___ = not measured; HR = heart rate; D = day; § = also exposed to heat stress; ¥ = ewe lambs; (NS) = not significant; ∩ = also cold exposed; £ = not cold exposed; US= ultrasound; (NS) = not significant; a.l. = ad libitum; Ø = circumference; GI = gastrointestinal.

Realimentation in pregnant sheep

Only a handful of studies have analyzed separately the effects of nutrient restriction, specified them and then reported if realimentation was able to counteract the effects of such restriction. Most of the studies restricted the dam during various periods of time and measured the results towards the end of pregnancy or at lambing (Tables 1.2, 1.3 and 1.4). A majority of the studies that nutrient restricted sheep during mid-gestation and refed them during a later period of gestation showed no difference in lamb or placental weights at birth (Gilbert et al., 2005; Sebert et al., 2008; Kotsampasi et al., 2009; Sharkey et al., 2009; Ma et al., 2011; Table 1.3).

One of the first studies that investigated the direct effects of realimentation in the sheep focused on the last 60 days of pregnancy (Mellor and Matheson, 1979). They found that fetal growth stops during the last third of pregnancy under severe undernourished conditions in ewes, and it continues after realimentation (Mellor and Matheson, 1979). It is possible that placental efficiency is enhanced in mothers that were previously nutrient restricted and realimented (Symonds et al., 2010). When mothers are nutrient restricted during mid-gestation, glucose levels drop (Ma et al., 2011). This glucose drop can vary the phenotype of the placental nutrient transport, for example, it increases the capacity of the placenta to transport triglycerides (Ford et al., 2007; Ma et al., 2011). Upon realimentation, glucose levels normalize; however, some of the effects of nutrient restriction on the placental phenotype remain, changing the fetal body composition with an increased body fat deposition (Ma et al., 2011). Similarly, variations in nutrient availability during mid-gestation have consequences in glucose and fat metabolism later in life as well as in the cardiovascular and urinary systems of the offspring (Gilbert et al., 2005; Sharkey et al., 2009; Ma et al., 2011; Hou et al., 2013; Table 1.3).

Studies of the effects of nutrient restriction and realimentation on umbilical and uterine artery blood flows in farm animals are limited. In cows, a 60% nutrient restriction during mid-gestation does not decrease uterine blood flow (Camacho et al., 2014). However, realimentation increases total and ipsilateral uterine artery blood flow (Camacho et al., 2014). To our knowledge, there are no studies that investigate the effects of nutrient restriction and realimentation in umbilical blood flow in sheep and it remains unknown if an increase in fetal body weight after realimentation is secondary to an increase in UBF in sheep. Table 1.5 displays a review of studies showing the effects of nutrient restriction and realimentation on fetal, placental and blood measurements.

Table 1.5. Ovine nutrient restriction and realimentation

Study	Amount of restriction	Period of restriction	Period of realimentation	Fetal weight after restriction	Fetal weight after realimentation	Placental weight after restriction	Placental weight after realimentation	Blood hormones and metabolites after restriction	Blood hormones and metabolites after realimentation
Ma et al., 2011	50% of requirements	28 - 78	78 - 135	↓	ND	↓	ND	↓ fetal and maternal glucose	ND in maternal and fetal glucose
Mellor and Matheson, 1979	Maternal glucose concentration 1.20 – 1.80 $mmol.l^{-1}$ (Control 2.5 – 3.20 $mmol.l^{-1}$)	116 – 124; 122 – 130.	124 to Birth; 130 to Birth.	↓ growth rate	Recovered growth rate	——	——	——	——
McMullen et al., 2005	Completely withdrawn	85 - 90	90 - 135	ND in total weight; ↓ thoracic girth and lung weight.	ND in total weight; ↓ lung weight.	↓	ND	ND in maternal glucose	ND in maternal glucose

ND = No difference; ↓= decreased; ___ = Not measured; HR = heart rate; D = day

Parity effects in sheep

In sheep, conception rate increases and fetal loss decreases as parity increases (Lafi et al., 2009). Similarly, the likelihood of twins increases as the number of parity increases (Lafi et al., 2009). In cattle, sheep, and mares, birth weight increases as parity increases (Kayisiz et al., 2010; Symonds et al., 2010; Yakubu et al., 2014; Abdel-Mageed and Abd El-Gawad, 2015; Klewitz et al., 2015; Lv et al., 2015). These adaptations are mediated at least in part by the changes in maternal physiology and uterine function after completion of a first pregnancy (Wilsher and Allen, 2003; Symonds et al., 2010).

In the human placenta, the microscopic surface area for exchange is bigger in normal pregnancies when compared to pregnancies with small-for-date fetuses (Robinson et al., 1995). In addition, these pregnancies have a proportionate reduction of parenchymal and non-parenchymal tissues of the placenta (Robinson et al., 1995). In mares, the increased foal birth weight of multiparous mothers has been related to a greater volume of the chorion, greater microcotyledon surface density, greater total microscopic area of feto-maternal contact and greater mass of the chorioallantois in young and middle-age multiparous mares compared to first parity mares (Wilsher and Allen, 2003). Similarly, foal birth weights are strongly correlated with greater microscopic area of feto-maternal contact (Wilsher and Allen, 2003). These changes are thought to be necessary for a "priming" of the mare's uterus by a first pregnancy before it can achieve its full potential in terms of promoting placental and fetal growth (Wilsher and Allen, 2003).

In mares, uterine artery diameter is more increased during all pregnancy in multiparous (3 to 8 foalings) when compared to first and second parity mares (Klewitz et al., 2015). Similarly, blood flow is increased in the uterine artery during the third period of gestation in multiparous mares when compared to first and second parity mares (Klewitz et al., 2015). In women PI

measurements taken on the uterine artery have shown that primiparous mothers have a greater PI value during mid-gestation (17 to 18 weeks) when compared to multiparous mothers (Suzuki et al., 2006). In the same way, many studies show that nulliparous women have greater blood pressure, pregnancy induced hypertension and greater risk of preeclampsia than multiparous women (Duckitt and Harrington, 2005; Rurangirwa et al., 2011). As far as we know, parity effects on the umbilical blood flow of nutrient restricted and realimented sheep have not been studied.

Statement of the problem

Current practice in the sheep industry is to get ewes pregnant as young as possible. However, there is also a desire to increase reproductive longevity within the flock. There currently is a lack of information on how age, parity, and nutritional status during pregnancy impacts the fetus, the reproductive success and longevity of the dam and overall profitability. The outcome of the following research studies establishes a foundation on how UBF is affected by parity and an inadequate nutrition during mid gestation. Continued efforts in this area could ultimately determine management practices.

This literature review exposes the importance of an adequate nutrition during all the periods of gestation. It also reviews the importance of the feto-placental circulatory physiology stating the period (mid-gestation) in which major development of this system is accomplished in sheep. Through indirectly measuring the amount of nutrients that are delivered to the fetus, UBF, via Doppler ultrasonography, can be used as an assessment of fetal growth without the necessity of terminating pregnant animals or performing major surgery. Several studies have analyzed the correlation between IUGR and a decreased UBF during several periods of gestation; however, none that we know of have attempted to analyze how realimentation and parity influence UBF during and after nutrient restriction in mid-gestation. The following chapters comprehend two

experiments through which we attempted to address these questions. The objectives were to investigate if nutrient restriction alone or with an additional effect of parity can cause a decrease in UBF during mid-gestation, and if realimentation could counteract this hypothetical effect. Our hypothesis was that the restriction, if not by itself, with the added effect of parity would decrease UBF, and that this decrease would recover control values upon realimentation.

Literature cited

Abdel-Mageed, I. and M. A. El-Gawad. 2015. Effects of breed, parity and post-mating nutrition on reproductive wastage and pregnancy outcomes of Egyptian sheep. Small Ruminant Research 130:171-177.

Aksoy, A. N., G. Batmaz, B. Dane, S. K. Kucur, and İ. Gözükara. 2015. Effects of altitude changes on Doppler flow parameters for uterine, umbilical, and mid-cerebral arteries in term pregnancy: A pilot study. Journal of the Turkish German Gynecological Association 16(4):237.

Andrade, L. P., S. M. Rhind, M. T. Rae, C. E. Kyle, J. Jowett, and R. G. Lea. 2013. Maternal undernutrition does not alter Sertoli cell numbers or the expression of key developmental markers in the mid-gestation ovine fetal testis. Journal of Negative Results in Biomedicine 12(1):1.

Anthony, R., A. Scheaffer, C. Wright, and T. Regnault. 2003. Ruminant models of prenatal growth restriction. Reproduction-Cambridge-Supplement:183-194.

Assali, N. and D. Vaughn. 1977. Blood volume in pre-eclampsia: fantasy and reality. American Journal of Obstetrics and Gynecology 129(4):355-359.

Barcroft, J., J. Kennedy, and M. Mason. 1939. The blood volume and kindred properties in pregnant sheep. The Journal of Physiology 95(1):159-172.

Barry, J. S. and R. V. Anthony. 2008. The pregnant sheep as a model for human pregnancy. Theriogenology 69(1):55-67.

Behrman, R., M. Lees, E. Peterson, C. W. de Lannoy, and A. Seeds. 1970. Distribution of the circulation in the normal and asphyxiated fetal primate. American Journal of Obstetrics and Gynecology 108(6):956-969.

Bell, A., B. McBride, R. Slepetis, R. Early, and W. Currie. 1989. Chronic heat stress and prenatal development in sheep: I. Conceptus growth and maternal plasma hormones and metabolites. Journal of Animal Science 67(12):3289-3299.

Bell, A., R. Wilkening, and G. Meschia. 1987. Some aspects of placental function in chronically heat-stressed ewes. Journal of Developmental Physiology 9(1):17-29.

Bjarnegård, N., E. Morsing, M. Cinthio, T. Länne, and J. Brodszki. 2013. Cardiovascular function in adulthood following intrauterine growth restriction with abnormal fetal blood flow. Ultrasound in Obstetrics and Gynecology 41(2):177-184.

Brace, R. A. 1983. Fetal blood volume responses to intravenous saline solution and dextran. American Journal of Obstetrics and Gynecology 147(7):777-781.

Burrage, D., L. Braddick, J. Cleal, P. Costello, D. Noakes, M. Hanson, and L. Green. 2009. The late gestation fetal cardiovascular response to hypoglycaemia is modified by prior peri-implantation undernutrition in sheep. The Journal of Physiology 587(3):611.

Camacho, L., C. Lemley, L. Prezotto, M. Bauer, H. Freetly, K. Swanson, and K. Vonnahme. 2014. Effects of maternal nutrient restriction followed by realimentation during midgestation on uterine blood flow in beef cows. Theriogenology 81(9):1248-1256. e1243.

Campbell, D. M. and A. J. Campbell. 1983. Evans Blue disappearance rate in normal and pre-eclamptic pregnancy. Clinical and Experimental Hypertension. Part B: Hypertension in Pregnancy 2(1):163-169.

Caton, D., C. J. Wilcox, R. Abrams, and D. H. Barron. 1975. The circulating plasma volume of the foetal lamb as an index of its weight and rate of weight gain (g/day) in the last third of gestation. Quarterly Journal of Experimental Physiology and Cognate Medical Sciences 60(1):45-54.

Courtice, F. 1943. The blood volume of normal animals. The Journal of Physiology 102(3):290.

Daniel, S., S. James, R. Stark, and P. Tropper. 1989. Prevention of the normal expansion of maternal plasma volume: a model for chronic fetal hypoxaemia. Journal of Developmental Physiology 11(4):225-233.

Dawes, G., J. C. Mott, and J. Widdicombe. 1954. The foetal circulation in the lamb. The Journal of Physiology 126(3):563.

Dien, K. and C. Lentner. 1970. Documenta Geigy Scientific Tables. Ciba-Geigy, Basel, Switzerland.

Duckitt, K. and D. Harrington. 2005. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. British Medical Journal 330(7491):565.

Duvekot, J. J., E. C. Cheriex, F. A. Pieters, P. P. Menheere, H. J. Schouten, and L. L. Peeters. 1995. Maternal volume homeostasis in early pregnancy in relation to fetal growth restriction. Obstetrics and Gynecology 85(3):361-367.

Edelstone, D. I. and A. M. Rudolph. 1979. Preferential streaming of ductus venosus blood to the brain and heart in fetal lambs. American Journal of Physiology-Heart and Circulatory Physiology 237(6):H724-H729.

Everett, N. B., B. Simmons, and E. P. Lasher. 1956. Distribution of blood (Fe59) and plasma (I131) volumes of rats determined by liquid nitrogen freezing. Circulation Research 4:419-424.

Fahey, A., J. Brameld, T. Parr, and P. Buttery. 2005. The effect of maternal undernutrition before muscle differentiation on the muscle fiber development of the newborn lamb. Journal of Animal Science 83(11):2564-2571.

Ferrazzi, E., M. Bozzo, S. Rigano, M. Bellotti, A. Morabito, G. Pardi, F. Battaglia, and H. Galan. 2002. Temporal sequence of abnormal Doppler changes in the peripheral and central circulatory systems of the severely growth-restricted fetus. Ultrasound in Obstetrics and Gynecology 19(2):140-146.

Ford, S., B. Hess, M. Schwope, M. Nijland, J. Gilbert, K. Vonnahme, W. Means, H. Han, and P. Nathanielsz. 2007. Maternal undernutrition during early to mid-gestation in the ewe results in altered growth, adiposity, and glucose tolerance in male offspring. Journal of Animal Science 85(5):1285-1294.

Galan, H. L., M. J. Hussey, A. Barbera, E. Ferrazzi, M. Chung, J. C. Hobbins, and F. C. Battaglia. 1999. Relationship of fetal growth to duration of heat stress in an ovine model of placental insufficiency. American Journal of Obstetrics and Gynecology 180(5):1278-1282.

Galan, H. L., M. J. Hussey, M. Chung, J. K. Chyu, J. C. Hobbins, and F. C. Battaglia. 1998. Doppler velocimetry of growth-restricted fetuses in an ovine model of placental insufficiency. American Journal of Obstetrics and Gynecology 178(3):451-456.

Galan, H. L., S. Rigano, J. Chyu, B. Beaty, M. Bozzo, J. C. Hobbins, and E. Ferrazzi. 2000. Comparison of low-and high-altitude Doppler velocimetry in the peripheral and central circulations of normal fetuses. American Journal of Obstetrics and Gynecology 183(5):1158-1161.

Gao, F., X. Hou, and Y. Liu. 2007. Effect of hormonal status and metabolic changes of restricted ewes during late pregnancy on their fetal growth and development. Science in China Series C: Life Sciences 50(6):766-772.

Gilbert, J. S., A. L. Lang, A. R. Grant, and M. J. Nijland. 2005. Maternal nutrient restriction in sheep: hypertension and decreased nephron number in offspring at 9 months of age. The Journal of Physiology 565(1):137-147.

Goodlin, R., M. Quaife, and J. Dirksen. 1981. The significance, diagnosis, and treatment of maternal hypovolemia as associated with fetal/maternal illness. Obstetrical and Gynecological Survey 36(10):541-542.

Graham, J. D. and C. L. Clarke. 1997. Physiological action of progesterone in target tissues 1. Endocrine Reviews 18(4):502-519.

Grünberger, W., S. Leodolter, and O. Parschalk. 1979. Maternal hypotension: fetal outcome in treated and untreated cases. Gynecologic and Obstetric Investigation 10(1):32-38.

Hou, L., A. H. Kongsted, S. M. Ghoreishi, T. K. Takhtsabzy, M. Friedrichsen, L. I. Hellgren, H. N. Kadarmideen, A. Vaag, and M. O. Nielsen. 2013. Pre-and early-postnatal nutrition modify gene and protein expressions of muscle energy metabolism markers and phospholipid fatty acid composition in a muscle type specific manner in sheep. PloS one 8(6):e65452.

Huhta, J. C., M. Cohen, and H. P. Gutgesell. 1984. Patency of the ductus arteriosus in normal neonates: two-dimensional echocardiography versus Doppler assessment. Journal of the American College of Cardiology 4(3):561-564.

Hyatt, M., E. Butt, H. Budge, T. Stephenson, and M. Symonds. 2008. Effects of maternal cold exposure and nutrient restriction on the ghrelin receptor, the GH–IGF axis, and metabolic regulation in the postnatal ovine liver. Reproduction 135(5):723-732.

Ireland, J., R. Roberts, G. H. Palmer, D. E. Bauman, and F. W. Bazer. 2008. A commentary on domestic animals as dual-purpose models that benefit agricultural and biomedical research. Journal of Animal Science 86(10):2797-2805.

Jellyman, J. K., D. S. Gardner, A. L. Fowden, and D. A. Giussani. 2004. Effects of dexamethasone on the uterine and umbilical vascular beds during basal and hypoxemic conditions in sheep. American Journal of Obstetrics and Gynecology 190(3):825-835.

Jobe, A. H., N. Wada, L. M. Berry, M. Ikegami, and M. G. Ervin. 1998. Single and repetitive maternal glucocorticoid exposures reduce fetal growth in sheep. American Journal of Obstetrics and Gynecology 178(5):880-885.

Kaygisiz, A., G. Bakir, I. Yilmaz, and Y. Vanli. 2011. Estimation of variance components and genetic parameters for direct and maternal effects on birth weight in Brown Swiss cattle. Pakistan Veterinary Journal 31(1):70-74.

Kiserud, T. 2005. Physiology of the fetal circulation. Pages 493-503 in Proc. Seminars in Fetal and Neonatal Medicine. Elsevier.

Kiserud, T., S. Rasmussen, and S. Skulstad. 2000. Blood flow and the degree of shunting through the ductus venosus in the human fetus. American Journal of Ostetrics and Gynecology 182(1):147-153.

Klewitz, J., C. Struebing, K. Rohn, A. Goergens, G. Martinsson, F. Orgies, J. Probst, F. Hollinshead, H. Bollwein, and H. Sieme. 2015. Effects of age, parity, and pregnancy abnormalities on foal birth weight and uterine blood flow in the mare. Theriogenology 83(4):721-729.

Kotsampasi, B., C. Balaskas, G. Papadomichelakis, and S. Chadio. 2009. Reduced Sertoli cell number and altered pituitary responsiveness in male lambs undernourished in utero. Animal Reproduction Science 114(1):135-147.

Lafi, S., A. Talafha, N. Giadinis, E. Kalaitzakis, K. Pourliotis, and N. Panousis. 2009. Factors affecting the reproductive performance of Awassi sheep flocks in north-east of Jordan: An epidemiological study. Tropical Animal Health and Production 41(8):1755-1764.

Lechat, P., J. Mas, G. Lascault, P. Loron, M. Theard, M. Klimczac, G. Drobinski, D. Thomas, and Y. Grosgogeat. 1988. Prevalence of patent foramen ovale in patients with stroke. New England Journal of Medicine 318(18):1148-1152.

Lekatz, L., J. Caton, J. Taylor, L. Reynolds, D. Redmer, and K. Vonnahme. 2010. Maternal selenium supplementation and timing of nutrient restriction in pregnant sheep: effects on maternal endocrine status and placental characteristics. Journal of Animal Science 88(3):955-971.

Lekatz, L., J. Luther, J. Caton, and K. Vonnahme. 2013. Impacts of maternal nutritional plane on umbilical artery hemodynamics, fetal and placentome growth in sheep. Animal Reproduction 10(2):99-105.

Lemley, C. O., A. M. Meyer, L. E. Camacho, T. L. Neville, D. J. Newman, J. S. Caton, and K. A. Vonnahme. 2012. Melatonin supplementation alters uteroplacental hemodynamics and fetal development in an ovine model of intrauterine growth restriction. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 302(4):R454-R467.

Lind, J. and C. Wegelius. 1949. Angiocardiographic studies on the human foetal circulation: A preliminary report. Pediatrics 4(4):391-400.

Longo, L. 1983. Maternal blood volume and cardiac output during pregnancy: a hypothesis of endocrinologic control. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 245(5):R720-R729.

Lund, C. J. and J. C. Donovan. 1967. Blood volume during pregnancy. American Journal of Obstetrics and Gynecology 98(3):393-403.

Luther, J. S., D. A. Redmer, L. P. Reynolds, and J. M. Wallace. 2005. Nutritional paradigms of ovine fetal growth restriction: implications for human pregnancy. Human Fertility 8(3):179-187.

Lv, S.-J., Y. Yang, C. Dwyer, and F.-K. Li. 2015. Pen size and parity effects on maternal behaviour of Small-Tail Han sheep. Animal 9(07):1195-1202.

Ma, Y., M. J. Zhu, A. B. Uthlaut, M. J. Nijland, P. W. Nathanielsz, B. W. Hess, and S. P. Ford. 2011. Upregulation of growth signaling and nutrient transporters in cotyledons of early to mid-gestational nutrient restricted ewes. Placenta 32(3):255-263.

Mann, D. L., D. P. Zipes, P. Libby, and R. O. Bonow. 2014. Braunwald's heart disease: a textbook of cardiovascular medicine. Elsevier Health Sciences.

McMullen, S., J. Osgerby, J. Milne, J. Wallace, and D. Wathes. 2005. The effects of acute nutrient restriction in the mid-gestational ewe on maternal and fetal nutrient status, the expression of placental growth factors and fetal growth. Placenta 26(1):25-33.

Mellor, D. and I. Matheson. 1979. Daily changes in the curved crown-rump length of individual sheep fetuses during the last 60 days of pregnancy and effects of different levels of maternal nutrition. Quarterly Journal of Experimental Physiology and Cognate Medical Sciences 64(2):119-131.

Mellor, D. and L. Murray. 1982. Effects of long term undernutrition of the ewe on the growth rates of individual fetuses during late pregnancy. Research in Veterinary Science 32(2):177-180.

Metcalfe, J. and J. Parer. 1966. Cardiovascular changes during pregnancy in ewes. American Journal of Physiology--Legacy Content 210(4):821-825.

Meza-Herrera, C. A., A. Vicente-Pérez, Y. Osorio-Marín, B. S. Girón-Gómez, E. Beltran-Calderon, L. Avendaño-Reyes, A. Correa-Calderon, and U. Macías-Cruz. 2015. Heat stress, divergent nutrition level, and late pregnancy in hair sheep: effects upon cotyledon development and litter weight at birth. Tropical Animal Health and Production 47(5):819-824.

Milchunas, D. G., J. Y. King, A. R. Mosier, J. C. Moore, J. A. Morgan, M. H. Quirk, and J. R. Slusser. 2004. UV radiation effects on plant growth and forage quality in a shortgrass steppe ecosystem. Photochemistry and Photobiology 79(5):404-410.

Muñoz, C., A. Carson, M. McCoy, L. Dawson, N. O'Connell, and A. Gordon. 2009. Effect of plane of nutrition of 1-and 2-year-old ewes in early and mid-pregnancy on ewe reproduction and offspring performance up to weaning. Animal 3(05):657-669.

Murdoch, W. J., E. A. Van Kirk, K. A. Vonnahme, and S. P. Ford. 2003. Ovarian responses to undernutrition in pregnant ewes, USA. Reproductive Biology and Endocrinology 1(6).

Murphy, P. J. 2005. The fetal circulation. Continuing Education in Anaesthesia, Critical Care and Pain 5(4):107-112.

Nuwayhid, B. 1979. Hemodynamic changes during pregnancy in the rabbit. American Journal of Obstetrics and Gynecology 135(5):590-596.

Parraguez, V. H., M. Atlagich, R. Díaz, M. E. Bruzzone, C. Behn, and L. A. Raggi. 2005. Effect of hypobaric hypoxia on lamb intrauterine growth: comparison between high-and low-altitude native ewes. Reproduction, Fertility and Development 17(5):497-505.

Parraguez, V. H., S. Mamani, E. Cofré, G. Castellaro, B. Urquieta, M. De los Reyes, S. Astiz, and A. Gonzalez-Bulnes. 2015. Disturbances in maternal steroidogenesis and appearance of

intrauterine growth retardation at high-altitude environments are established from early pregnancy: effects of treatment with antioxidant vitamins. PloS one 10(11):e0140902.

Pritchard, J. A. 1965. Changes in the blood volume during pregnancy and delivery. The Journal of the American Society of Anesthesiologists 26(4):393-399.

Redmer, D., J. Wallace, and L. Reynolds. 2004. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domestic Animal Endocrinology 27(3):199-217.

Reed, J., M. Ward, K. Vonnahme, T. Neville, S. Julius, P. Borowicz, J. Taylor, D. Redmer, A. Grazul-Bilska, and L. Reynolds. 2007. Effects of selenium supply and dietary restriction on maternal and fetal body weight, visceral organ mass and cellularity estimates, and jejunal vascularity in pregnant ewe lambs. Journal of Animal Science 85(10):2721-2733.

Reynolds, L. P., J. S. Caton, D. A. Redmer, A. T. Grazul-Bilska, K. A. Vonnahme, P. P. Borowicz, J. S. Luther, J. M. Wallace, G. Wu, and T. E. Spencer. 2006. Evidence for altered placental blood flow and vascularity in compromised pregnancies. The Journal of Physiology 572(1):51-58.

Reynolds, L. P., P. P. Borowicz, K. A. Vonnahme, M. L. Johnson, A. T. Grazul-Bilska, D. A. Redmer, and J. S. Caton. 2005. Placental angiogenesis in sheep models of compromised pregnancy. The Journal of Physiology 565(1):43-58.

Reynolds, L., C. Ferrell, J. Nienaber, and S. Ford. 1985. Effects of chronic environmental heat stress on blood flow and nutrient uptake of the gravid bovine uterus and foetus. The Journal of Agricultural Science 104(02):289-297.

Rhodes, P., J. Craigon, C. Gray, S. M. Rhind, P. T. Loughna, and D. S. Gardner. 2009. Adult-onset obesity reveals prenatal programming of glucose-insulin sensitivity in male sheep nutrient restricted during late gestation. PloS one 4(10):e7393.

Rigano, S., M. Bozzo, E. Ferrazzi, M. Bellotti, F. C. Battaglia, and H. L. Galan. 2001. Early and persistent reduction in umbilical vein blood flow in the growth-restricted fetus: a longitudinal study. American Journal of Obstetrics and Gynecology 185(4):834-838.

Robinson, J., S. Chidzanja, K. Kind, F. Lok, P. Owens, and J. Owens. 1995. Placental control of fetal growth. Reproduction, Fertility and Development 7(3):333-344.

Royal, C. R., H. Ma, R. Walker, and R. E. White. 2011. Estrogen signaling in microvascular arteries: Parturition reduces vasodilation by reducing 17β-estradiol and nNOS. Steroids 76(10):991-997.

Rudolph, A. M. and M. A. Heymann. 1968. The fetal circulation. Annual Review of Medicine 19(1):195-206.

Rumball, C., F. Bloomfield, and J. Harding. 2008. Cardiovascular adaptations to pregnancy in sheep and effects of periconceptional undernutrition. Placenta 29(1):89-94.

Rurangirwa, A. A., R. Gaillard, E. A. Steegers, A. Hofman, and V. W. Jaddoe. 2012. Hemodynamic adaptations in different trimesters among nulliparous and multiparous pregnant women; the Generation R study. American Journal of Hypertension 25(8):892-899.

Satterfield, M. C., K. A. Dunlap, D. H. Keisler, F. W. Bazer, and G. Wu. 2013. Arginine nutrition and fetal brown adipose tissue development in nutrient-restricted sheep. Amino acids 45(3):489-499.

Scheaffer, A., J. Caton, D. Redmer, and L. Reynolds. 2004. The effect of dietary restriction, pregnancy, and fetal type in different ewe types on fetal weight, maternal body weight, and visceral organ mass in ewes. Journal of Animal Science 82(6):1826-1838.

Schrier, R. W. and V. A. Briner. 1991. Peripheral arterial vasodilation hypothesis of sodium and water retention in pregnancy: implications for pathogenesis of preeclampsia-eclampsia. Obstetrics and Gynecology 77(4):632-639.

Schwab, M., T. Coksaygan, and P. W. Nathanielsz. 2006. Betamethasone effects on ovine uterine and umbilical placental perfusion at the dose used to enhance fetal lung maturation. American Journal of Obstetrics and Gynecology 194(2):572-579.

Sebert, S., M. Hyatt, L. Chan, N. Patel, R. Bell, D. Keisler, T. Stephenson, H. Budge, M. Symonds, and D. Gardner. 2009. Maternal nutrient restriction between early and midgestation and its impact upon appetite regulation after juvenile obesity. Endocrinology 150(2):634-641.

Sharkey, D., D. S. Gardner, M. E. Symonds, and H. Budge. 2009. Maternal nutrient restriction during early fetal kidney development attenuates the renal innate inflammatory response in obese young adult offspring. American Journal of Physiology-Renal Physiology 297(5):F1199-F1207.

Shukla, P., S. Ghatta, N. Dubey, C. O. Lemley, M. L. Johnson, A. Modgil, K. Vonnahme, J. S. Caton, L. P. Reynolds, and C. Sun. 2014. Maternal nutrient restriction during pregnancy impairs an endothelium-derived hyperpolarizing factor-like pathway in sheep fetal coronary arteries. American Journal of Physiology-Heart and Circulatory Physiology 307(2):H134-H142.

Silver, H. M., M. Seebeck, and R. Carlson. 1998. Comparison of total blood volume in normal, preeclamptic, and nonproteinuric gestational hypertensive pregnancy by simultaneous measurement of red blood cell and plasma volumes. American Journal of Obstetrics and Gynecology 179(1):87-93.

Stegeman, J. H. 1974. Placental development in the sheep and its relation to fetal development. A qualitative and quantitative anatomic and histologic study. Bijdragen tot de Dierkunde 44(1):3-72.

Sutera, S. P. and R. Skalak. 1993. The history of Poiseuille's law. Annual Review of Fluid Mechanics 25(1):1-20.

Suzuki, S. 2006. Influence of parity on second-trimester uterine artery Doppler waveforms in twin pregnancy. The Journal of Maternal-Fetal and Neonatal Medicine 19(3):193-194.

Symonds, M., S. Sebert, and H. Budge. 2010. Nutritional regulation of fetal growth and implications for productive life in ruminants. Animal 4(07):1075-1083.

Torgersen, C. K. L. and C. A. Curran. 2006. A systematic approach to the physiologic adaptations of pregnancy. Critical Care Nursing Quarterly 29(1):2-19.

Tygesen, M. P., M. O. Nielsen, P. Norgaard, H. Ranvig, A. P. Harrison, and A.-H. Tauson. 2008. Late gestational nutrient restriction: Effects on ewes' metabolic and homeorhetic adaptation, consequences for lamb birth weight and lactation performance. Archives of Animal Nutrition 62(1):44-59.

Vonnahme, K. 2012. How the maternal environment impacts fetal and placental development: implications for livestock production. Animal Reproduction 9:789-797.

Vonnahme, K., C. Lemley, P. Shukla, and S. O'Rourke. 2013a. 2011 and 2012 Early careers achievement awards: Placental programming: How the maternal environment can impact placental function. Journal of Animal Science 91(6):2467-2480.

Vonnahme, K., T. Neville, G. Perry, D. Redmer, L. Reynolds, and J. Caton. 2013b. Maternal dietary intake alters organ mass and endocrine and metabolic profiles in pregnant ewe lambs. Animal Reproduction Science 141(3):131-141.

Wallace, J. M., D. A. Bourke, R. P. Aitken, N. Leitch, and W. W. Hay. 2002. Blood flows and nutrient uptakes in growth-restricted pregnancies induced by overnourishing adolescent sheep. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 282(4):R1027-R1036.

Wallace, J. M., J. S. Milne, and R. P. Aitken. 2010. Effect of weight and adiposity at conception and wide variations in gestational dietary intake on pregnancy outcome and early postnatal performance in young adolescent sheep. Biology of Reproduction 82(2):320-330.

Wallace, J., D. Bourke, and R. Aitken. 1998. Nutrition and fetal growth: paradoxical effects in the overnourished adolescent sheep. Journal of Reproduction and Fertility. Supplement 54:385-399.

White, R. E. 2002. Estrogen and vascular function. Vascular pharmacology 38(2):73-80.

Wilsher, S. and W. Allen. 2003. The effects of maternal age and parity on placental and fetal development in the mare. Equine Veterinary Journal 35(5):476-483.

Wu, G., F. W. Bazer, T. A. Cudd, C. J. Meininger, and T. E. Spencer. 2004. Maternal nutrition and fetal development. The Journal of Nutrition 134(9):2169-2172.

Yakubu, D., A. Mostyn, V. Wilson, S. Pearce, M. Alves-Guerra, C. Pecqueur, B. Miroux, H. Budge, T. Stephenson, and M. Symonds. 2007. Different effects of maternal parity, cold exposure and nutrient restriction in late pregnancy on the abundance of mitochondrial proteins in the kidney, liver and lung of postnatal sheep. Reproduction 133(6):1241-1252.

Yakubu, H., P. Barje, and G. Iyeghe-Erakpotobor. 2014. Influence of calf parity number, season of calving and period of calving on birth and weaning weights of Friesian-Bunaji calves. World Journal of Life Sciences and Medical Research 3(2):59.

Zhou, Y., M. Nijland, M. Miller, S. Ford, P. W. Nathanielsz, and J. T. Brenna. 2008. The influence of maternal early to mid-gestation nutrient restriction on long chain polyunsaturated fatty acids in fetal sheep. Lipids 43(6):525-531.

CHAPTER 2. EFFECTS OF REALIMENTATION ON UMBILICAL BLOOD FLOW, FETAL AND PLACENTAL MEASUREMENTS, AND BIRTH WEIGHT IN NUTRIENT RESTRICTED EWES

Abstract

Nutritional restriction (60% of total nutritional requirement) from d 50 to 130 applied in nulliparous ewes has shown to reduce umbilical blood flow (UBF). We hypothesized that during restriction, UBF and fetal and placentome dimensional measurements would be lower as compared to adequately fed ewes, but upon realimentation, ewes would have similar UBF as ewes that were never restricted. Second parity Dorset ewes were assigned either to an adequate nutrition group (CON, n = 7) or a restricted (60% of CON) group (RES, n = 8), from d 50 to 90 of gestation. On d 90, all ewes were fed 100% of nutritional recomendations according to body weight. Ewe body weight and conceptus measurements via ultrasonography were recorded every 10 days from d 50 to 130 of gestation. Every 10 days, we calculated average length and average width by measuring 10 random placentomes. Placentome area was calculated. Fetal biparietal and abdominal length, as well as kidney length and width were recorded. Doppler mode was used to obtain UBF, pulsatility index (PI) and resistance index (RI). At birth, weights of lambs and placenta were obtained. Cotyledon numer was also recorded. Data were analyzed using the Proc Mixed procedure of SAS. Treatment and day were treated as fixed effects, ewe as random. By d 70, RES ewes were lighter ($P \leq 0.05$), and remained lighter than CON ewes throughout the experiment. While there were no treatment-by-day interactions or main effects of treatment ($P > 0.13$) for any measurements obtained by ultrasonography, there were some interesting observations. On d 80, UBF was decreased ($P \leq 0.05$, means separation of unprotected F test), placentome area tended to be decreased ($P \leq 0.10$), and PI and RI tended to increase in RES vs CON ewes ($P \leq 0.10$, means

separation of unprotected F test). On d 90, prior to the realimentation, all ultrasound measurements were similar between treatments. After realimentation, there was no effect of treatment on any of the ultrasound measurements. Hematocrit (Ht) was greater in RES vs CON ewes ($P \leq 0.05$) from d 50 until d 80. By d 90, Ht was similar between RES and CON ewes and remained similar until d 130. At birth, lambs and placental measurements were similar ($P > 0.43$). Perhaps the increased resistance indices in RES on d 80 were a trigger to the dam to enhance UBF to the growing fetus. Further studies are needed to determine the impact of maternal age and parity in the face of nutrient restriction on UBF.

Introduction

Sheep are normally managed within grazing systems; forage availability and quality are dependent upon seasonal conditions such as drought and humidity. Forages with lower quality not only have less nutrient components but also have lower digestibility (Milchunas et al, 2004). Non-digestible fibers are higher in low quality forages; therefore, these forages stay for a longer period of time in the rumen, reducing feed intake (Dickhoefer et al., 2013). Hence, we can see that seasonal conditions impact forage quality, which in turn impacts animal nutrient availability, particularly during pregnancy.

Various environmental factors affect fetal and placental development. Nutrition is the most important among these factors (Wu et al., 2004). During times of drought, ewes may experience deficient total energy requirements. Furthermore, during different stages of conceptus development, ewes may need additional nutritional supplements. Therefore, it is important for producers to know when during gestation it is critical to supplement animals with additional feed.

The majority of placental growth happens in the first two-thirds of pregnancy, with the placenta reaching its maximum weight by d 90 in sheep (Stegeman, 1974). Impairing placental

growth or utero placental blood supply affects fetal growth trajectory (Chapter 1; Reynolds et al., 2005; Vonnahme et al., 2014). Late gestation nutrient restriction results in decreased growth and development of the fetus (Redmer et al., 2004). Low birth weight is a common result of late gestation restriction with several studies reporting intrauterine growth restriction (Ferrazzi et al., 2002; Redmer et al., 2004; Lemley et al., 2012).

Ultrasonography has been successfully used to assess abnormal pregnancies in sheep and humans (Galan et al., 1998). Abnormal Doppler velocimetry measurements from a variety of fetal blood vessels have been correlated with intrauterine growth restriction, fetal and neonatal mortality, and developmental abnormalities in the offspring (Galan et al., 1998; Rigano et al. 2001; Ferrazzi et al., 2002).

Early studies in sheep focused on the pulsatility index (PI) of fetal blood vessels because this value is least affected by changes in fetal heart rate (Galan et al., 1998). As technology in ultrasound equipment advanced, researchers discovered that umbilical vein and artery blood flows are the earliest Doppler abnormalities detected in intrauterine growth restriction (Rigano et al., 2001; Ferrazzi et al., 2002).

An increased blood volume is necessary during pregnancy in order to meet the metabolic demands of an enlarged uterus and to protect the mother and the fetus from the deleterious effects of a decreased venous blood return and cardiac output (Chapter 1; Pritchard, 1965). In normal pregnancies in humans, blood volume moderately increases by the end of the first third of pregnancy, has a greater increase during the second third of pregnancy, and a slight increase during the third trimester (Hytten, 1985). During the first two-thirds of the pregnancy, blood volume increases faster than red cell mass (Hematocrit, Ht), the opposite is true during the last third (Pritchard, 1965). Little is known about the physiologic mechanisms that trigger this increase in

blood volume (Pritchard, 1965), and it is unknown how nutrient restriction could affect Ht in the pregnant ewe.

Few studies have been done analyzing the effects of realimentation in pregnant animals. In beef cows, a 40% nutrient restriction applied from d 30 to 140 of gestation did not change uterine blood flow (Camacho et al., 2014). Upon realimentation (d 140 to 198 of gestation) ipsilateral blood flow was increased in the previously restricted cows, compared to cows that never experienced a nutrient restriction (Camacho et al., 2014). Some studies in the sheep have shown that a nutrient restriction from d 30 to 80 of gestation does not affect fetal or placental growth (Anthony et al., 2003); however, our laboratory has demonstrated that a 40% nutrient restriction beginning on d 50 reduces umbilical blood flow (UBF) by d 70 and UBF does not recover to control values until d 130 in which ewes were euthanized (Lemley et al., 2012). To our knowledge there is limited information on how realimentation impacts UBF in the ewe. The objective of the present study was to determine if realimenting previously restricted pregnant ewes would restore UBF to control levels during mid-gestation. We hypothesized that during restriction, UBF and fetal and placentome measurements would be lower as compared to adequately fed ewes, but upon realimentation, ewes would have similar UBF as ewes that were never restricted. In addition, we investigated the changes in Ht during this period.

Materials and methods

Animals and experimental design

Animal care and use were according to protocols approved by the North Dakota State University Animal Care and Use Committee (#A15076).

Forty two second parity Dorset ewes were randomly selected from the NDSU Sheep Unit. Estrus was synchronized using progesterone containing Controlled Internal Drug Release (CIDR)

devices. After synchronization ewes were transported to the NDSU Animal Nutrition and Physiology Center (ANPC). At ANPC ewes were fed a diet of pellets (Table 2.1) and hay for ad libitum intake. All ewes were bred to one ram and breeding dates were recorded. Three ewes were rebred to the same ram 17 days days later. Pregnancy diagnosis and fetal enumeration was performed on d 30 of gestation via ultrasonography (US, Aloka Prosound Alpha 6). Fifteen singleton carrying ewes were randomly divided into two treatment groups: Control (CON; n=7) and Restricted (RES; n=8). All ewes received a pelleted diet only once daily (Table 2.1) from d 30 to 50 at 100% of NRC recommendations (NRC, 1985). On d 50 CON ewes continued to receive 100% of NRC recommendations throughout the duration of the study while RES ewes received 60% of requirements from d 50 to d 90 of gestation. On d 91, ewes were realimented to 100% of NRC requirements. Body weight, blood samples and ultrasonography scans were performed every 10 days until d 130 (see below). Diets were adjusted every 10 days for body weight gain or loss. After samples were obtained on d 130 of gestation, ewes were transported back to the NDSU Sheep Unit and received hay and water for ad libitum intake. Birth was monitored and placentas were collected. Lamb birth weights, placental weights, and number of cotyledons were recorded.

Table 2.1. Diet composition

Ingredients	**Percentage**
Corn	9.3%
SBM	4.0%
Beet pulp	28.9%
Alfalfa meal	33.4%
Wheat Midds	24.4%
Total	100%

Diet per kg: 2.659 Mcal; CP=169.8g; MP=118.86g; NDF= 369.5g; Starch=294.24g; Ca=7.33g; P=4.084g; Cu=104.44ppm; Se=3.813ppm.
Diet based on NRC recommendations (NRC, 1985).

Gestational measurements

Beginning on d 50, and every 10 days until d 110, ewes were restrained so that conceptus measurements and umbilical hemodynamics could be obtained. All measurements were obtained prior to feeding. Conceptus measurements included the length and width from 10 random placentomes. Fetal biparietal and abdominal lengths, and kidney length and width in duplicate every 10 days. For umbilical hemodynamic measurements, Doppler mode was used to obtain UBF, fetal heart rate, PI and resistance index (RI) as previously described (Lemley et al., 2012).

Blood collection

Prior to feeding and US, 20 mL of blood (serum and EDTA containing tubes; BD Vacutainer) was collected every 10 days from d 50 to 130 via jugular venipuncture. Hematocrit was measured immediately after collection. After centrifugation (3010 g for 20 min), serum and plasma were collected and stored at – 20 °C until further analyses.

Statistical analyses

The research was conducted as a completely randomized design with repeated measures. Repeated data were analyzed using the MIXED procedure of SAS (SAS software version 9.4, SAS Institute, Cary, NC). Ewe was treated as a random independent variable; treatment and day were treated as fixed effects. UBF, PI, RI, placentome area, fetal biparietal and abdominal lengths, kidney length and width, ewe body weight and Ht were the dependent variables. Coding included ewe, treatment and day in the class statement; day was included in the repeated statement, dependent variables (treatment, day and their interaction) were included in the model statement, LSmeans were separated using the PDIFF option of the LSMEANS statement. Birth data were analyzed using the GLM procedure, class statement included treatment and the model statement included placentome weight, cotyledon number, cotyledon weight, fetal membrane and birth

weight, means were separated using the LSMEANS statement. *P* values ≤ 0.05 are considered significant. Tendencies are described when *P* values are >0.05 but ≤ 0.10.

Results

On d 50 of gestation, both treatment groups had similar (*P* = 0.45) weight (62.08 ± 1.88 kg; appendix table A.1.) and US measurements (Appendix tables A.2. and A.3.). By d 70, RES ewes were lighter (*P* < 0.05) or remained lighter *P* ≤ 0.10; Fig. 2.1; appendix table A.1.) than CON ewes throughout the experiment. There was no treatment by day, nor main effect of treatment (*P* ≥ 0.15; Figs. 2.2, 2.3 and 2.4; appendix table A.2.), on fetal abdominal girth, biparital distance, or kidney lengths. As expected, there was a main effect of day (*P* < 0.01) where all measurements increased as gestation advanced (Figs. 2.2, 2.3 and 2.4; appendix tables A.2. and A.3.).

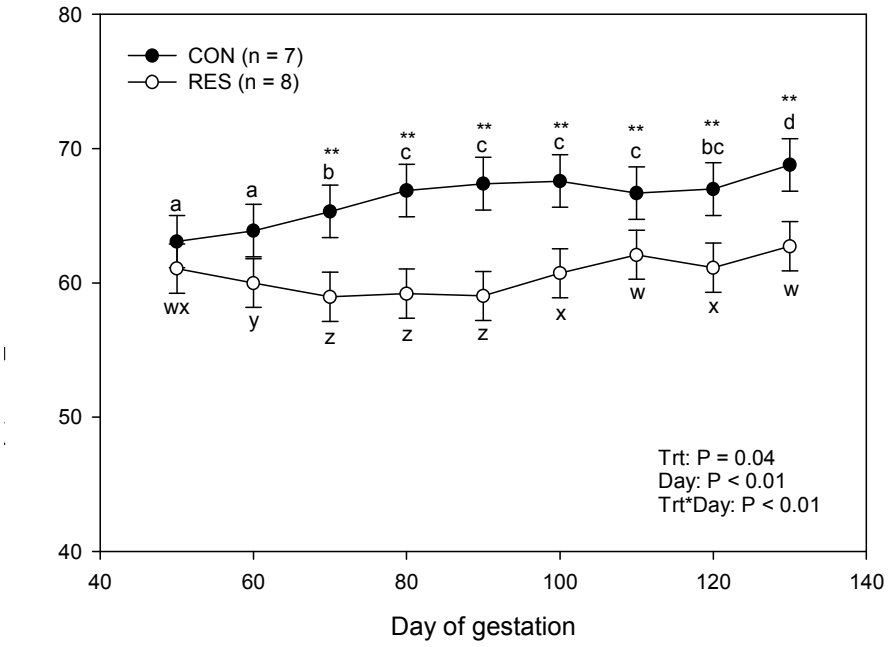

Figure 2.1. Impacts of maternal nutrition on ewe weight from d 50 to 130 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. [abcd]LSMEANS ± SEM within CON differ *P* < 0.05; [wxyz]LSMEANS ± SEM within RES differ *P*< 0.05. Differences between CON and RES are denoted by ** P ≤ 0.05, * P ≤ 0.10 within a day.

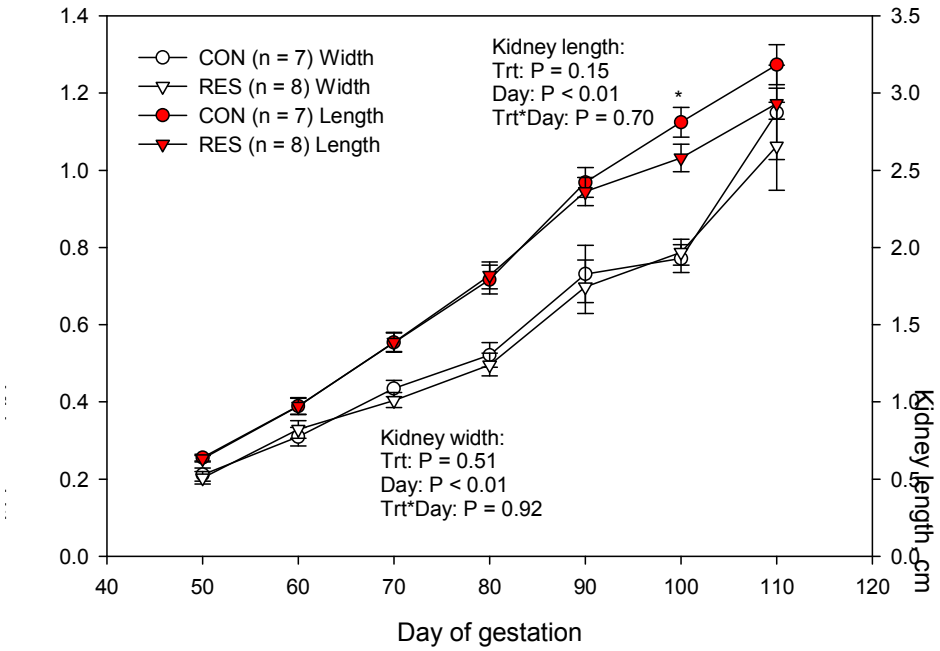

Figure 2.2. Impacts of maternal nutrition on fetal kidney length and width from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by * $P \leq 0.10$ within a day.

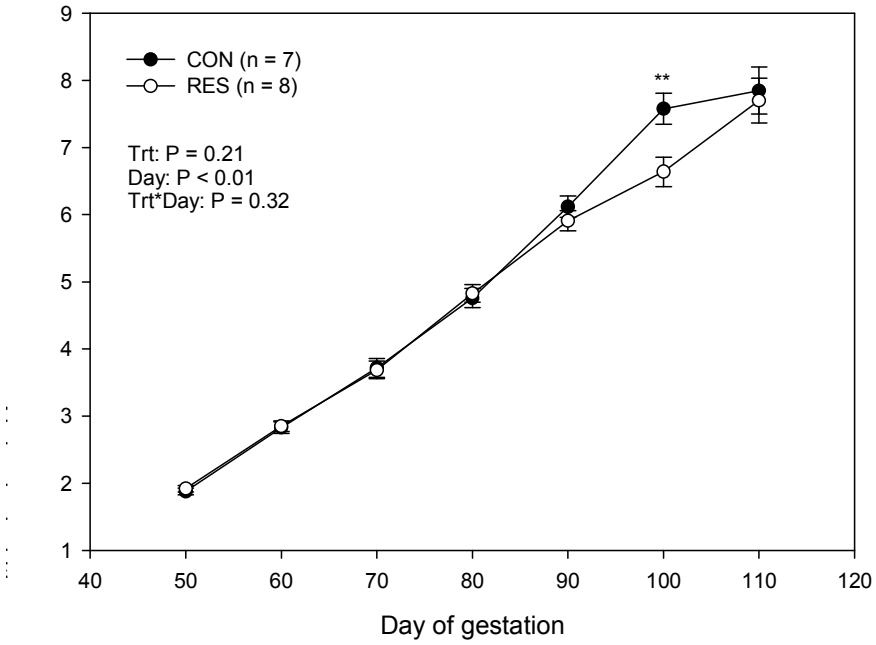

Figure 2.3. Impacts of maternal nutrition on fetal abdominal width from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by ** $P \leq 0.05$.

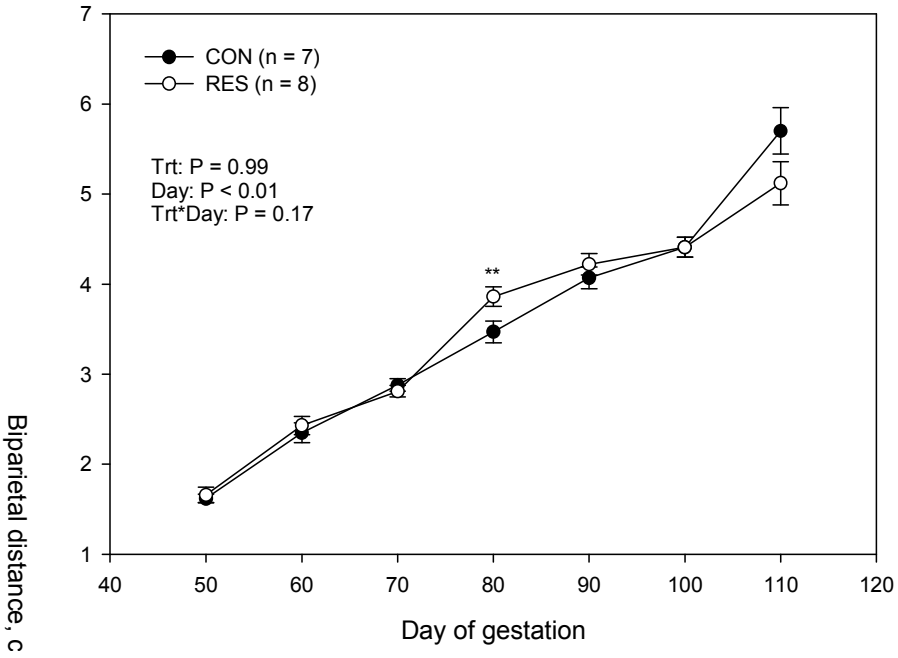

Figure 2.4. Impacts of maternal nutrition on fetal biparietal distance from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by ** $P \leq 0.05$.

There was no interaction of treatment and day on placentome area ($P = 0.49$). While there were no treatment by day interactions or main effects of treatment ($P > 0.19$; Fig. 2.6, 2.7 and 2.8) for any measurements obtained in UBF, PI and RI. There were some interesting observations. On d 80, UBF was decreased (unprotected *F*: $P \leq 0.05$; Fig. 2.8; appendix table A.3.), placentome area tended to be decreased ($P \leq 0.10$; Fig. 2.5; appendix table A.2.), and PI and RI ($P \leq 0.10$; Figs. 2.6 and 2.7; appendix table A.3.) tended to be increased in RES compared to CON. On d 90, prior to the realimentation, all these measurements were similar to CON (Appendix tables A.2. and A.3.). At birth, lambs and placental measurements were similar ($P > 0.43$, Table 2.2).

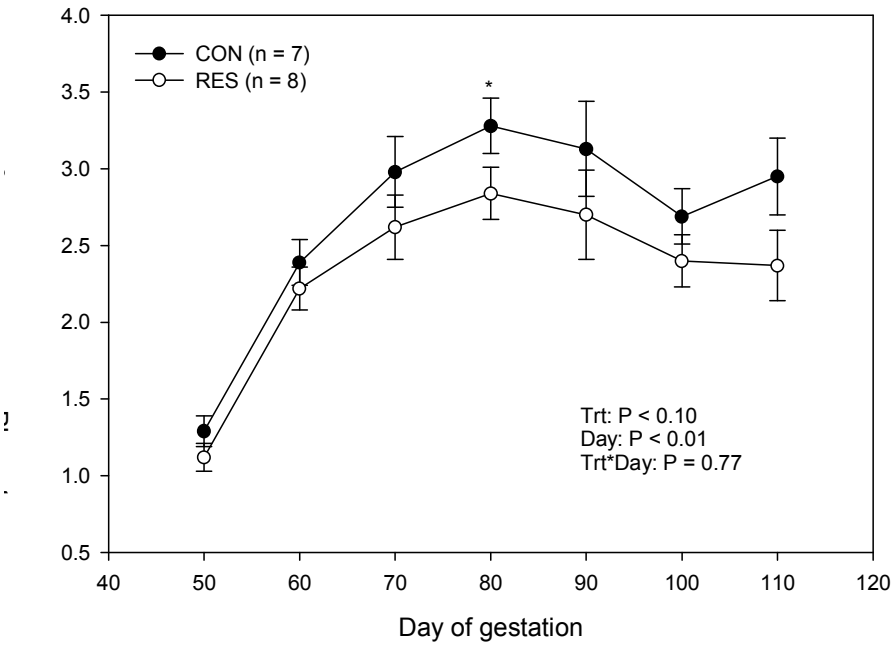

Figure 2.5. Impacts of maternal nutrition on maternal and fetal placentome area from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by * $P \leq 0.10$ within a day.

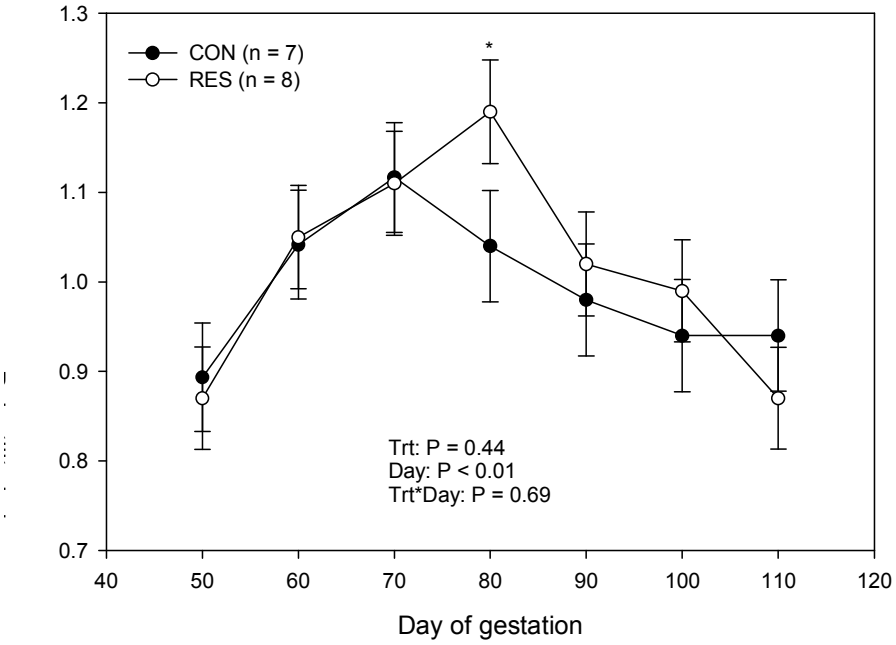

Figure 2.6. Impacts of maternal nutrition on umbilical pulsatility index (PI) from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by * $P \leq 0.10$ within a day.

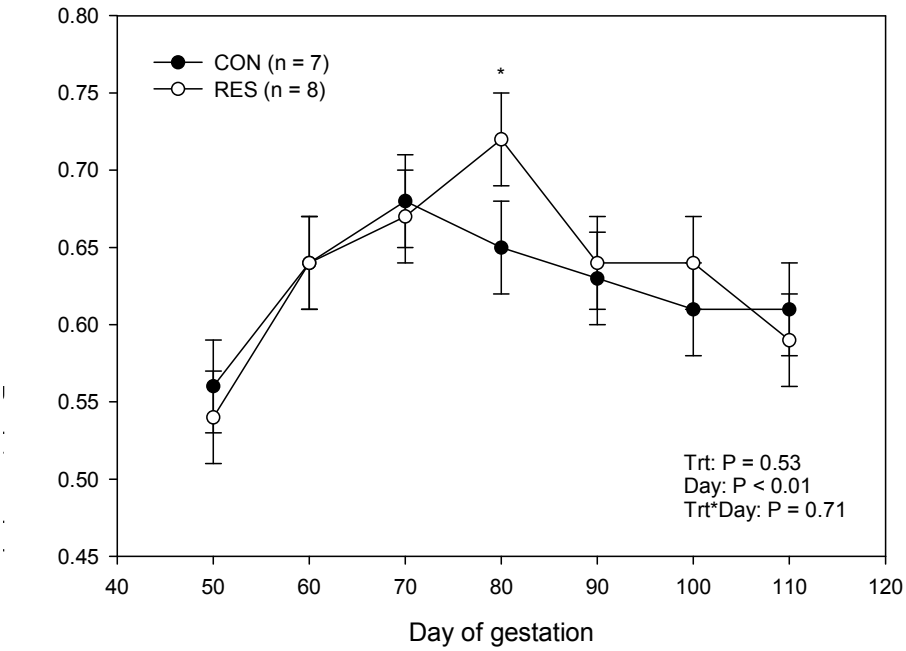

Figure 2.7. Impacts of maternal nutrition on umbilical resistance index (RI) from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. * $P \leq 0.10$ within a day.

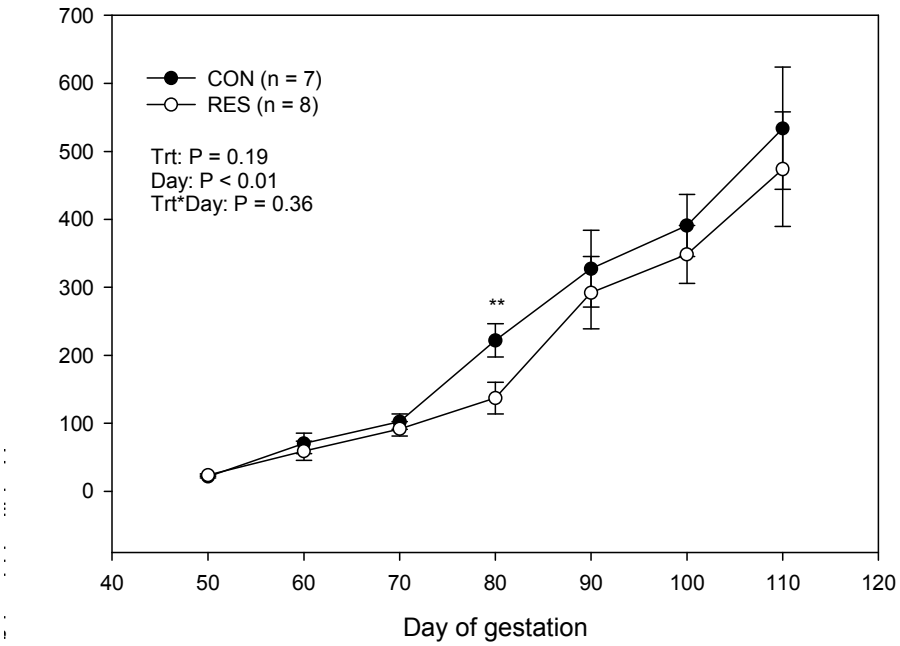

Figure 2.8. Impacts of maternal nutrition on umbilical blood flow from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by ** $P \leq 0.05$.

Table 2.2. Placental weight, cotyledon weight, birth weight, fetal membrane weight and cotyledon number

	CON	RES	SEM	P-value
Placenta wt, g	433.57	478.58	51.41	0.52
Cotyledon wt, g	117.42	101.09	10.33	0.26
Fetal membrane wt, g	272.95	326.18	33.29	0.25
Birth wt, g	5258.49	4972.02	266.26	0.43
Cotyledon number	93.67	94.63	8.78	0.93

P < 0.05 are considered significant

Blood analysis

There was a treatment by day interaction ($P < 0.01$; Fig. 2.9) for Ht. While Ht was similar ($P = 0.40$) on d 50, by d 60 and continuing through d 80 RES Ht was greater or tended to be greater (days 60 and 80: $P \leq 0.05$, d 70: $P \leq 0.10$; appendix table A.4.) compared to CON ewes. From d 90 until 130, Ht was similar ($P \geq 0.13$; appendix table A.4.) again between RES and CON ewes.

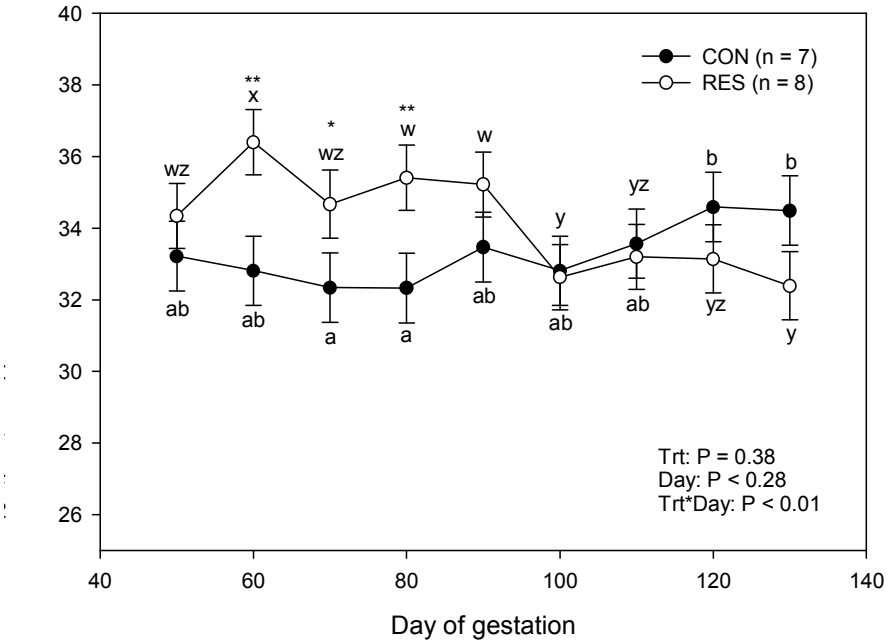

Figure 2.9. Impacts of maternal nutrition on maternal hematocrit (Ht) from d 50 to 110 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. [ab]LSMEANS ± SEM within CON differ $P < 0.05$; [wxyz]LSMEANS ± SEM within RES differ P< 0.05. Differences between CON and RES are denoted by ** $P \leq 0.05$, * $P \leq 0.10$ within a day.

Discussion

Umbilical BF in RES ewes returned to CON values by d 90 of gestation, before the effects of realimentation could be determined. Therefore our hypothesis was unable to be adequately tested in this study.

The placenta is essential for endocrine production and nutrient exchange between the dam and fetus. In ruminants, including the sheep, the fetal-maternal exchange occurs in structures called the placentome. Placentome size has been previously used as an indirect measurement of nutrient delivery to the fetus (Redmer et al., 2004). The umbilical cord is the structure that transports the nutrients and oxygen from the placentomes to the fetal circulatory system and transports carbon dioxide and waste products from the fetus to the placentomes (Chapter 1). Assessment of UBF is

used as an index of nutrient delivery (Kiserud, 2005; Lemley et al., 2012). While we failed to reach significance in our Doopler velocimetry measurements due to treatment, we did have some interesting observations. Using differences from unprotected *F* tests, there were interesting observations on d 80 of gestation. In RES ewes, there were smaller placentomes, reduced UBF, and increased measurements of resistance (i.e. PI and RI). Taken together, these measurements suggest in other studies (and other species) that UBF and nutrient exchange may be compromised (Chapter 1). As mentioned above, these measurements were similar prior to realimentation; therefore, we were unable to determine how diet impacts these measurements. The question now becomes, why didn't we continue to observe the differences until realimentation was implemented.

A previous study performed in our laboratory reported that in primiparous ewes experiencing a 40% restriction from d 50 to 130 of gestation resulted in decreased UBF by d 80 of gestation (Lemley et al., 2012). The present study was done with multiparous ewes. We preserved the same housing conditions, breed, and experimental protocol of Lemley et al. (2012). We speculate that the fact that our RES group had already reached CON values of UBF to the fetus before realimentation could be explained by the number of parities of the dam.

In the pregnant adult sheep, nutrient partitioning prioritizes the placenta and fetus (Redmer et al., 2004). However, hierarchy of nutrient partitioning in adolescent pregnancy has a higher priority for maternal tissue growth and fat deposition (Redmer et al., 2004). Few studies have analyzed the influence of the number of parities in the reproductive performance. In sheep, conception rate increases and fetal loss decreases as parity increases (Lafi et al., 2009), and the likelihood of twins increases as the number of parity increases (Lafi et al., 2009). The effects of parity on uterine microanatomy and UBF in sheep remain unknown.

Perhaps our hemodynamic measurements can be further explained by changes in blood volume. Hematocrit is the principal component of blood viscosity (Birchard, 1997). If Ht increases, blood viscosity increases (Melkumyants et al., 1989; Birchard, 1997). Blood viscosity and blood flow are responsible for the amount of shear stress exercised on a blood vessel (Birchard, 1997). Shear stress is the tangential force that blood causes in the walls of blood vessels. An increased shear stress causes vasodilation via NO release (Melkumyants et al., 1989; Markos et al., 2002). If blood viscosity is increased, shear stress is increased; therefore vasodilation is increased (Melkumyants et al., 1989). In our experiment, Ht increased in RES on d 60 of gestation. By d 90, Ht was similar between both treatment groups. The increase in Ht in RES could have had an effect on the umbilical artery, producing vasodilation, therefore increasing blood flow before realimentation.

An acute increase in Ht in RES could potentially be explained by a slight decrease of plasma proteins after nutrient restriction, which in turn could slightly decrease plasma oncotic pressure, producing a decrease in plasma volume (Valenzuela, 1989; Birchard, 1997). These effects could result in an increased hematocrit with a theoretic decrease in blood volume. To explain a more permanent change in Ht, baroreceptors along the circulatory system would have had to adapt to the new protein concentration (Birchard, 1997), therefore oncotic pressure, maintaining a slightly higher Ht than the one previous to nutrient restriction. Changes in Ht after d 110 could be explained by an increase in plasma proteins. Due to an increase in plasma proteins, oncotic pressure would have increased; thus, increasing plasma volume and total blood volume. Baroreceptors, again, would have to adapt to the new plasma protein concentration, therefore oncotic pressure.

By d 70 RES group had lower body weight than CON. By d 130, the RES group was still behind CON group in terms of body weight. The fact that body weights were different between treatment groups demonstrates that treatments had the desired effect of mimicking the limited nutrition that can happen due to seasonal conditions. The results of this study were similar to other studies that have shown significantly decreased ewe body weight when there is a 40% reduced feed intake (Muñoz et al., 2009; Lemley et al., 2012).

Regarding fetal body measurements we focused on kidney area, abdominal width and biparietal distance. In previous studies kidney length and width have not been affected by nutrient restriction in mid and late pregnancies (Lemley et al., 2012). Those results were corroborated in our study. The kidneys, unlike other organs of the developing fetus (e.g. small intestine, spleen), seem not to be affected by a lower nutrient availability in mid to late gestation (less than 130 days of gestation; Osgerby et al., 2002; Lemley et al., 2012). Abdominal width and biparietal distance are used jointly as indirect measurements of fetal size and weight (Araujo et al., 2014; Rueda et al., 2014). Since biparietal distance and abdominal width are used jointly to assess fetal size and weight, we think the differences on d 80 and 100 in this study are not an accurate reflection of size and weight of the fetuses.

At birth, placental weight, cotyledon weight, cotyledon number, fetal membrane weight (placenta without cotyledons), and lamb birth weights were similar between both treatment groups. The results of the placental measurements indicate that the majority of the placental development happens during an early stage of gestation, more accurately, during the first 50 days of gestation. Moreover, we have observed that fetal weight measurements are determined by maternal plane of nutrition from d 90 to 130 (Lekatz et al., 2010). We saw a tendency for placentome areas to be

reduced in RES ewes on d 80, but we are unable to conclude if we had a reduced placental size, placentome numbers, or total placentome weight as all ewes were allowed to lamb.

Our lab is currently investigating the influence of parity in nutrient restricted pregnant ewes placental and blood parameters. Preliminarily, it would appear that older or multiparous ewes have a greater resistance to nutrient restriction than nulliparous ewes, and this should be tested.

Literature cited

Anthony, R., A. Scheaffer, C. Wright, and T. Regnault. 2003. Ruminant models of prenatal growth restriction. Reproduction-Cambridge-Supplement:183-194.

Birchard, G. F. 1997. Optimal hematocrit: theory, regulation and implications. American Zoologist 37(1):65-72.

Camacho, L., C. Lemley, L. Prezotto, M. Bauer, H. Freetly, K. Swanson, and K. Vonnahme. 2014. Effects of maternal nutrient restriction followed by realimentation during midgestation on uterine blood flow in beef cows. Theriogenology 81(9):1248-1256. e1243.

Dickhoefer, U., J. Hao, B. M. Bösing, L. Lin, M. Gierus, F. Taube, and A. Susenbeth. 2014. Feed intake and performance of sheep grazing semiarid grassland in response to different grazing systems. Rangeland Ecology and Management 67(2):145-153.

Ferrazzi, E., M. Bozzo, S. Rigano, M. Bellotti, A. Morabito, G. Pardi, F. Battaglia, and H. Galan. 2002. Temporal sequence of abnormal Doppler changes in the peripheral and central circulatory systems of the severely growth-restricted fetus. Ultrasound in Obstetrics and Gynecology 19(2):140-146.

Galan, H. L., M. J. Hussey, M. Chung, J. K. Chyu, J. C. Hobbins, and F. C. Battaglia. 1998. Doppler velocimetry of growth-restricted fetuses in an ovine model of placental insufficiency. American Journal of Obstetrics and Gynecology 178(3):451-456.

Hytten, F. 1986. Blood volume changes in normal pregnancy. Obstetrical and Gynecological Survey 41(7):426-428.

Kiserud, T. 2005. Physiology of the fetal circulation. Pages 493-503 in Proc. Seminars in Fetal and Neonatal Medicine. Elsevier.

Lafi, S., A. Talafha, N. Giadinis, E. Kalaitzakis, K. Pourliotis, and N. Panousis. 2009. Factors affecting the reproductive performance of Awassi sheep flocks in north-east of Jordan: An epidemiological study. Tropical Animal Health and Production 41(8):1755-1764.

Lemley, C. O., A. M. Meyer, L. E. Camacho, T. L. Neville, D. J. Newman, J. S. Caton, and K. A. Vonnahme. 2012. Melatonin supplementation alters uteroplacental hemodynamics and fetal development in an ovine model of intrauterine growth restriction. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 302(4):R454-R467.

Markos, F., B. Hennessy, M. Fitzpatrick, J. O'Sullivan, and H. Snow. 2002. Reverse arterial wall shear stress causes nitric oxide-dependent vasodilatation in the anaesthetised dog. Pflügers Archiv 445(1):51-54.

Melkumyants, A. M., S. A. Balashov, and V. M. Khayutin. 1989. Endothelium dependent control of arterial diameter by blood viscosity. Cardiovascular Research 23(9):741-747.

Milchunas, D. G., J. Y. King, A. R. Mosier, J. C. Moore, J. A. Morgan, M. H. Quirk, and J. R. Slusser. 2004. UV radiation effects on plant growth and forage quality in a shortgrass steppe ecosystem. Photochemistry and Photobiology 79(5):404-410.

Osgerby, J., D. Wathes, D. Howard, and T. Gadd. 2002. The effect of maternal undernutrition on ovine fetal growth. Journal of Endocrinology 173(1):131-141.

Pritchard, J. A. 1965. Changes in the blood volume during pregnancy and delivery. The Journal of the American Society of Anesthesiologists 26(4):393-399.

Redmer, D., J. Wallace, and L. Reynolds. 2004. Effect of nutrient intake during pregnancy on fetal and placental growth and vascular development. Domestic Animal Endocrinology 27(3):199-217.

Rigano, S., M. Bozzo, E. Ferrazzi, M. Bellotti, F. C. Battaglia, and H. L. Galan. 2001. Early and persistent reduction in umbilical vein blood flow in the growth-restricted fetus: a longitudinal study. American Journal of Obstetrics and Gynecology 185(4):834-838.

Rueda, S., S. Fathima, C. L. Knight, M. Yaqub, A. T. Papageorghiou, B. Rahmatullah, A. Foi, M. Maggioni, A. Pepe, and J. Tohka. 2014. Evaluation and comparison of current fetal ultrasound image segmentation methods for biometric measurements: a grand challenge. Medical Imaging, IEEE transactions on 33(4):797-813.

Stegeman, J. H. 1974. Placental development in the sheep and its relation to fetal development. A qualitative and quantitative anatomic and histologic study. Bijdragen tot de Dierkunde 44(1):3-72.

National Research Council, Subcommittee on Sheep Nutrition, Committee on Animal Nutrition, Board on Agriculture. 1985. Nutrient Requirements of Sheep. Sixth Revised Edition. National Academy Press, Washington, D.C.

Sutera, S. P. and R. Skalak. 1993. The history of Poiseuille's law. Annual Review of Fluid Mechanics 25(1):1-20.

Valenzuela, G. J. 1989. Is a decrease in plasma oncotic pressure enough to explain the edema of pregnancy?. American Journal of Obstetrics and Gynecology 161(6):1624-1627

CHAPTER 3. EFFECTS OF PARITY AND MID-GESTATION NUTRIENT RESTRICTION ON UMBILICAL BLOOD FLOW, FETAL AND PLACENTAL MEASUREMENTS, AND BIRTH WEIGHT IN SHEEP

Abstract

We recently reported that mid-gestation (d 50 to 90) nutrient-restriction tended to decrease umbilical blood flow (UBF) and placentome area (PA), and increases pulsatility index (PI) and resistance index (RI) on d 80 of gestation in multiparous Dorset ewes (Chapter 2). The same nutritional restriction applied in nulliparous ewe lambs decreased UBF by d 70 (Chapter 1). We hypothesized that multiparous ewes would be more resilient to restriction compared to nulliparous ewes. On d 50 of gestation, adult (15 mo) nulliparous (NUL; n = 12) and multiparous (MUL; n = 16) Dorset ewes carrying singletons were randomly assigned to receive 100% of NRC recommendations (CON) or 60% of CON (RES). On d 91, RES ewes were realimented to 100% of NRC recommendations. On d 50, and every 10 days until d 110, fetal and placental measurements and umbilical hemodynamics were obtained via ultrasonography. Lamb birth weights were recorded. The study was conducted as a 2 by 2 factorial arrangement of treatments with repeated measures. Data were analyzed using the MIXED procedure of SAS. By d 60 RES ewes were lighter than CON ewes ($P < 0.01$), and remained lighter throughout the experiment. There were no three way interactions or main effects of treatments on UBF, PI, RI and PA ($P \geq 0.57$). There was a parity by day interaction ($P < 0.05$) for RI, but UBF was not affected by parity or diet. At birth no differences were observed in lamb weight ($P \geq 0.78$). Restriction from d 50 to 90 does not appear to impact umbilical hemodynamics or conceptus growth in adults, regardless of parity. Our laboratory's previous observation that reduced UBF in young ewes (6 mo old)

resulting from nutrient restriction may be due to maternal age. Future studies investigating age, parity, and body fat reserves of the dam on umbilical hemodynamics are underway.

Introduction

In several species, parity can influence litter size as well as birth weight. In the common vole, parity is influenced by age (Tkadlec and Krejcova, 2001). When voles breed at an early age, litter size is greater in the first parity compared to the second. On the other hand, when females are first bred at an older age, the first parity results in fewer pups compared to the second parity (Tkadlec and Krejcova, 2001). In short-lived small mammals this is thought to be related to the fact that these animals may not have numerous chances to mate, therefore the body invests significant resources in their first parity when they are young (Jacquot and Vessey, 1998; Tkadlec and Krejcova, 2001). The reason why this does not happen in older animals is not fully understood, but it could be related to a decreased mortality rate in older mothers (Tkadlec and Krejcova, 2001). Environment could also influence the effect of parity in short-lived small mammals. In laboratory conditions, with greater energy resources, number of offspring increases as parity increases (Jacquot and Vessey, 1998).

The reproductive effect of parity, in small mammals under laboratory conditions resembles the effects of parity seen in larger, long-lived mammals (Jacquot and Vessey, 1998). Number of pigs born from multiparous sows is greater than from primiparous sows (Mahan, 1997; Whitley et al., 2001). In sheep the conception rate increases and fetal loss decreases as parity increases (Lafi et al., 2009). Similarly, the likelihood of twins increases as the number of parity increases in sheep (Lafi et al., 2009). In cattle, sheep, and mares, birth weight increases as parity increases (Kayisiz et al., 2010; Yakubu et al., 2014; Abdel-Mageed and Abd El-Gawad, 2015; Klewitz et al., 2015; Lv et al., 2015).

In humans and several domesticated animals, uterine and umbilical blood flows have been related to litter size and birth weight. In sows and cows, uterine blood flow per fetus decreases as the number of fetuses increase; however, total uterine blood flow increases as the number of fetuses increase (Ferrell and Reynolds, 1992; Pere and Etiene, 2000). In humans and sheep, lesser fetal and offspring birth weights are consistently related with lower umbilical blood flow (UBF; Galan et al., 1997; Rigano et al., 2001; Ferrazi et al., 2002; Reynolds et al., 2005; Lemley at al., 2012).

In mares, the diameter of the uterine artery increases more throughout pregnancy in multiparous (3 to 8 foalings) compared to first and second parity mares (Klewitz et al., 2015). Similarly, blood flow is increased in the uterine artery during the third period of gestation in multiparous mares when compared to first and second parity mares (Klewitz et al., 2015). In women, pulsatility index (PI) measurements taken on the uterine artery are greater in primiparous mothers compared to multiparous mothers during mid-gestation (17 to 18 weeks; Suzuki et al., 2006). Similarly, many studies show that nulliparous women have greater blood pressure, pregnancy induced hypertension and greater risk of preeclampsia than multiparous women (Duckitt and Harrington, 2005; Rurangirwa et al., 2011). Pulsatility index, an indirect measurement of blood vessel resistance, is correlated with the resistance index (RI; Suzuki et al., 2006), and both measurements are usually increased when blood flow is decreased.

Umbilical blood flows are the earliest Doppler abnormalities detected in intrauterine growth restriction (Rigano et al., 2001; Ferrazzi et al., 2002). Our laboratory has demonstrated that in young primiparous ewes (6 to 12 months of age), a 40% nutrient restriction during mid to late gestation reduced umbilical blood flow (UBF) from d 80 until d 130 of gestation (Lemley et al., 2012). A good proportion of the studies analyzing the influence of parity in reproductive traits do not report maternal age (Abdel-Magged and Abd El-Gawad, 2015; Klewitz et al., 2015). To our

knowledge, in sheep, only one study has analyzed the effect of maternal age, controlling for parity, in placental development (Borowicz et al., 2005). Several studies in humans and other mammals suggest that there could be an independent effect of parity in the maternal utero-placental physiology that could enhance the reproductive capacity of the multiparous mother (Kelly et al., 1992; Whitley et al., 2001; Wilsher and Allen, 2003; Elliot et al., 2009). Furthermore, we recently reported that in multiparous ewes receiving a 40% nutrient restriction, UBF spontaneously recovered at d 90 of gestation (Chapter 2), suggesting that this recovery could be a result of the influence of parity. Studies in animals and humans show no difference in the reproductive effects of parity after the second parturition (Wilsher and Allen, 2003; Zaborski et al., 2008), suggesting that possible adaptive physiological changes of parity happen after the first pregnancy (Wilsher and Allen, 2003; Zaborski et al., 2008).

Our laboratory has demonstrated that multiparous pregnant ewes show an increased hematocrit (Ht) during the period of restriction and that these values recover to control values by d 90 of gestation, when UBF spontaneously recovered (Chapter 2). Our hypothesis is that Ht may be increased due to nutrient restriction, increasing shear stress and potentially driving uterine blood flow (Birchard, 1997; Salazar-Vazquez et al., 2010).

The aim of this study was to investigate if parity, independent of maternal age, influences the effect of nutrition on umbilical blood flow in sheep. To our knowledge, this is the first study done analyzing these effects. We investigated the effects of parity and nutrient availability during mid-gestation on the UBF, PI, RI, body and placental measurements of the fetus. We also analyzed Ht of the dam.

Materials and methods

Animals and experimental design

Animal care and use were according to protocols approved by the North Dakota State University Animal Care and Use Committee (#A15076). Ninety-one multiparous (more than one previous parity) and thirty-seven adult nulliparous (approximately 1.5 years of age) Dorset ewes were randomly selected from the NDSU Sheep Unit. Estrus was synchronized using progesterone containing Controlled Internal Drug Release (CIDR) devices. After synchronization all ewes were bred to three different rams and breeding dates were recorded via use of rams with chest markers. Thirty-eight ewes were rebred by the same rams 17 d later. Pregnancy diagnosis and fetal enumeration was performed from d 30 to d 40 of gestation via ultrasonography (US, Aloka Prosound Alpha 6). After pregnancy was confirmed, 12 singleton carrying nulliparous ewes and 16 singleton carrying multiparous ewes (one to three previous parities) were transported to the NDSU Animal Nutrition and Physiology Center (ANPC), where they were housed in individual pens. At ANPC, ewes were fed a diet of pellets (Table 1) and hay for ad libitum intake for five days. After this period, all ewes were fed a pelleted diet only once daily (Table 2.1, chapter 2) at 100% of NRC recommendations until d 50 of gestation. On d 50 of gestation, 12 nulliparous (NUL) ewes were randomly divided into two treatment groups: Control (CON; n=6) and restricted (RES; n=6) and 16 multiparous (MUL) ewes were randomly divided into two treatment groups: Control (CON; n=8) and restricted (RES; n=8). Control ewes continued to receive 100% of NRC recommendations throughout the duration of the study while RES ewes received 60% of requirements from d 50 to d 90 of gestation. On d 91, RES ewes were realimented to 100% of NRC requirements. Body weight, blood samples and ultrasonography scans were performed every 10 days until d 130 (see below). Diets were adjusted every 10 days for body weight gain or loss.

After samples were obtained on d 130 of gestation, ewes were transported back to the NDSU Sheep Unit and received hay and water ad libitum. Birth was monitored, lamb birth weights and birth problems (dystocia, placental retention, lamb vitality, and early lamb mortality) were recorded.

Gestational measurements

Beginning on d 50, and every 10 days until d 110, ewes were restrained so that conceptus measurements and umbilical hemodynamics could be obtained. All measurements were obtained in the morning, prior to feeding. Conceptus measurements included the length and width from 10 random placentomes. Fetal biparietal, abdominal lengths, and kidney length and width were recorded in duplicate every 10 days. For umbilical hemodynamic measurements, Doppler mode was used to obtain UBF, PI and RI as previously described (Chapter 2).

Blood collection

Prior to feeding and ultrasonography, 10 mL of blood (EDTA containing tubes; BD Vacutainer) was collected every 10 days from d 50 to 130 via jugular venipuncture. Hematocrit was measured immediately after collection. After centrifugation (3010 g for 20 min), plasma were collected and stored at -20 °C until further analysis.

Statistical analyses

The research was conducted as a completely random design with a 2 by 2 factorial arrangement and repeated measures. Data were analyzed using the MIXED procedure of SAS (SAS software version 9.4, SAS Institute, Cary, NC). Ewe was treated as a random independent variable; parity (NUL or MUL) was modeled as factor one, diet (CON or RES) was modeled as factor two. Both factors and day were treated as fixed effects. Umbilical blood flow, PI, RI, placentome area, fetal biparietal and abdominal lengths, kidney width and length, ewe body weight and hematocrit, were the dependent variables. Coding included ewe, ram, parity, diet and day of

gestation in the class statement; the model statement included parity, diet, day of gestation and all their interactions. Day of gestation was included in the repeated statement; LSmeans were separated using the PDIFF option of the LSMEANS statement. Ewe initial body weight was added as a covariate for all the dependent variables, after testing, the influence of the covariate was significant in ewe body weight, Ht, kidney length and width. For these variables, the covariate was included in the model. Birth data were analyzed using the GLM procedure, class statement included the treatments and the model statement included birth weight, and birth related problems, means were separated using the LSMEANS option. P values ≤ 0.05 were considered significant. P values > 0.05 but ≤ 0.10 were considered as a tendency.

Results

There was no three interaction or main effect of parity in body weights throughout the experiment ($P = 0.46$). However, nutrient restriction, day of gestation and their interaction had a significant effect in MUL and NUL ewes (diet: $P < 0.01$; day: $P < 0.01$; diet*day: $P < 0.01$). On d 50 of gestation, MUL and NUL, CON and RES groups had similar body weights (Appendix table B.1; figure 3.1). By d 60, MUL and NUL restricted (MUL-RES, NUL-RES) animals had lesser body weights when compared to MUL and NUL control (MUL-CON, NUL-CON) animals (Appendix table B.1; Figure 3.1). Restricted animals maintained lesser body weights until the end of the experiment (Appendix table B.1; Figure 3.1). Multiparous-RES animals had lesser weights than MUL-CON animals by d 60 and maintained this difference throughout the experiment (Appendix table B.1; Figure 3.1), whereas NUL-RES were lesser than NUL-CON by d 70 and only tended to be lesser on d 90 (Appendix table B.1; Figure 3.1). The remaining days of the experiment NUL-RES were lesser than NUL-CON (Appendix table B.1; Figure 3.1). Multiparous-RES means tended to be less than NUL-RES on d 80 (Appendix table B.1).

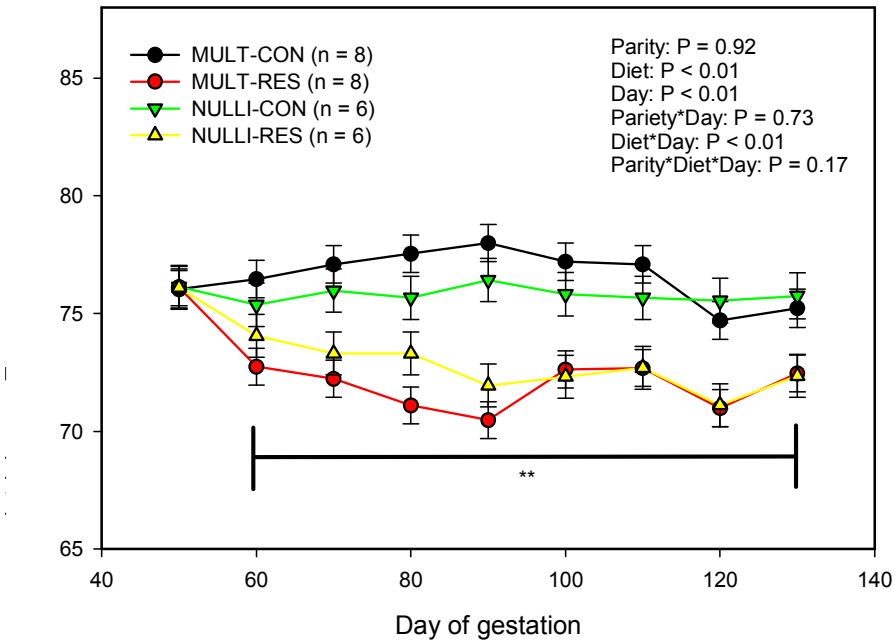

Figure 3.1. Impacts of maternal nutrition and parity on ewe weight from d 50 to 130 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by ** $P \leq 0.05$.

In all treatment groups with the exception of fetal biparietal distance (Figure 3.1) no three way interactions or main effects for parity or diet, were significant for any of the US fetal and placental measurements (abdominal width, placentome area, Figures 3.3 and 3.4 respectively; kidney length: $P = 0.29$; kidney width: $P = 0.76$). The day main effect was significant ($P < 0.01$; figures 3.2, 3.3 and 3.4) for all US fetal measurements, with sizes increasing as gestation advanced. Placentome size increased until d 80 of gestation (Figure 3.4), and then remained similar until the end of the experiment (Figure 3.4).

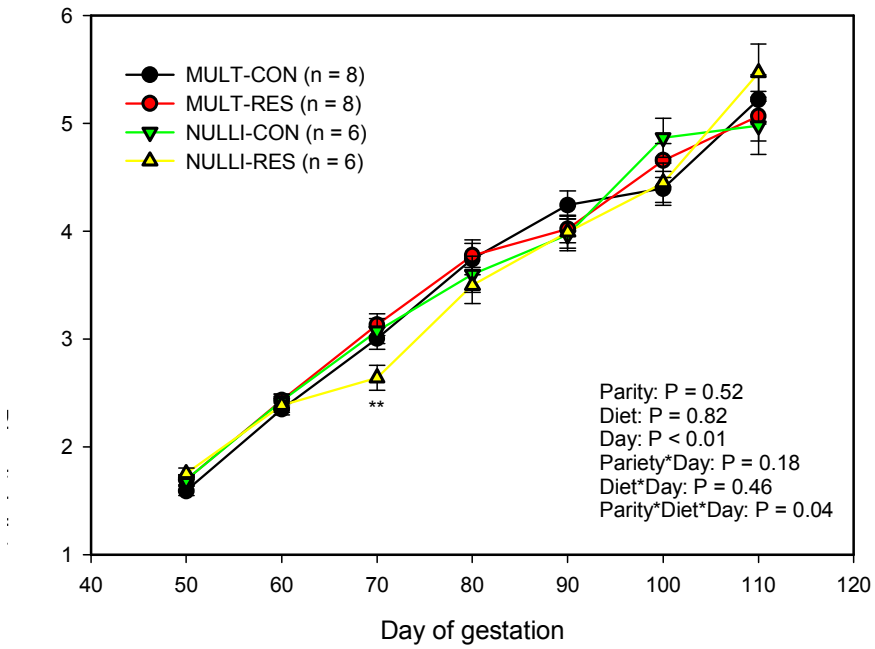

Figure 3.2. Impacts of maternal nutrition and parity on fetal biparietal distance from d 50 to 110 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. Differences between CON and RES are denoted by ** $P \leq 0.05$.

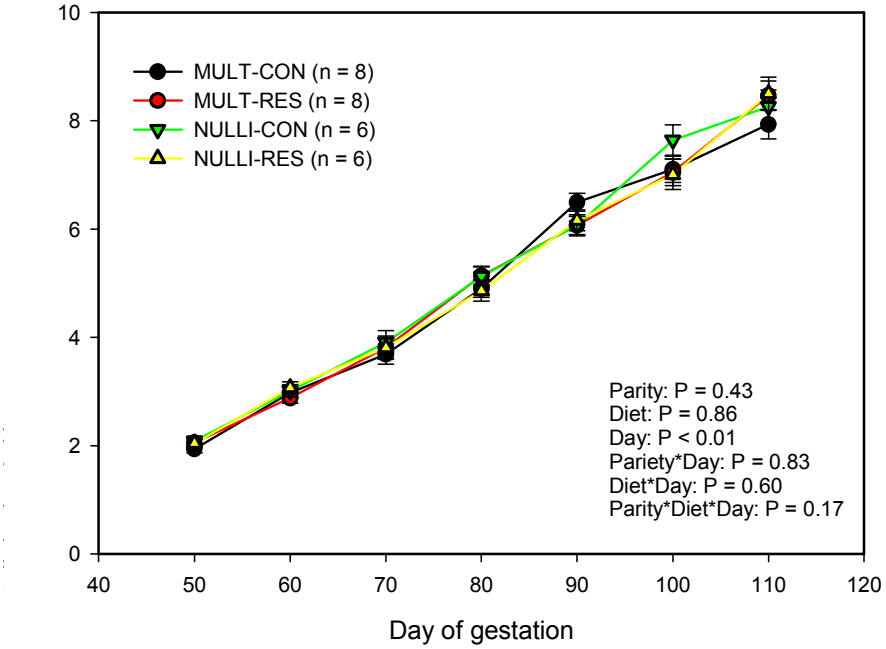

Figure 3.3. Impacts of maternal nutrition and parity on fetal abdominal width from d 50 to 110 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90.

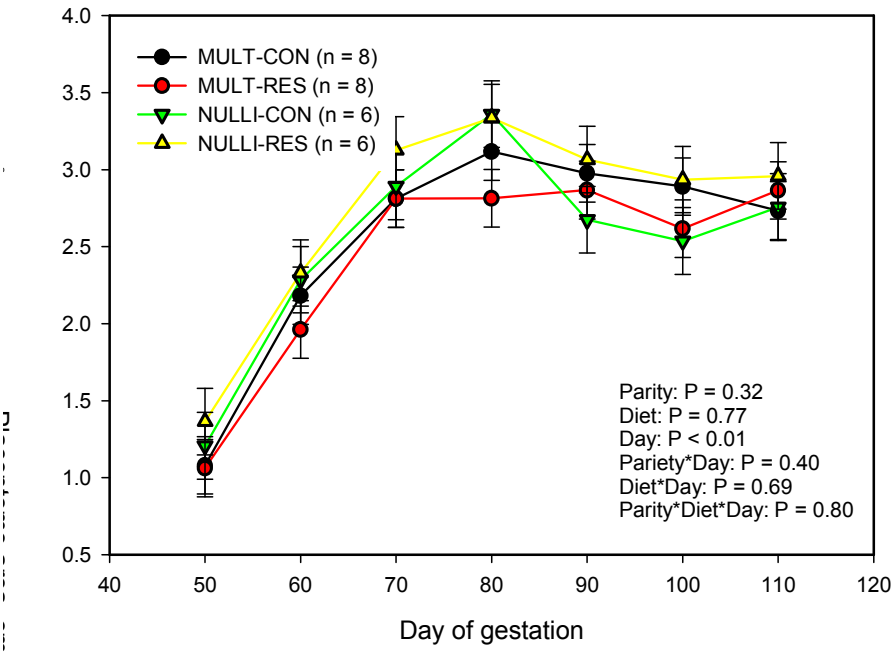

Figure 3.4. Impacts of maternal nutrition and parity on fetal and maternal placentome area from d 50 to 110 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90.

The three way interaction and main effects for parity and diet were not significant for UBF, PI and RI (Appendix tables B.2., B.3. and B.4.; Figures 3.5, 3.6, and 3.7). Multiparous-CON animals had similar UBF, PI and RI values when compared to MUL-RES (Appendix tables B.2., B.3. and B.4.). Similarly, NUL-CON were not different than NUL-RES (Appendix tables B.2., B.3. and B.4.). An unprotected mean separation of UBF shows that MUL-CON ewes had similar values to NUL-CON during the length of the study (Appendix table B.2.). However, UBF means of NUL-RES tended to be greater (P = 0.09) than MUL-RES animals on d 90 and were greater on d 110 (P = 0.01; Appendix table B.2.). Resistance index showed an interaction effect of parity by day (Figures 3.7, and 3.8). Pulsatility index and RI means, respectively were greater (P = 0.04) and tended to be greater (P = 0.10) in MUL-CON vs. NUL-CON on d 50 and 100 (Appendix tables B.3. and B.4.). Pulsatility index tended to be greater (P = 0.08) on NUL-CON vs. MUL-CON on

d 60 (Appendix table B.3.). Resistance index and PI respectively were greater (P = 0.02) and tended to be greater (P = 0.07) in NUL-RES vs. MUL-RES on d 70 of gestation (Appendix table B.4). On the other hand, PI and RI were lesser (P =0.03) on NUL-RES vs. MUL-RES on d 90 (Appendix tables B.3. and B.4.). The day main effect was significant (P < 0.01) for all three Doppler US measurements, with UBF values increasing as gestation advanced, and PI and RI reaching their peak on d 80 of gestation (Appendix tables B.2., B.3. and B.4.; Figures 3.5, 3.6, and 3.7).

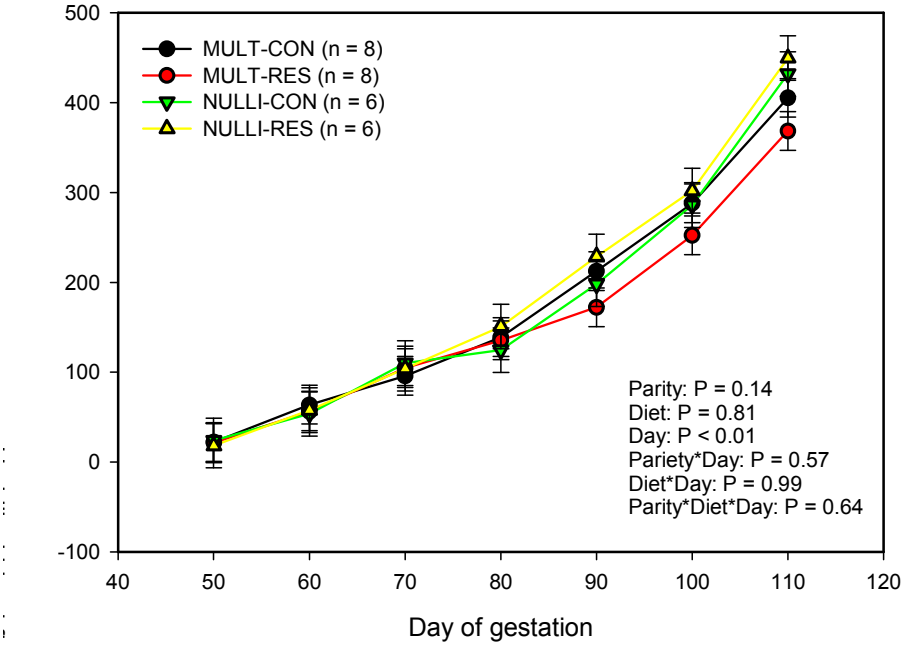

Figure 3.5. Impacts of maternal nutrition and parity on umbilical blood flow from d 50 to 110 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90.

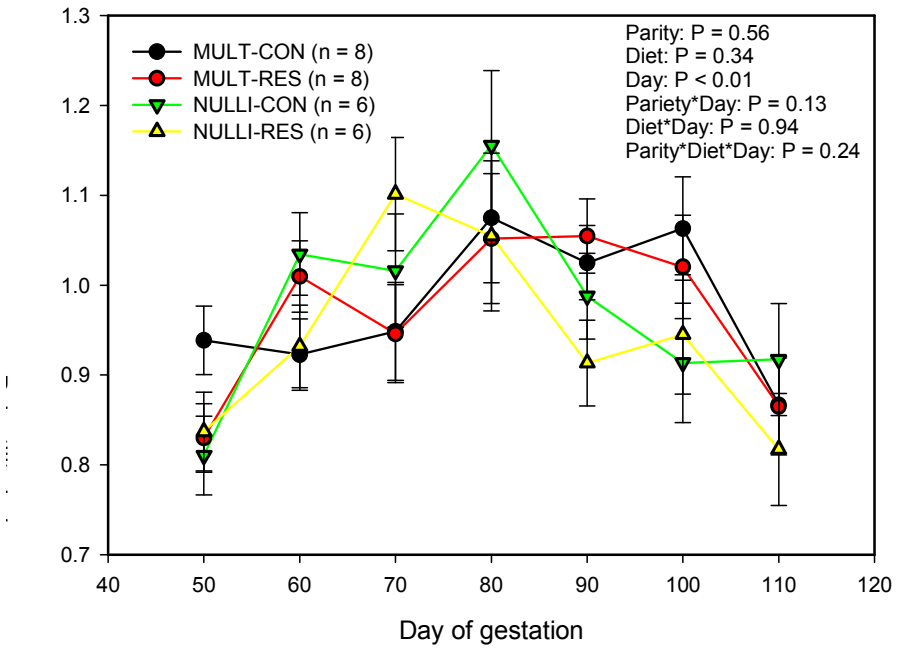

Figure 3.6. Impacts of maternal nutrition and parity on pulsatility index (PI) from d 50 to 110 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90.

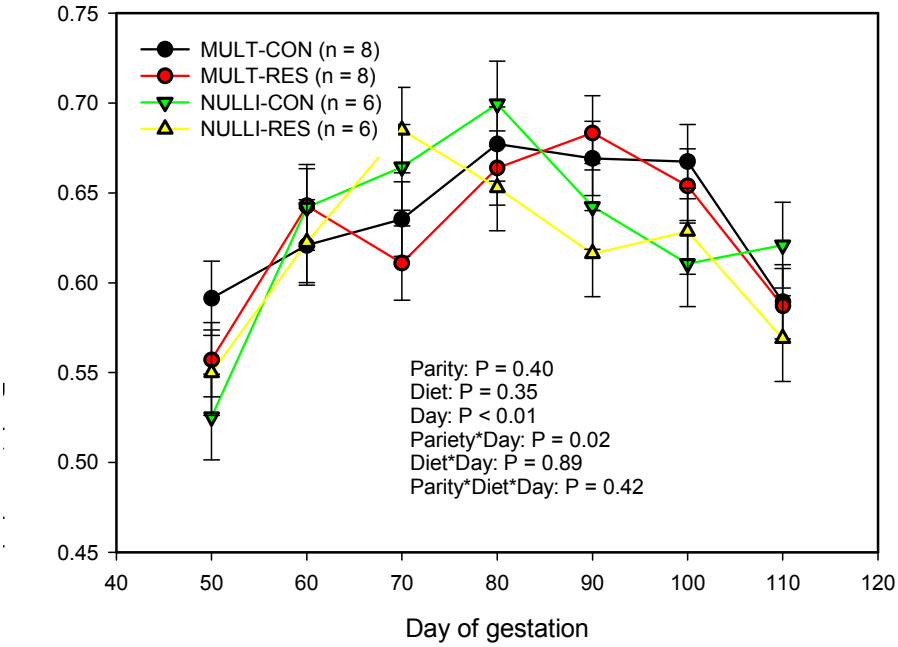

Figure 3.7. Impacts of maternal nutrition and parity on resistance index (RI) from d 50 to 110 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90.

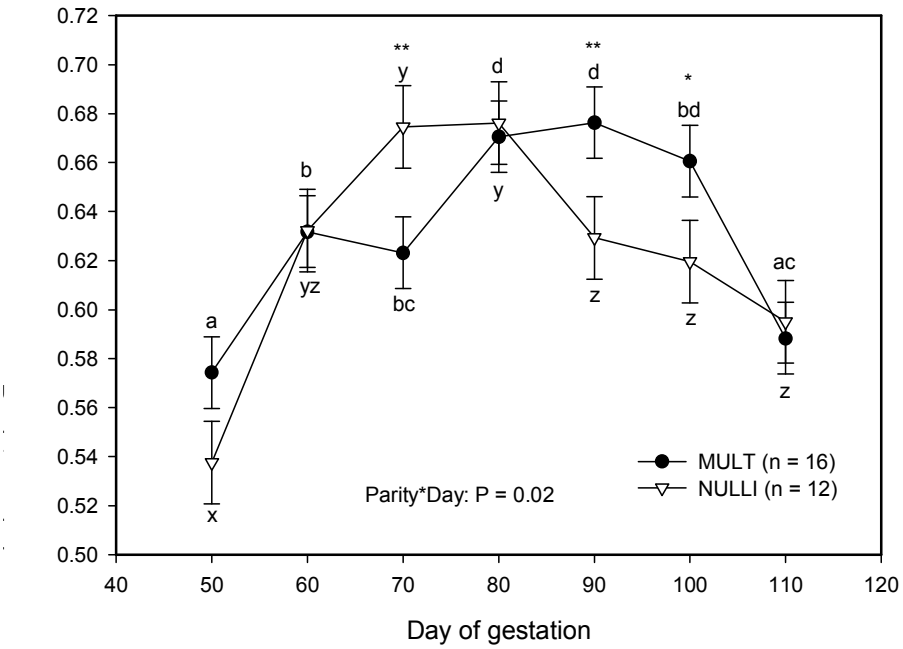

Figure 3.8. Impacts of parity on resistance index (RI) from d 50 to 110 of gestation. MULT = 1 to 3 previous parities. NULLI = no previous parities. [abcd]LSMEANS ± SEM within MULT differ P < 0.05; [xyz]LSMEANS ± SEM within NULLI differ $P < 0.05$. Differences between MULT and NULLI are denoted by ** $P \leq 0.05$, * $P \leq 0.10$ within a day.

Hematocrit was not influenced by the parity by diet by day interaction or the main effects for parity and diet ($P = 0.62$). However, the parity by day and diet by day interactions were significant ($P = 0.01$ and $P < 0.01$, respectively). A mean separation of CON and RES animals shows a greater Ht in RES on d 60 of gestation (Appendix table B.5; Figure 3.9). In the remaining days of the study, Ht was similar between these two groups (Appendix table B.5; Figure 3.9). Although there was a parity by day interaction, mean separations of MUL vs. NUL animals did not show any differences or tendencies between these two groups (Appendix table B.5; Figure 3.10). There was a day effect ($P < 0.01$) with Ht values varying independently in all treatment groups as gestation advanced (Appendix table B.5).

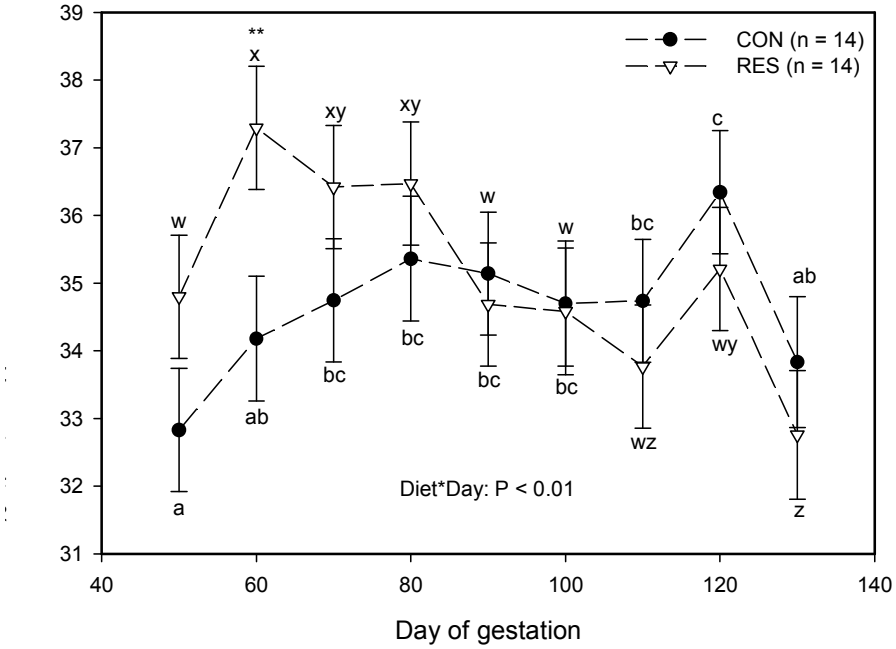

Figure 3.9. Impacts of maternal nutrition on maternal hematocrit (Ht) from d 50 to 130 of gestation. CON = 100% of NRC recommendations. RES = 60% of CON. Restriction was applied from d 50 until d 90. ^{abc}LSMEANS ± SEM within CON differ $P < 0.05$; ^{wxyz}LSMEANS ± SEM within RES differ $P< 0.05$. Differences between CON and RES are denoted by $^{**}P \leq 0.05$.

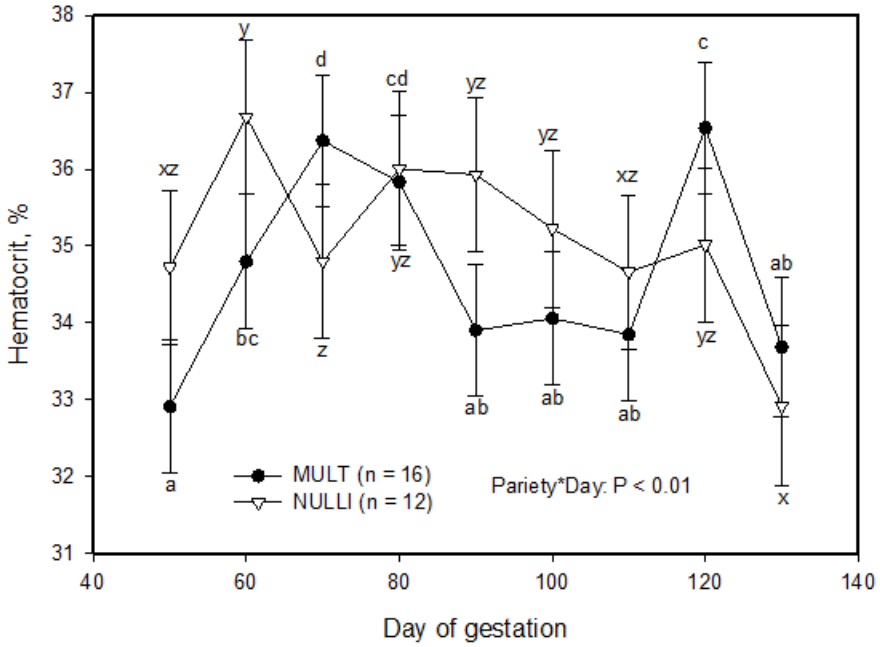

Figure 3.10. Impacts of parity on maternal hematocrit (Ht) from d 50 to 130 of gestation. MUL = 1 to 3 previous parities. NUL = no previous parities. [abc]LSMEANS ± SEM within MULT differ $P < 0.05$; [xyz]LSMEANS ± SEM within NULLI differ $P < 0.05$.

No differences in birth weight were observed among any treatment groups (Table 3.1). Maternal and lamb birth problems [placental retention, dystocia, low lamb viability and/or earlylamb mortality (first 24 hours)] were analyzed. Although not significant, NUL animals appear to have more birth problems than MUL (Table 3.1). An LSMEAN separation (data not shown) demonstrates that NUL-RES animals showed a near-tendency ($P = 0.11$) to have greater birth problems when compared to MUL-RES and MUL-CON (Table 3.1). Restricted animals had no difference in birth problems when compared to CON (Table 3.1).

Table 3.1. Birth weight means (kg) and number of birth related problems (Number of ewe and/or lamb per treatment)

	Multiparous		Nulliparous			P value		
	CON	RES	CON	RES	SEM	Parity	Diet	Parity*Diet
Birth weight	5.10	5.16	5.29	5.05	0.33	0.91	0.78	0.64
Birth problems	1/8	1/8	1/6	3/6		0.20	0.30	0.30

Total n=28 (CON=14, RES=14; MUL=16, NUL=12); $P < 0.05$ are considered significant
Birth problems = Placental retention, dystocia, low lamb viability and/or lamb mortality (first 24 hours)

Discussion

We previously reported that in multiparous ewes, a mid-gestation nutrient restriction has a detrimental effect on UBF, PI, RI and placentome area on d 80 of gestation (Chapter 2), and that on d 90 all these values returned to control levels (Chapter 2). We also suggested that these four measurements were indirectly related to nutrient transport (blood flow) through the placenta and the umbilical cord (Kiserud, 2005; Lemley et al., 2012). In the present study we hypothesized that parity could have a protective effect on these measurements on d 90. However, none of the effects of nutrient restriction could be mirrored in our MUL or NUL animals in this experiment. Moreover, NUL-RES animals tended to have and had a greater UBF on days 90 and 110, respectively, when compared to MUL-RES. We also found no relation between PI, RI and UBF. On d 50 and 100, in which PI and RI were greater and tended to be greater in MUL-CON vs. NUL-CON, UBF was not different between them (Appendix tables B.3 and B.4). Similarly, on d 70 RI and PI, respectively, were greater and tended to be greater when comparing NUL-RES vs. MUL-RES, presenting again a similar UBF (Appendix tables B.3 and B.4). The only day in which PI and RI values were related to UBF was on d 90, in the comparison between NUL-RES vs MUL-RES. These findings suggest that the generalized idea of PI and RI being inversely related to UBF is not always true. There could be other factors such as tissue specific vessel properties that influence these results.

Multiparous restricted animals appeared to lose more body weight than NUL-RES animals. This is opposed to another study in which early-gestating restricted multiparous ewes seemed to

be more resistant in weight and body condition score (BCS) when compared to nulliparous (Abdel-Mageed and Abd El-Gawad, 2015). In our study, we did not measure BCS. It is possible that BCS prior to mating, breed differences, period of gestation and/or severity of the restriction could have influenced body weight loss.

Our lab has previously demonstrated that a 40% nutrient restriction in mid and mid-to-late gestation does not influence US kidney measurements in sheep (Chapter 2). Those results were corroborated in the present study; therefore, we conclude that there was no effect of any of the treatments in the kidney size of the developing fetus. The kidneys, unlike other organs of the developing fetus (e.g. small intestine, spleen), seem not to be affected by a lower nutrient availability in mid to late gestation (50 to 130 days of gestation) (Osgerby et al., 2002; Lemley et al., 2012). Abdominal width and biparietal distance are used jointly as indirect measurements of fetal size and weight (Araujo et al., 2014; Rueda et al., 2014). We saw a three way interaction in biparietal distance. This interaction effect was probably driven by the lesser biparietal distance present in NUL-RES animals on d 70 of gestation. Placental growth is particular to the first two-thirds of pregnancy (Redmer et al., 2004), with fetal growth being exponential during the last third of pregnancy (Redmer et al., 2004). Nevertheless, some studies have shown that when a severe nutrient restriction is applied during mid-gestation, and the fetuses are collected at the end of the restriction period [before the last period of gestation (no realimentation)], fetal weight can be affected (Murdoch et al., 2003; Zhou et al., 2008; Ma et al., 2011). Abdominal width however, as well as the remaining days of biparietal width measurements were not affected by nutritional treatment. Similarly, these results are analogous to what we have formerly reported (Chapter 2), and appear to demonstrate that fetal growth in adult white face ewes seems to be protected against a 40% nutrient restriction during mid-gestation.

Hematocrit results are peculiar. We previously showed that Ht increases in restricted animals, proposing a relationship between low plasma proteins and decreased oncotic pressure that could result in a decreased plasma volume, ultimately increasing Ht (Chapter 2). In the present study we see no such difference in Ht between the two dietary treatments. Moreover, a statistical analysis of the percentage increase in CON vs. RES groups (data not shown) shows a greater Ht increase in CON animals than in RES animals. We could speculate that the decreased nutrient availability in the RES ewes could be decreasing red blood cell synthesis in the bone marrow of these animals; however, the explanation of the difference seen in the results between this and our previous study is not clear. Plasma protein analysis could help answer this question.

We found that a 40% nutrient restriction on multiparous and nulliparous animals during mid-gestation did not affect birth weights. This is similar to what we previously found in multiparous animals (Chapter 2). Furthermore, others have demonstrated that moderate to severe nutrient restriction applied during early-to-mid and mid-to-late gestation periods have also shown no effects on birth weight (Gilbert et al., 2005; Sebert et al., 2008; Kotsampasi et al., 2009; Sharkey et al., 2009; Lekatz et al., 2013). These results prove that the majority of the fetal development occurs during the last third of pregnancy (Redmer et al., 2004). Studies in cows and mares have shown an influence of parity in birth weight (Kayisiz et al., 2010; Yakubu et al., 2014; Abdel-Mageed and Abd El-Gawad, 2015; Klewitz et al., 2015; Lv et al., 2015). In this study, parity did not have an effect on birth weight. This is opposite to another study done in cross bred sheep in which nulliparous dam lambs had lower birth weights than multiparous animals (Yakubu et al., 2007). Breed differences could be influencing the dissimilar results.

Although the number of animals in this study is not enough to make inferences between birth related problems and treatments, it is interesting that NUL animals seemed to have more

parturition problems than MUL animals, and that NUL-RES animals seemed to be more prone to these problems than any of the other treatment groups. Across species, a parity effect on parturition appears to be variable. In cattle, it is accepted that heifers have higher probability of presenting dystocia than multiparous animals (Zaborski et al., 2008). In sheep, some studies show higher lambing difficulty and early lamb mortality in nulliparous ewes (Kelly et al., 1992; Southey et al., 2004; McHugh et al., 2015), while others show no difference between nulliparous and multiparous animals (Leontides et al., 2000).

A nutrient restriction during mid-gestation in multiparous cows showed that uterine blood flow did not decrease during the restriction period; however, it increased upon realimentation (Camacho et al., 2014). Our findings suggest that adult white face sheep are resistant to a 40% mid-gestation nutrient restriction, and they do not have an increase in UBF upon realimentation. The decreased UBF seen in restricted adolescent nulliparous ewes (Lemley et al., 2012) is almost certainly an effect of age of gestation. Adolescent pregnant ewes compete for nutrient resources with the developing fetus (Redmer et al., 2004). Additionally, nulliparous adult animals have greater fetal, cotyledonary and caruncular weights as well as a greater cotyledonary angiogenic factor expression than nulliparous adolescent ewes (Borowicz et al., 2005). Nutrient restriction during mid-pregnancy could be enhancing these differences. However, this has to be specifically analyzed. Furthermore, mid-gestation nutrient-restriction effects seen in other studies in sheep vary in breed, period of restriction and severity of the restriction (Kelly et al., 1992; Reynolds et al., 2005). These aspects have to be noted when analyzing the results.

Literature cited

Abdel-Mageed, I. and M. A. El-Gawad. 2015. Effects of breed, parity and post-mating nutrition on reproductive wastage and pregnancy outcomes of Egyptian sheep. Small Ruminant Research 130:171-177.

Araujo Júnior, E., R. Ruano, P. Javadian, W. P. Martins, J. Elito Júnior, C. R. Pires, and S. M. Zanforlin Filho. 2014. Reference charts for fetal biometric parameters in twin pregnancies according to chorionicity. Prenatal Diagnosis 34(4):382-388.

Borowicz, P., K. Vonnahme, A. Grazul-Bilska, D. Redmer, M. Johnson, and L. Reynolds. 2005. The effect of maternal age (age at first pregnancy) on placental expression of the major angiogenic factors and their receptors. Pages 327A-328A in proc. Journal of the Society for Gynecologic Investigation. Elsevier science Inc. 360 park ave. south, NY 10010-1710 USA.

Duckitt, K. and D. Harrington. 2005. Risk factors for pre-eclampsia at antenatal booking: systematic review of controlled studies. British Medical Journal 330(7491):565.

Elliott, C., J. Morton, and J. Chopin. 2009. Factors affecting foal birth weight in thoroughbred horses. Theriogenology 71(4):683-689.

Ferrazzi, E., M. Bozzo, S. Rigano, M. Bellotti, A. Morabito, G. Pardi, F. Battaglia, and H. Galan. 2002. Temporal sequence of abnormal Doppler changes in the peripheral and central circulatory systems of the severely growth-restricted fetus. Ultrasound in Obstetrics and Gynecology 19(2):140-146.

Ferrell, C. and L. Reynolds. 1992. Uterine and umbilical blood flows and net nutrient uptake by fetuses and uteroplacental tissues of cows gravid with either single or twin fetuses. Journal of Animal Science 70(2):426-433.

Galan, H. L., M. J. Hussey, M. Chung, J. K. Chyu, J. C. Hobbins, and F. C. Battaglia. 1998. Doppler velocimetry of growth-restricted fetuses in an ovine model of placental insufficiency. American Journal of Obstetrics and Gynecology 178(3):451-456.

Gilbert, J. S., A. L. Lang, A. R. Grant, and M. J. Nijland. 2005. Maternal nutrient restriction in sheep: hypertension and decreased nephron number in offspring at 9 months of age. The Journal of Physiology 565(1):137-147.

Jacquot, J. J. and S. H. Vessey. 1998. Recruitment in white-footed mice (Peromyscus leucopus) as a function of litter size, parity, and season. Journal of Mammalogy 79(1):312-319.

Kaygisiz, A., G. Bakir, I. Yilmaz, and Y. Vanli. 2011. Estimation of variance components and genetic parameters for direct and maternal effects on birth weight in Brown Swiss cattle. Pakistan Veterinary Journal 31(1):70-74.

Kelly, R., E. Speijers, I. Ralph, and J. Newnham. 1992. Lambing performances and wool production of maiden and adult Merino ewes fed different amounts of lupin seed in mid-pregnancy. Crop and Pasture Science 43(2):339-354.

Kiserud, T. 2005. Physiology of the fetal circulation. Pages 493-503 in Proc. Seminars in Fetal and Neonatal Medicine. Elsevier.

Klewitz, J., C. Struebing, K. Rohn, A. Goergens, G. Martinsson, F. Orgies, J. Probst, F. Hollinshead, H. Bollwein, and H. Sieme. 2015. Effects of age, parity, and pregnancy abnormalities on foal birth weight and uterine blood flow in the mare. Theriogenology 83(4):721-729.

Kotsampasi, B., C. Balaskas, G. Papadomichelakis, and S. Chadio. 2009. Reduced Sertoli cell number and altered pituitary responsiveness in male lambs undernourished in utero. Animal Reproduction Science 114(1):135-147.

Lafi, S., A. Talafha, N. Giadinis, E. Kalaitzakis, K. Pourliotis, and N. Panousis. 2009. Factors affecting the reproductive performance of Awassi sheep flocks in north-east of Jordan: An epidemiological study. Tropical Animal Health and Production 41(8):1755-1764.

Lekatz, L., J. Luther, J. Caton, and K. Vonnahme. 2013. Impacts of maternal nutritional plane on umbilical artery hemodynamics, fetal and placentome growth in sheep. Animal Reproduction 10(2):99-105.

Lemley, C. O., A. M. Meyer, L. E. Camacho, T. L. Neville, D. J. Newman, J. S. Caton, and K. A. Vonnahme. 2012. Melatonin supplementation alters uteroplacental hemodynamics and fetal development in an ovine model of intrauterine growth restriction. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology 302(4):R454-R467.

Lv, S.-J., Y. Yang, C. Dwyer, and F.-K. Li. 2015. Pen size and parity effects on maternal behaviour of Small-Tail Han sheep. Animal 9(07):1195-1202.

Ma, Y., M. J. Zhu, A. B. Uthlaut, M. J. Nijland, P. W. Nathanielsz, B. W. Hess, and S. P. Ford. 2011. Upregulation of growth signaling and nutrient transporters in cotyledons of early to mid-gestational nutrient restricted ewes. Placenta 32(3):255-263.

Mahan, D. 1998. Relationship of gestation protein and feed intake level over a five-parity period using a high-producing sow genotype. Journal of Animal Science 76(2):533-541.

Murdoch, W. J., E. A. Van Kirk, K. A. Vonnahme, and S. P. Ford. 2003. Ovarian responses to undernutrition in pregnant ewes, USA. Reprodive Biology Endocrinology 1(6).

Osgerby, J., D. Wathes, D. Howard, and T. Gadd. 2002. The effect of maternal undernutrition on ovine fetal growth. Journal of Endocrinology 173(1):131-141.

Père, M.-C. and M. Etienne. 2000. Uterine blood flow in sows: effects of pregnancy stage and litter size. Reproduction Nutrition Development 40(4):369-382.

Reynolds, L. P., P. P. Borowicz, K. A. Vonnahme, M. L. Johnson, A. T. Grazul-Bilska, D. A. Redmer, and J. S. Caton. 2005. Placental angiogenesis in sheep models of compromised pregnancy. The Journal of Physiology 565(1):43-58.

Rigano, S., M. Bozzo, E. Ferrazzi, M. Bellotti, F. C. Battaglia, and H. L. Galan. 2001. Early and persistent reduction in umbilical vein blood flow in the growth-restricted fetus: a longitudinal study. American Journal of Obstetrics and Gynecology 185(4):834-838.

Rueda, S., S. Fathima, C. L. Knight, M. Yaqub, A. T. Papageorghiou, B. Rahmatullah, A. Foi, M. Maggioni, A. Pepe, and J. Tohka. 2014. Evaluation and comparison of current fetal ultrasound image segmentation methods for biometric measurements: a grand challenge. Medical Imaging, IEEE transactions on 33(4):797-813.

Rurangirwa, A. A., R. Gaillard, E. A. Steegers, A. Hofman, and V. W. Jaddoe. 2012. Hemodynamic adaptations in different trimesters among nulliparous and multiparous pregnant women; the Generation R study. American Journal of Hypertension 25(8):892-899.

Salazar-Vazquez, B. Y., P. Cabrales, A. G. Tsai, P. C. Johnson, and M. Intaglietta. 2008. Lowering of blood pressure by increasing hematocrit with non–nitric oxide–scavenging red blood cells. American Journal of Respiratory Cell and Molecular Biology 38(2):135-142.

Sebert, S., M. Hyatt, L. Chan, N. Patel, R. Bell, D. Keisler, T. Stephenson, H. Budge, M. Symonds, and D. Gardner. 2009. Maternal nutrient restriction between early and midgestation and its impact upon appetite regulation after juvenile obesity. Endocrinology 150(2):634-641.

Sharkey, D., D. S. Gardner, M. E. Symonds, and H. Budge. 2009. Maternal nutrient restriction during early fetal kidney development attenuates the renal innate inflammatory response in obese young adult offspring. American Journal of Physiology-Renal Physiology 297(5):F1199-F1207.

Southey, B., S. Rodriguez-Zas, and K. Leymaster. 2004. Competing risks analysis of lamb mortality in a terminal sire composite population. Journal of Animal Science 82(10):2892-2899.

Suzuki, S. 2006. Influence of parity on second-trimester uterine artery Doppler waveforms in twin pregnancy. The Journal of Maternal-Fetal and Neonatal Medicine 19(3):193-194.

Tkadlec, E. and P. Krejčová. 2001. Age-specific effect of parity on litter size in the common vole (Microtus arvalis). Journal of Mammalogy 82(2):545-550.

Whitley, N. C., M. Thomas, J. Ramirez, A. Moore, and N. Cox. 2002. Influences of parity and level of feed intake on reproductive response to insulin administration after weaning in sows. Journal of Animal Science 80(4):1038-1043.

Yakubu, D., A. Mostyn, V. Wilson, S. Pearce, M. Alves-Guerra, C. Pecqueur, B. Miroux, H. Budge, T. Stephenson, and M. Symonds. 2007. Different effects of maternal parity, cold exposure and nutrient restriction in late pregnancy on the abundance of mitochondrial proteins in the kidney, liver and lung of postnatal sheep. Reproduction 133(6):1241-1252.

Yakubu, H., P. Barje, and G. Iyeghe-Erakpotobor. 2014. Influence of calf parity number, season of calving and period of calving on birth and weaning weights of Friesian-Bunaji calves. World Journal of Life Sciences and Medical Research 3(2):59.

Yotov, S. 2012. Ultrasound diagnostics of late embryonic and foetal death in three sheep breeds. Journal of Veterinary Advances 2(3):120-125.

Zhou, Y., M. Nijland, M. Miller, S. Ford, P. W. Nathanielsz, and J. T. Brenna. 2008. The influence of maternal early to mid-gestation nutrient restriction on long chain polyunsaturated fatty acids in fetal sheep. Lipids 43(6):525-531.

CHAPTER 4. GENERAL DISCUSSION AND FUTURE DIRECTIONS

The results found in both studies suggest that umbilical blood flow (UBF) is not only due to nutritional plane of nutrition, but perhaps, the age of the animal. Perhaps parity could influence this phenomenon as well, but our initial findings may point to age, or attaining a mature body size, to be more specific. This however has to be specifically confirmed in a future study. This could be accomplished by having an adult multiparous an adult nulliparous and a younger (6 – 12 mo.) group of pregnant sheep under a mid-gestation nutrient restriction. The rest of the experiment would be similar to both studies presented in this thesis.

Another interesting and conflicting result that we observed between the two projects was for hematocrit (Ht). In Chapter 2, restricted ewes had increased Ht during the nutrient restriction, whereas in Chapter 3 Ht increased in the control ewes compared to restricted ewes during the same period. First, we will analyze the serum protein levels of those blood samples. This will help us further understand if the decrease in Ht in the first study could be related to a decrease in oncotic pressure due to a decrease in serum protein levels. This decrease would in turn decline the water amount in the blood with a subsequent decrease in blood volume, therefore increasing Ht. On the other hand, if protein levels are normal, it could mean that a decreased nutrient intake in restricted animals can produce a decrease in the nutrients necessary for erythrocyte production of these animals, which would decrease Ht. Perhaps the ewes were able to mobilize their body stores to maintain adequate blood volume. Therefore, the assessment of protein intake/mobilization via urea nitrogen analysis and fat mobilization via non-esterified fatty acid analysis should be performed.

Finally, it would also be interesting to analyze how fetal Ht varies under the nutrient restriction of the ewe. It is possible that a decreased amount of nutrients to the dam could cause a decrease in the amount of nutrients to the bone marrow and other erythropoietic tissues of the fetus.

This would cause a decrease in the fetal Ht. On the other side, if nutrient restriction causes an increase in the Ht of the ewe, and the same happens to the Ht of the fetus, this would increase the viscosity of the blood, increasing the shear stress on the fetal vessels. This in turn could produce an increase in the diameter of the umbilical arteries thus increasing blood flow. It would also be interesting to know if and how oxygen and carbon dioxide vary in the fetal blood as Ht changes in the fetus, when a nutritional insult is present in the dam.

In summary, chapters 2 and 3 seem to indicate that the decrease in umbilical blood flow observed previously by our laboratory upon mid-gestation nutrient restriction (Chapter 1) is not solely a consequence of the restriction itself or an additional effect of parity. Moreover, it appears very probable that those previous results are also an effect of maternal age. In order to address this question a future experiment is needed. This experiment would have to account for maternal age, parity and nutrient restriction during mid-gestation. Additionally, the physiological relationships between maternal nutrient-restriction and fetal and maternal Ht and blood volume, with its consequences in fetal blood flow need to be further investigated.

APPENDIX A

Table A.1. Ewe body weight, means separation

CON			RES			CON*RES
Day	**Mean**	**St. E.**	**Day**	**Mean**	**St. E.**	***P***
20	64.24	1.96	20	61.97	1.84	0.41
25	64.92	1.94	25	62.44	1.83	0.36
30	64.72	1.94	30	61.62	1.81	0.26
40	63.62	1.94	40	61.00	1.81	0.34
50	63.10	1.94	50	61.05	1.81	0.45
60	63.88	1.94	60	59.98	1.81	0.16
70	65.30	1.94	70	58.96	1.81	≤ 0.05**
80	66.86	1.94	80	59.18	1.81	≤ 0.01**
90	67.38	1.94	90	59.01	1.81	≤ 0.01**
100	67.57	1.94	100	60.71	1.81	≤ 0.05**
110	66.66	1.94	110	62.07	1.81	≤ 0.10*
120	66.97	1.96	120	61.12	1.82	≤ 0.05**
130	68.77	1.94	130	62.72	1.83	≤ 0.05**

Table A.2. Fetal abdominal width, biparietal distance, kidney length and width, and placentome area, means separation

CON			RES			CON*RES
Day	**Mean**	**St. E.**	**Day**	**Mean**	**St. E.**	***P***
Abdominal width (cm).						
50	1.88	0.05	50	1.92	0.05	0.57
60	2.84	0.09	60	2.85	0.08	0.90
70	3.72	0.14	70	3.70	0.13	0.90
80	4.76	0.14	80	4.83	0.13	0.72
90	6.13	0.16	90	5.91	0.15	0.36
100	7.59	0.24	100	6.64	0.22	≤ 0.01**
110	7.85	0.36	110	7.70	0.33	0.77
Biparietal distance (cm).						
50	1.62	0.05	50	1.66	0.05	0.57
60	2.35	0.11	60	2.44	0.10	0.58
70	2.89	0.07	70	2.82	0.06	0.47
80	3.47	0.13	80	3.86	0.12	≤ 0.05**
90	4.08	0.13	90	4.23	0.12	0.41
100	4.41	0.12	100	4.42	0.11	0.98
110	5.74	0.27	110	5.13	0.25	0.12
Kidney length (cm).						
50	0.64	0.02	50	0.63	0.02	0.79
60	0.97	0.05	60	0.97	0.05	0.98
70	1.39	0.06	70	1.39	0.06	0.99
80	1.79	0.09	80	1.82	0.09	0.83
90	2.42	0.10	90	2.36	0.09	0.66
100	2.81	0.10	100	2.58	0.09	≤ 0.10*
110	3.18	0.13	110	2.93	0.10	0.15
Kidney width (cm).						
50	0.21	0.02	50	0.20	0.02	0.72
60	0.31	0.02	60	0.33	0.02	0.58
70	0.44	0.02	70	0.40	0.02	0.30
80	0.52	0.03	80	0.50	0.03	0.57
90	0.73	0.07	90	0.70	0.07	0.75
100	0.77	0.04	100	0.79	0.03	0.75
110	1.15	0.12	110	1.06	0.11	0.61
Placentome area (cm^2).						
50	1.29	0.10	50	1.12	0.09	0.22
60	2.39	0.15	60	2.22	0.14	0.43
70	2.98	0.23	70	2.62	0.21	0.27
80	3.28	0.18	80	2.84	0.17	≤ 0.10*
90	3.14	0.31	90	2.71	0.29	0.33
100	2.69	0.18	100	2.40	0.17	0.23
110	2.95	0.25	110	2.37	0.23	0.11

Table A.3. Umbilical blood flow, pulsatility index and resistance index, means separation

CON			RES			CON*RES
Day	**Mean**	**St. E.**	**Day**	**Mean**	**St. E.**	***P***
Umbilical blood flow (mL/min)						
50	21.49	2.14	50	23.60	2.00	0.49
60	70.30	15.13	60	59.39	14.16	0.61
70	102.42	11.35	70	91.92	10.62	0.51
80	221.91	24.70	80	137.14	23.11	≤ 0.05**
90	327.41	56.68	90	292.12	53.02	0.66
100	390.91	45.49	100	348.45	42.55	0.51
110	533.82	89.98	110	473.69	84.17	0.63
Pulsatility index (mL/min)						
50	0.89	0.06	50	0.87	0.06	0.80
60	1.04	0.06	60	1.05	0.06	0.86
70	1.11	0.06	70	1.11	0.06	0.99
80	1.04	0.06	80	1.19	0.06	≤ 0.10*
90	0.98	0.06	90	1.02	0.06	0.64
100	0.94	0.06	100	0.99	0.06	0.58
110	0.94	0.06	110	0.87	0.06	0.42
Resistance index (mL/min)						
50	0.56	0.03	50	0.55	0.03	0.74
60	0.64	0.03	60	0.65	0.03	0.95
70	0.68	0.03	70	0.68	0.03	0.92
80	0.65	0.03	80	0.72	0.03	≤ 0.10*
90	0.63	0.03	90	0.64	0.03	0.72
100	0.62	0.03	100	0.64	0.03	0.56
110	0.61	0.03	110	0.59	0.03	0.65

Table A.4. Ewe hematocrit, means separation

CON			RES			CON*RES
Day	**Mean**	**St. E.**	**Day**	**Mean**	**St. E.**	***P***
Hematocrit (%).						
50	33.22	0.97	50	34.34	0.91	0.40
60	32.81	0.97	60	36.40	0.91	≤ 0.01**
70	32.34	0.97	70	34.67	0.95	≤ 0.10*
80	32.33	0.97	80	35.41	0.91	≤ 0.05**
90	33.47	0.97	90	35.22	0.91	0.20
100	32.81	0.97	100	32.63	0.91	0.89
110	33.57	0.97	110	33.20	0.91	0.79
120	34.59	0.97	120	33.14	0.95	0.29
130	34.49	0.97	130	32.39	0.95	0.13

APPENDIX B

Table B.1. Ewe weights, means separation

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	CON			**RES**		**CON*RES**
Ewe weights (kg)						
50	76.08	0.60	50	76.10	0.60	0.98
60	75.91	0.60	60	73.40	0.60	0.004**
70	76.53	0.60	70	72.76	0.60	< 0.001**
80	76.60	0.60	80	72.20	0.60	<0.001**
90	77.21	0.60	90	71.20	0.60	<0.001**
100	76.51	0.60	100	72.47	0.60	<0.001**
110	76.38	0.60	110	72.69	0.60	<0.001**
120	75.12	0.61	120	71.04	0.60	<0.001**
130	75.49	0.63	130	72.40	0.60	<0.001**
	MUL-CON			**MUL-RES**		**MC*MR**
50	76.03	0.79	50	76.11	0.79	0.95
60	76.46	0.79	60	72.74	0.79	0.001**
70	77.08	0.79	70	72.23	0.79	<0.001**
80	77.54	0.79	80	71.09	0.79	<0.001**
90	77.99	0.79	90	70.47	0.79	<0.001**
100	77.20	0.79	100	72.62	0.79	<0.001**
110	77.08	0.79	110	72.68	0.79	<0.001**
120	74.70	0.79	120	70.98	0.79	0.001**
130	75.22	0.82	130	72.45	0.79	0.02**
	NUL-CON			**NUL-RES**		**NC*NR**
50	76.12	0.92	50	76.09	0.91	0.98
60	75.37	0.92	60	74.05	0.91	0.31
70	75.97	0.92	70	73.30	0.91	0.04**
80	75.67	0.92	80	73.30	0.91	0.07*
90	76.43	0.92	90	71.94	0.91	<0.001**
100	75.82	0.92	100	72.31	0.91	0.008**
110	75.67	0.92	110	72.69	0.91	0.02**
120	75.55	0.96	120	71.11	0.91	0.001**
130	75.75	0.98	130	72.35	0.91	0.01**
	MUL-CON			**NUL-CON**		**MC*NC**
50	76.03	0.79	50	76.12	0.92	0.94
60	76.46	0.79	60	75.37	0.92	0.38
70	77.08	0.79	70	75.97	0.92	0.37
80	77.54	0.79	80	75.67	0.92	0.13
90	77.99	0.79	90	76.43	0.92	0.21
100	77.20	0.79	100	75.82	0.92	0.27
110	77.08	0.79	110	75.67	0.92	0.25
120	74.70	0.79	120	75.55	0.96	0.50
130	75.22	0.82	130	75.75	0.98	0.69
	MUL-RES			**NUL-RES**		**MR*NR**
50	76.11	0.79	50	76.09	0.91	0.99

Table B.1. Ewe weights, means separation (continued)

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	MUL-RES			**NUL-RES**		**MR*NR**
60	72.74	0.79	60	74.05	0.91	0.28
70	72.23	0.79	70	73.30	0.91	0.38
80	71.09	0.79	80	73.30	0.91	0.07*
90	70.47	0.79	90	71.94	0.91	0.23
100	72.62	0.79	100	72.31	0.91	0.80
110	72.68	0.79	110	72.69	0.91	0.99
120	70.98	0.79	120	71.11	0.91	0.92
130	72.45	0.79	130	72.35	0.91	0.93

Table B.2. Umbilical blood flow, means separation

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	MUL			**NUL**		**MUL*NUL**
UBF (mL/min)						
50	21.72	15.25	50	21.08	17.61	0.98
60	60.10	15.25	60	55.68	17.61	0.85
70	100.20	15.25	70	106.94	17.61	0.77
80	137.21	15.25	80	137.68	17.61	0.98
90	192.26	15.25	90	213.42	17.61	0.37
100	270.15	15.25	100	293.92	17.61	0.31
110	386.88	15.25	110	440.78	17.61	0.02**
	MUL-CON			**MUL-RES**		**MC*MR**
50	22.05	21.57	50	21.39	21.57	0.98
60	63.87	21.57	60	56.33	21.57	0.81
70	95.91	21.57	70	104.49	21.57	0.78
80	138.89	21.57	80	135.53	21.57	0.91
90	212.41	21.57	90	172.10	21.57	0.19
100	287.98	21.57	100	252.32	21.57	0.24
110	405.39	21.57	110	368.38	21.57	0.23
	NUL-CON			**NUL-RES**		**NC*NR**
50	23.95	24.91	50	18.20	24.91	0.87
60	53.66	24.91	60	57.70	24.91	0.91
70	109.96	24.91	70	103.91	24.91	0.86
80	124.46	24.91	80	150.90	24.91	0.45
90	197.93	24.91	90	228.91	24.91	0.38
100	285.94	24.91	100	301.89	24.91	0.65
110	431.95	24.91	110	449.60	24.91	0.62
	MUL-CON			**NUL-CON**		**MC*NC**
50	22.05	21.57	50	23.95	24.91	0.95
60	63.87	21.57	60	53.66	24.91	0.76
70	95.91	21.57	70	109.96	24.91	0.67
80	138.89	21.57	80	124.46	24.91	0.66
90	212.41	21.57	90	197.93	24.91	0.66
100	287.98	21.57	100	285.94	24.91	0.95
110	405.39	21.57	110	431.95	24.91	0.42
	MUL-RES			**NUL-RES**		**MR*NR**
50	21.39	21.57	50	18.20	24.91	0.92
60	56.33	21.57	60	57.70	24.91	0.97
70	104.49	21.57	70	103.91	24.91	0.99
80	135.53	21.57	80	150.90	24.91	0.64
90	172.10	21.57	90	228.91	24.91	0.09*
100	252.32	21.57	100	301.89	24.91	0.13
110	368.38	21.57	110	449.60	24.91	0.01**

Table B.3. Pulsatility index, means separation

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	MUL			**NUL**		**MUL*NUL**
PI (mL/min)						
50	0.88	0.03	50	0.82	0.03	0.15
60	0.97	0.03	60	0.98	0.03	0.70
70	0.95	0.04	70	1.06	0.04	0.07*
80	1.06	0.05	80	1.11	0.06	0.60
90	1.04	0.03	90	0.95	0.03	0.06*
100	1.04	0.04	100	0.93	0.05	0.08*
110	0.87	0.04	110	0.87	0.04	0.98
	MUL-CON			**MUL-RES**		**MC*MR**
50	0.94	0.04	50	0.83	0.04	0.05**
60	0.92	0.04	60	1.01	0.04	0.14
70	0.95	0.05	70	0.95	0.05	0.97
80	1.07	0.07	80	1.05	0.07	0.82
90	1.02	0.04	90	1.05	0.04	0.61
100	1.06	0.06	100	1.02	0.06	0.61
110	0.87	0.05	110	0.87	0.05	0.98
	NUL-CON			**NUL-RES**		**NC*NR**
50	0.81	0.04	50	0.84	0.04	0.67
60	1.03	0.05	60	0.93	0.05	0.13
70	1.02	0.06	70	1.10	0.06	0.35
80	1.16	0.08	80	1.06	0.08	0.40
90	0.99	0.05	90	0.91	0.05	0.28
100	0.91	0.07	100	0.95	0.07	0.74
110	0.92	0.06	110	0.82	0.06	0.27
	MUL-CON			**NUL-CON**		**MC*NC**
50	0.94	0.04	50	0.81	0.04	0.04**
60	0.92	0.04	60	1.03	0.05	0.08*
70	0.95	0.05	70	1.02	0.06	0.43
80	1.07	0.07	80	1.16	0.08	0.47
90	1.02	0.04	90	0.99	0.05	0.56
100	1.06	0.06	100	0.91	0.07	0.10*
110	0.87	0.05	110	0.92	0.06	0.55
	MUL-RES			**NUL-RES**		**MR*NR**
50	0.83	0.04	50	0.84	0.04	0.90
60	1.01	0.04	60	0.93	0.05	0.21
70	0.95	0.05	70	1.10	0.06	0.07*
80	1.05	0.07	80	1.06	0.08	0.98
90	1.05	0.04	90	0.91	0.05	0.03**
100	1.02	0.06	100	0.95	0.07	0.40
110	0.87	0.05	110	0.82	0.06	0.57

Table B.4. Resistance index, means separation

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	MUL			**NUL**		**MUL*NUL**
RI (mL/min)						
50	0.57	0.01	50	0.54	0.02	0.10*
60	0.63	0.01	60	0.63	0.02	0.98
70	0.62	0.01	70	0.67	0.02	0.02**
80	0.67	0.01	80	0.68	0.02	0.80
90	0.68	0.01	90	0.63	0.02	0.04**
100	0.66	0.01	100	0.62	0.02	0.07*
110	0.59	0.01	110	0.60	0.02	0.76
	MUL-CON			**MUL-RES**		**MC*MR**
50	0.59	0.02	50	0.56	0.02	0.24
60	0.62	0.02	60	0.64	0.02	0.45
70	0.64	0.02	70	0.61	0.02	0.40
80	0.68	0.02	80	0.66	0.02	0.65
90	0.67	0.02	90	0.68	0.02	0.63
100	0.67	0.02	100	0.65	0.02	0.65
110	0.59	0.02	110	0.59	0.02	0.94
	NUL-CON			**NUL-RES**		**NC*NR**
50	0.53	0.02	50	0.55	0.02	0.47
60	0.64	0.02	60	0.62	0.02	0.56
70	0.66	0.02	70	0.68	0.02	0.54
80	0.70	0.02	80	0.65	0.02	0.17
90	0.64	0.02	90	0.62	0.02	0.44
100	0.61	0.02	100	0.63	0.02	0.59
110	0.62	0.02	110	0.57	0.02	0.13
	MUL-CON			**NUL-CON**		**MC*NC**
50	0.59	0.02	50	0.53	0.02	0.04**
60	0.62	0.02	60	0.64	0.02	0.50
70	0.64	0.02	70	0.66	0.02	0.36
80	0.68	0.02	80	0.70	0.02	0.48
90	0.67	0.02	90	0.64	0.02	0.40
100	0.67	0.02	100	0.61	0.02	0.07*
110	0.59	0.02	110	0.62	0.02	0.34
	MUL-RES			**NUL-RES**		**MR*NR**
50	0.56	0.02	50	0.55	0.02	0.82
60	0.64	0.02	60	0.62	0.02	0.52
70	0.61	0.02	70	0.68	0.02	0.02**
80	0.66	0.02	80	0.65	0.02	0.73
90	0.68	0.02	90	0.62	0.02	0.03**
100	0.65	0.02	100	0.63	0.02	0.42
110	0.59	0.02	110	0.57	0.02	0.57

Table B.5. Hematocrit, means separation

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	CON			**RES**		**CON*RES**
Hematocrit (%).						
50	32.83	0.91	50	34.80	0.91	0.13
60	34.18	0.92	60	37.29	0.91	0.02**
70	34.75	0.91	70	36.42	0.91	0.20
80	35.36	0.92	80	36.47	0.91	0.39
90	35.14	0.91	90	34.69	0.91	0.72
100	34.70	0.92	100	34.58	0.94	0.93
110	34.74	0.91	110	33.77	0.91	0.45
120	36.34	0.91	120	35.21	0.91	0.38
130	33.84	0.97	130	32.76	0.95	0.43
	MUL			**NUL**		**MUL*NUL**
50	32.91	0.86	50	34.72	1.00	0.18
60	34.80	0.87	60	36.68	1.00	0.17
70	36.37	0.86	70	34.80	1.00	0.25
80	35.83	0.87	80	36.00	1.00	0.90
90	33.90	0.86	90	35.93	1.00	0.14
100	34.06	0.87	100	35.22	1.02	0.40
110	33.85	0.86	110	34.66	1.00	0.55
120	36.54	0.86	120	35.02	1.00	0.26
130	33.68	0.90	130	32.91	1.04	0.59
	MUL-CON			**MUL-RES**		**MC*MR**
50	31.47	1.21	50	34.35	1.19	0.09*
60	33.84	1.25	60	35.76	1.19	0.27
70	35.06	1.21	70	37.68	1.19	0.12
80	34.69	1.25	80	36.96	1.19	0.19
90	33.92	1.21	90	33.88	1.19	0.98
100	34.55	1.25	100	33.57	1.19	0.57
110	34.52	1.21	110	33.17	1.19	0.43
120	37.24	1.21	120	35.84	1.19	0.41
130	34.23	1.30	130	33.13	1.24	0.54
	NUL-CON			**NUL-RES**		**NC*NR**
50	34.19	1.40	50	35.25	1.39	0.59
60	34.53	1.40	60	38.83	1.39	0.03**
70	34.43	1.40	70	35.16	1.39	0.71
80	36.03	1.40	80	35.98	1.39	0.98
90	36.36	1.40	90	35.49	1.39	0.66
100	34.85	1.40	100	35.60	1.45	0.71
110	34.96	1.40	110	34.36	1.39	0.76
120	35.45	1.40	120	34.58	1.39	0.66
130	33.44	1.47	130	32.39	1.45	0.61
	MUL-CON			**NUL-CON**		**MC*NC**
50	31.47	1.21	50	34.19	1.40	0.15
60	33.84	1.25	60	34.53	1.40	0.72
70	35.06	1.21	70	34.43	1.40	0.74
80	34.69	1.25	80	36.03	1.40	0.49

Table B.5. Hematocrit, means separation (continued)

DAY	MEAN	ST.E.	DAY	MEAN	ST.E.	*P*
	MUL-CON			**NUL-CON**		**MC*NC**
90	33.92	1.21	90	36.36	1.40	0.20
100	34.55	1.25	100	34.85	1.40	0.87
110	34.52	1.21	110	34.96	1.40	0.82
120	37.24	1.21	120	35.45	1.40	0.35
130	34.23	1.30	130	33.44	1.47	0.69
	MUL-RES			**NUL-RES**		**MR*NR**
50	34.35	1.19	50	35.25	1.39	0.62
60	35.76	1.19	60	38.83	1.39	0.10*
70	37.68	1.19	70	35.16	1.39	0.18
80	36.96	1.19	80	35.98	1.39	0.60
90	33.88	1.19	90	35.49	1.39	0.39
100	33.57	1.19	100	35.60	1.45	0.29
110	33.17	1.19	110	34.36	1.39	0.52
120	35.84	1.19	120	34.58	1.39	0.50
130	33.13	1.24	130	32.39	1.45	0.70