Spring 5-11-2013

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May 2013
College of Agriculture and Natural Resources
Department of Animal Science
Honors Thesis

Acknowledgements

I would like to express my appreciation to Dr. Zinn for his support and guidance both in the laboratory and in writing this thesis. I would like to acknowledge Dr. Govoni for acting as an additional mentor during this process. My special thanks are extended to Kristen Peck and Michelle Forella for their contributions to this project. I would also like to thank the members of the Sheep Project for assistance with sample collection.

I am particularly grateful to the Office of Undergraduate Research for the Summer Undergraduate Research Fund (SURF) grant, which allowed me to work on this project over the summer.

Finally, I wish to thank my parents for their un-ending support throughout this study.

Introduction

Growth of an animal results from the interaction of its genetic potential with environmental conditions (Bauer et al., 1995). The environment, in regards to fetal growth, consists of many variables. This study focuses on in-utero conditions, which largely depend on placental transfer (Longtine et al., 2011). Normal placental growth, which is an important precursor to adequate placental function, is essential, as the placenta is responsible for the transfer of all nutrients, including oxygen, from the mother to the fetus (Longtine et al., 2011). Specifically, placental insufficiency results in reduced nutrient transfer, increased potential for hypoxic conditions, and increased occurrence of intrauterine growth retardation (IUGR) of the fetus (Bell and Ehrhardt, 2002; Gluckman and Hanson, 2004; Wallace et al., 2004). However, if nutrients are not plentiful in maternal circulation, or altered concentrations of hormones are present, fetal growth can still be hindered (Wu et al., 2006).

Intrauterine Growth Retardation

Intrauterine growth retardation is defined as impaired growth and development of the mammalian embryo/fetus or its organs during pregnancy (Wu et al., 2006). According to Wu et al (2006), from a statistical standpoint, an organism must weigh at least two standard deviations less than the mean to be considered IUGR. It results in smaller offspring and negative long-term consequences in the offspring such as insulin resistance, Type-2 diabetes, obesity, hypertension, altered organ size, and poor meat quality due to an increased ratio of adipose tissue to muscle tissue (Ong et al., 2000; Wu et al., 2006; Tosh et al., 2010).

In the livestock industry, this results in animals with poor meat quality, with increased adipose tissue accretion at the expense of muscle tissue (Boersma and Wit, 1997). This produces a product that is less healthy, and less palatable, and therefore is less in demand by the consumer. Additionally, the cost to raise this animal to slaughter is greater, as its feed efficiency, or the amount of feed required for the animal to gain muscle, is reduced. There are also implications of IUGR in humans. Metabolic syndrome is prevalent, and perhaps maternal diet during gestation is responsible. It is hypothesized that these symptoms of metabolic syndrome stem from modifications made to the somatotropic axis, the hormonal system responsible for the regulation of growth, and modifications to other metabolic pathways, to enhance fetal survival (Thorn et al., 2011).

The focus of my research was on IUGR as a result of poor maternal nutrition, due to both under-feeding and over-feeding of the mother. Sheep were used as a model to study concerns to both the livestock industry and to human health. As sheep are ruminants, they model the effects to production animals. Before weaning, however, the rumen is not fully developed, and the sheep can model effects of a monogastric animal, such as humans.

Vonnahme et al. (2003) used sheep as a model, and determined that nutrient restriction of 50% of the nutrient requirements suggested by the National Research Council (National Research Council, 1985) for a pregnant ewe from days 28 to 78 of gestation results in IUGR of the fetus, as displayed by a 105 g decrease in fetal weight on day 78 of development. Over-nutrition of the ewes also imparted negative effects. Over-nourished ewes had a placenta that was reduced in size by 40%, and in addition, carried lambs of low-birth-weight from 128 days of gestation to term (Wallace et al., 1996, 1997,

2000). This suggests that the over-nourished, pregnant ewe partitions nutrients toward maternal fat deposition, rather than toward placental and fetal development (Wallace et al., 2000).

In this literature review, I will discuss the somatotropic axis, insulin signaling pathways, as well as the leptin pathway during normal fetal development and postnatal growth. I will also be discussing the role each pathway may have in the phenomenon of IUGR, and the specifics of these hypotheses.

The Somatotropic Axis

The somatotropic axis includes hormones secreted from the hypothalamus, the anterior pituitary gland, the liver, and other body tissues as well as their receptors; it is responsible for the regulation of growth (Renaville et al., 2002). Growth hormone releasing hormone (GHRH) is released from the hypothalamus in response to reduced circulating concentrations of growth hormone (GH), a 191 amino acid protein hormone (Secci and Borromeo, 1997). Growth hormone is secreted in a constant pulsatile manner from the anterior pituitary gland (Zinn et al., 1994; Tuggle and Trenkle, 1996). Somatostatin (SRIF), on the other hand, is released from the hypothalamus in response to an excess of circulating GH; together, these act as a negative feedback loop to maintain homeostasis in circulating GH and insulin-like growth factor -1 (IGF-1) concentrations (Zinn et al., 1994; Tuggle and Trenkle, 1996).

Growth hormone has both direct and indirect effects on body tissues. It directly results in triglyceride breakdown, and prevention of lipid accumulation in adipocytes (Garten et al., 2012). Its actions are mediated through binding to GH receptors (GHR), which bind to cell-surface receptors of the tyrosine kinase JAK2 families, to initiate its

numerous cellular functions (Baumann, 1994; Wojcik et al., 1995; Renaville et al., 2002). Increased GH concentrations also cause the liver and local tissues to release IGF-1 (Bloomfield et al., 2006), which acts to directly stimulate growth of target tissues bone and muscle, while acting as a negative control of GH (Yakar et al., 1999). Insulin-like growth factor-1 also indirectly inhibits GH by stimulation of SRIF secretion (Zinn et al., 1994; Tuggle and Trenkle, 1996).

Placental Lactogen

During gestation, maternal GH is gradually replaced by placental (p)GH (Igout et al., 1995). Placental GH is responsible for reducing maternal insulin sensitivity by 45 to 70%, which serves to prevent normal nutrient partitioning to maintain the fetal nutrient supply (Freemark, 2006). This means that insulin resistance is induced by pGH to ensure that not all maternal nutrients are transported into cells, but that enough are left in circulation to support a growing fetus (Newbern and Freemark, 2011). The increased nutrient transfer increases fetal insulin and IGF-1 concentrations, which promote fetal growth (Murphy et al., 2006). It does not appear that pGH is regulated by GHRH, but is secreted tonically (Baumann, 2009). Mannik et al. (2010) found decreased mRNA transcript for pGH associated with infants born small for gestational age (SGA), suggested dysregulation of the somatropic axis. Placental GH has not been found in the fetal circulation (Newbern and Freemark, 2011).

IGF-1

Insulin-like growth factor-1 is a 70 amino acid protein (Rinderknecht and Humbrel, 1978). Growth is stimulated when IGF-1 and IGF-2 bind to the IGF-1 receptor, which stimulates bone and muscle development through a tyrosine kinase signaling

cascade (Liu et al., 1993). Growth hormone directly stimulates prechondrocyte production while IGF-1 is directly responsible for development of new bone (Isaksson et al., 1987). Therefore, only IGF-1 and GH together result in normal bone growth. There is a similar mechanism of action in muscle accretion; GH stimulates amino acid uptake, the precursor to muscle accretion, while IGF-1 is responsible for actual protein synthesis (Norrelund, 2005).

For growth to occur, cooperation between GH and IGF-1 is required. It was found that IGF-1-null mice experience 40% reduced growth, whereas IGF-1 receptor-null mice experience 55% reduced growth, reduced organ size, reduced muscle development, and delayed bone development; they are not viable and die of respiratory failure (Liu et al., 1993). Inactive GH receptors result in a lack of production of IGF-1, causing Laron dwarfism (Laron et al., 1966) whereas overproduction of GH, and therefore IGF-1 is the cause of acromegaly (Melmed et al., 1983). The discrepancy in the amount of growth reduction means that GH is capable of inducing some growth on its own, but cooperation with IGF-1 is required for normal growth. Mice lacking only hepatic IGF-1, however, experience normal growth, which confirms the importance of the autrine/paracrine actions of local IGF-1 (Yakar et al., 1999).

Insulin-like growth factor-1 can also bind to the insulin receptor due to their structures being 70% homologous, and can stimulate insulin-like actions (Rinderknecht and Humbel, 1978; Zapf et al., 1986). These actions include stimulation of glucose transport via translocation of the GLUT4 glucose transporter to the cell surface (Zapf et al., 1986). The GLUT4 transporter is responsible for the uptake of glucose from the circulation into the cell. IGF-2 can also induce growth through the AKT and MAP-kinase

pathway by binding to the insulin receptor (Somwar et al., 2001). Postnatally, GH and IGF-1 are the ligands primarily responsible for growth, but IGF-2, which is responsive to PGH, rather than fetal GH, is the hormone responsible for fetal development (Gluckman and Pinal, 2003).

IGF Binding Proteins (IGFBP)

The IGFBP are a family of binding proteins produced by the liver (Govoni et al., 2005), that bind to IGF and modulate its activity (Breier, 1999). Insulin-like growth factor binding proteins -1, -2, -4, and -6 are known to inhibit the actions of IGF-1, and restrict growth by binding to IGF to prevent binding to receptors (Govoni et al., 2005). In contrast, IGF-3 and -5 are known to stimulate the action of IGF-1, and promote growth by binding in a ternary complex with an acid labile subunit (Govoni et al., 2005; Sabin et al., 2011). Osteoporosis, or reduced bone mineral density (BMD), is associated with an increase in inhibitory proteins, IGFBP-1, -2, -4, and -6, and a decrease in stimulatory proteins, IGFBP-3, and -5, as well as IGF-1 (Jehle et al., 2003).

The association with IUGR and the IGFBP lies in part, in the availability of IGF. The IGFBP have a greater binding affinity for IGF than do the IGF receptors, and only a small amount of circulating IGF is unbound and bioactive (Kawai and Rosen, 2012). In offspring born to nutrient-restricted or overfed mothers, it is expected that reduced growth, would predominate as a response to reduced IGF-1. However, increased concentrations of the inhibitory IGFBP would also work to reduce the amount of bioactive IGF, resulting in a similar outcome.

Insulin-like growth factor binding protein-3 also has IGF-independent actions. It can translocate to the nuclear membrane and bind to retinoid X receptor α (RXRα) and peroxisome proliferator-activated receptor γ (PPARγ), both of which modulate adipocyte differentiation (Chan et al., 2009). This inhibits the differentiation of adipocytes, preventing their maturation (Chan et al., 2009). Insulin-like growth factor binding protein-3 reduces insulin-stimulated glucose uptake by reducing glucose uptake by GLUT4 transporters, in part by inhibiting GLUT4 transporter translocation (Chan et al., 2005). Insulin resistance is also induced by a mutant form of IGFBP-3 with an altered nuclear localization sequence (NLS), that prevents it from being able to tanslocate to the nucleus or bind to RXRα (Chan et al., 2005). All of this results in insulin resistance and impaired glucose when IGFBP-3 is overexpressed (Silha et al., 2002).

During fetal development IGFBP-2 is the IGFBP primarily expressed (Shimasaki and Ling, 1991). However, postpartum IGFBP-2 concentrations are inversely related to body mass index (Mattsson et al., 2008). Therefore, it is inversely correlated with adiposity-related insulin resistance, or metabolic syndrome (Heald et al., 2006).

Our research focuses on IGFBP-2 and -3 because they have been noted to be impacted by both nutrition and stage of development in ruminants (Breier, 1999; Govoni et al., 2003).

Other Affected Metabolic Pathways

Other metabolic pathways affected by IUGR include glucose metabolism and leptin signaling. This alteration could be due to altered receptor number, altered metabolite concentration, or numerous other variables such as mRNA expression and epigenetic modifications.

The body responds to increased blood glucose concentrations by secreting insulin from pancreatic β cells (Yuan et al., 2011). Insulin aids in the facilitated diffusion of glucose from the blood into the cells by GLUT transporters; this provides energy for cellular respiration and keeps blood glucose concentrations relatively stable (Zapf et al., 1986). Insulin also works to stimulate glycogenesis, the process by which glucose is converted to glycogen, a compound that can be stored in the liver for later energy conversion (Zapf et al., 1986).

Leptin is a 16kDa protein secreted by adipose tissue and coded for the the *ob* gene (Zhang et al., 1994). Leptin acts on the hypothalamus to suppress appetite, inhibit feed intake, and increase energy expenditure (Yura et al., 2005). In ruminants, receptors are specifically expressed in the hypothalamus, anterior pituitary gland, and adipose tissue (Dyer et al., 1997). In well-fed ewes, there are fewer hypothalamic receptors for leptin expressed than in nutrient-restricted ewes (Dyer et al., 1997). It was also determined that plasma leptin concentration was positively correlated to body weight, body condition score, and adiposity, so it is therefore primarily known as a marker of organism adiposity (Delavaud et al., 2000). Thus, increased circulating leptin, either as a function of fewer receptors or as a function of leptin resistance is associated with obesity. This holds true for humans as well; infants born SGA who experience postnatal compensatory growth have greater total body fat, and increased circulating leptin concentrations as adults, than infants born at a normal weight (Phillips et al., 1999).

Nutrition-Induced Changes to Regulation of Growth and Metabolism

When a gestating mother is in a period of nutritional stress, IGF-1 concentrations decrease, resulting in increased pGH concentrations (Baumann, 2009); this results in

nutrient partitioning in favor of the mother, and a fetus with decreased circulating IGF-1 and reduced growth (Sjogren et al., 1999; Yakar et al., 1999; Tosh et al., 2010). Fetal nutrition increases both fetal IGF and fetal insulin, and the concentrations of the two are correlated (Baumann, 2009). Fetal pancreatectomy resulted in significantly reduced plasma IGF-1, while simultaneously increasing IGF-2 activity (Gluckman et al., 1987). Concentrations of GH are increased in response to reduced IGF-1 concentrations, as is consistent with a negative feedback loop (Giustina et al., 2008). IGF-1 concentrations are also positively correlated with BMD, so when IGF-1 is reduced, BMD should be reduced as well (Mohan et al., 2003; Govoni et al., 2005).

Over-nutrition of adolescent ewes can also lead to IUGR in their offspring (Wallace et al., 1996, 2004). This occurs through nutrient partitioning away from both placental and fetal growth, and toward maternal growth, resulting in reduced placental and fetal size (Wallace et al., 2004; Lea et al., 2005). Lea et al. (2005) found that on day 81 of gestation, overnourished adolescent ewes exhibited reduced placental growth, and increased level of the pro-apoptotic protein bax, when compared with the control. This means that at day 81 of gestation, placental cells from overnourished adolescent ewes are not growing as they should be, due to an increased ratio of cells undergoing apoptosis, to proliferating cells.

Hypotheses of the Mechanism of Action

Changes to the pregnant mother result in changes in the fetus. There are three hypotheses suggesting possible mechanisms behind these fetal changes: the Fetal Insulin Hypothesis, the Thrifty Phenotype Hypothesis, and the Predictive Adaptive Response Hypothesis. The Fetal Insulin Hypothesis suggests that insulin resistance, glucose

intolerance, diabetes, and hypertension are all phenotypes of the same insulin-resistant genotype (Hattersley and Tooke, 1999). A mutation in the glucokinase gene of the fetus resulted in reduced birth weight, suggesting that a mutation in this same gene in the mother would result in reduced maternal insulin secretion, and therefore hyperglycemia (Hattersley et al., 1998). This would then lead to a period of increased fetal growth and insulin secretion postpartum (Byrne et al., 1994). These hypotheses were proven valid at birth, when it was determined that the effects of these mutations were additive, but Vehlo et al. (2000) determined that these effects did not persist into adulthood.

An alternative insulin-resistant genotype results from an absent allele of a polymorphism in the promoter region of the IGF-1 gene, resulting in reduced insulin-secreting capacity as well as in reduced circulating IGF-1 concentrations, reduced adult height, and increased risk of heart attack and Type-2 diabetes (Vaessen et al., 2001, 2002). Larnkjaer et al. (2009), however, found an inverse relationship between IGF-1 concentrations at 9 months of age and 17 years of age between breast-fed and formula-fed humans. A greater IGF-1 serum concentration at 9 months in formula-fed individuals corresponded to a reduced IGF-1 concentration at 17 years old (Larnkjaer et al., 2009). This finding, that the mutation fixes itself, suggests that the changes that occur in hormonal axes are due to metabolic programming, rather than genetic differences. This is especially interesting because human babies that were nursed had reduced risk of obesity later in life than formula-fed babies (von Kries et al., 2000), suggesting that the mother regulates intake of the offspring. The increased IGF-1 serum concentration at 9 months in the formula-fed individuals would therefore correspond with increased nutrients and increased fetal insulin. Thus, this model is similar to that of a nutrient-restricted fetus

being introduced to an environment of adequate nutrition, in terms of metabolic programming. This finding lends credence to the Thrifty Phenotype Hypothesis.

The Thrifty Phenotype Hypothesis is the more accepted hypothesis, and was originally asserted by Hales and Barker (1992). This hypothesis suggests that permanent changes in glucose-insulin metabolism stem from effects of poor nutrition during fetal and early life (Hales and Barker, 1992). Prenatal modifications to metabolism selectively alter the growth rates of body tissues, sparing nutrient availability for the brain at the expense of other organs, including the pancreas, kidneys, liver, and lungs (de Boo et al., 2008; Yuan et al., 2011; Novitskaya et al., 2011). The metabolic setting of these organs is also altered by IUGR, which essentially programs the fetus to thrive in an environment with poor nutrient availability (Barker, 1997). Thus, poor gestational nutrition enhances the ability of the fetus to absorb and use nutrients (Hales and Barker, 1992). Whereas these modifications enhance fetal survival, they lead to negative health consequences as they persist into adulthood (Wu et al., 2006). This is known as fetal programming, which is defined by Lucas (1991) as when a provocation at a vital stage of development results in changes that last the lifetime of the individual. It is also known as the developmental origins of health and disease (Barker, 1997). The somatotropic axis, as well as glucose metabolism and leptin signaling are all potentially reprogrammed during gestation by IUGR (Thorn et al., 2011).

The Predictive Adaptive Response Hypothesis was asserted by Gluckman and Hanson (2006). It is an offshoot of the Thrifty Phenotype Hypothesis and suggests that the maternal uterine environment serves as a prediction of the postnatal nutritional environment (Gluckman and Hanson, 2006). While the Thrifty Phenotype Hypothesis

stresses the negative consequences that IUGR has on long-term animal health, the Predictive Adaptive Response Hypothesis views fetal programming as advantageous to the offspring. Depending on the external environment, either interpretation can be correct.

Compensatory Growth

Due to the thrifty phenotype and fetal programming, IUGR newborns are smaller at birth, but tend to undergo rapid postnatal growth, known as compensatory growth, once adequate nutrition becomes available (Ong et al., 2000; Tosh et al., 2010). However, due to alterations made during fetal programming of metabolism, nutrient partitioning, and endocrine regulation, this rapid growth often causes obesity in adulthood in IUGR animals (Tosh et al., 2010; Thorn et al., 2011). This results in increased body fat, decreased lean muscle mass, increased insulin resistance, and eventually in diabetes (Wu et al., 2006). Compensatory growth is defined as a growth rate above that which is statistically normal, during a period of time immediately following growth inhibition (Boersma and Wit, 1997). As a result, the individual resumes its original growth trajectory from before the restriction. Compensatory growth essentially occurs through increased feed intake, which increases IGF-1 concentrations above normal (Boersma and Wit, 1997). Once the normal trajectory has been achieved, intake decreases, and IGF-1 concentrations resume to normal (Boersma and Wit, 1997). For this reason, in animals undergoing compensatory growth, body weight is inversely correlated to IGF-1 concentrations (Fall et al., 1995).

Compensatory growth also results in obesity and other metabolic disorders (Boersma and Wit, 1997; Wu et al., 2006; Bol et al., 2008). An animal programmed to

best absorb nutrients in an environment not conducive to growth will be programmed for obesity and reduced health when nutrition is plentiful (Bol et al., 2008; Setia and Sridhar, 2009). When nutrient restriction is maintained postpartum, compensatory growth is postponed, and the negative consequences associated with metabolic syndrome do not occur (Tosh et al., 2010). It was found that IUGR animals had similar concentrations of hepatic IGF-1 mRNA to the control group when nutrient restriction was maintained postpartum (Tosh et al., 2010). Therefore, restriction of postpartum nutrition and regulation of the rate of compensatory growth may prevent any epigenetic modifications related to IUGR that may lead to metabolic syndrome and its negative impacts (Tosh et al., 2010).

Changes to Fetal Growth and Metabolism

During gestation, a fetus of an underfed ewe experiences reduced circulating concentrations of insulin, IGF-1, IGF-2, and IGFBP-3, along with increased concentrations of GH and IGFBP-1 and -2 (Giudice et al., 1995; de Zegher et al., 1997; Renaville et al., 2002). At 9 months of age in IUGR rats fed ad libitum postpartum, it was noted that an increased concentrations of plasma IGF-1 were present (Tosh et al., 2010). In another study, Vonnahme et al., (2003) found that increased concentrations of IGF-1 coincided with reduced body weight and size at birth in lambs. However, Coupé et al., (2012) saw differences in size of rats similar to the Vonnahme group, yet this difference was lost by 5 months of age. This is in part due to the effects of compensatory growth (Boersma and Wit, 1997).

Changes in Gene Expression

Compensatory growth results in increased hepatic IGF-1 mRNA expression (Tosh et al., 2010). It is suggested that increased amounts of mRNA are due to IGF-1 histone and chromatin structure modifications to the methylation of the fourth lysine residue of histone three (H3K4; Tosh et al., 2010). This suggests that compensatory growth results in epigenetic changes to chromatin and histone structure. Additionally, there are two IGF-1 isoforms, which stem from alternative splicing of the six IGF-1 exons; fasting, diabetes, and development all result in different transcriptional start sites through epigenetic modifications (Adamo et al., 1991; Tosh et al., 2010). Therefore, epigenetic modifications to histone structure can alter access of transcriptional machinery to the DNA and could therefore cause differential transcription of isoforms, both in terms of start site and amount of mRNA transcribed (Cheung and Lau, 2005; Feil, 2006). For example, trimethylation of H3K4 has been established as a marker on promoter regions of active genes, whereas dimethylation is associated with promoting expression and recruitment of transcriptional machinery (Santos-Rosa et al., 2002; Cheung and Lau, 2005).

Rats exhibiting IUGR given adequate nutrition postpartum, displayed increased mRNA expression of both isoforms of IGF-1 at 9 months of age (Tosh et al., 2010). This is associated with decreased H3K4 dimethylation at the IGF-1 promoters 1 and 5, and increased H3K4 at untranslated region 4 (Tosh et al., 2010). However, IUGR rats with postpartum feed restriction only displayed increased mRNA expression of IGF-1B at 9 months of age (Tosh et al., 2010). This coincided with reduced H3K4 demethylation at IGF-1 promoter 1 and increased dimethylation at both promoter 2 and untranslated region

3 (Tosh et al., 2010). At birth, there was no significant difference in mRNA expression between treatment groups, but there was increased H3K4 dimethylation at promoter 1, exon 5, and untranslated region 3 in nutrient restricted rats at this time (Tosh et al., 2010).

It has been determined in the laboratory that *Cited 1* gene knockout mice develop late gestational placental insufficiency, as the *Cited 1* gene is responsible for trophoblast development (Rodriguez et al., 2004). This is one model of experimentally inducing IUGR in a fetus. *Cited 1* is X-linked, allowing researchers to distinguish placental from embryonic effects (Novitskaya et al., 2011). In the *Cited 1* knockout model, there was a reduction of fetal IGF-1 receptors (IGF-1R) as well as fetal insulin receptors in the kidneys, and a reduced number of insulin receptors in the lungs and liver of mice pups born to *Cited 1* knockout dams (Novitskaya et al., 2011). There was no change in IGF-1R in the brain, which means that the change in phosphorylation is due solely to placental insufficiency (Novitskaya et al., 2011). The change in phosphorylation in the kidneys and lungs was due to alterations of IGF-1 and -2 expression, while liver changes were associated with decreased pancreatic insulin secretion (Novitskaya et al., 2011). This model of inducing IUGR allows researchers to determine which effects of IUGR are due to solely placental insufficiency, and which have more compound causes

In the sheep model, placental restriction induced by caruncle removal increased peri-renal leptin expression, but not receptor expression at day 43 postpartum (De Blasio et al., 2010). Plasma leptin concentrations at day 40 in fed lambs correlated with per-renal leptin expression in both IUGR and control lambs, but with receptor expression only in IUGR lambs (De Blasio et al., 2010), suggesting dysregulation between circulating leptin concentrations and receptor amounts. Thus, these animals are primed

for hyperleptinemia, and leptin insensitivity. In fasted lambs, such a change was not noted (De Blasio et al., 2010). Peri-renal leptin expression was positively correlated with visceral fat mass, as a percentage of body weight in IUGR lambs (De Blasio et al., 2010). Plasma leptin concentration, however, correlated positively with total visceral fat mass, and with total visceral fat mass as a percentage of body weight (De Blasio et al., 2010). It is also interesting to note that in a 1.5 hour observation period on day 15, leptin concentration correlated negatively with the total number of suckling events in lambs born to control-fed ewes (De Blasio et al., 2010). In contrast, leptin concentration correlated positively with total suckling time in lambs that experienced placental restriction (De Blasio et al., 2010). When adjusted for body weight, leptin concentration also correlated positively with total number of suckling events in placentally-restricted lambs (De Blasio et al., 2010). This points to leptin insensitivity, and the possibility that these lambs will become obese later in life.

Bol et al. (2008) altered protein composition of the diet of rat dams during gestation, and induced compensatory growth in pups by reducing the number of pups per litter. The group compared adiposity of rats born to dams fed low a protein diet with rats born to control-fed dams during gestation (LP rats) and found an increased number of preadipocytes in LP animals, and increased *Cyclin D1* mRNA expression (Bol et al., 2008). The preadipocytes from male rat pups at four weeks of age were harvested and cultured; it was found that total DNA and protein within the preadipocytes of LP rats at days 4, 7, and 9 of culture was increased, suggesting altered adipocyte growth/development (Bol et al., 2008). However, no difference was noted at later days of culture (Bol et al., 2008). At this time, LP rats also displayed decreased mRNA

expression of PPARγ and SREBP-1 mRNA, which are regulators of adipocyte differentiation (Hausman et al., 2001; Bol et al., 2008). This makes sense, since differentiation and proliferation cannot occur simultaneously (Bol et al., 2008). These results suggest that at 4 weeks in vivo, LP rats are experiencing increased proliferation of preadipocytes, which will eventually mature into adipocytes, resulting increased total fat mass.

Insulin Resistance/Glucose Intolerance/Type-2 Diabetes

Offspring that are IUGR due to maternal feed restriction, experience reduced insulin concentrations while in-utero, which presents a lack of stimulus for the development of the insulin-receptor system (Hales and Barker, 1992; Guidice et al., 1995). Fewer insulin receptors results in reduced glucose clearance from the blood, and therefore increased circulating glucose (Guidice et al., 1995). It is suggested that the GLUT4 transporter, which is responsible for glucose uptake into cells, is also impaired (Jaquet et al., 2001). This is because in peri-renal fat of lambs energy-restricted in utero, proteins for insulin-receptor β (Irβ) and the p110β isofrm of P13-kinase in the phosphotidylinositol pathway were increased (Gardner et al., 2005). These proteins are responsible for translocating GLUT4 transporters to the cell surface of adipocytes, so theoretically, GLUT4 protein should be increased; however, GLUT4 protein was decreased (Gardner et al., 2005). In muscle, only the Irβ protein was significantly decreased (Gardner et al., 2005). This demonstrates dysregulation between signaling for GLUT4 protein in adipose tissue, and its expression. Additionally, GLUT4 expression and its mRNA expression in adipose tissue was not stimulated by the presence of circulating insulin in humans born IUGR, as it was in controls (Jaquet et al., 2001).

Impaired GLUT4 transporter function means that adipose tissue is not as capable to clear glucose from the circulation and bring it into the cell.

Yuan et al. (2011) reported that from birth to 15 weeks of age, both glucose tolerance and insulin sensitivity decreased in IUGR rats when compared with controls. In this experimental design, IUGR was induced by reducing maternal caloric intake by 50% from days 11 to 21 (mid-late) gestation (Yuan et al., 2011). At birth, the IUGR group exhibited increased glucose tolerance and increased insulin sensitivity, as the fetus was programmed to maximize nutrient uptake as a result of a poor gestational environment; however, with age, this trend reversed (Yuan et al., 2011). In a glucose tolerance test, in which glucose is injected into the blood stream at birth to determine the time it takes for glucose clearance and the magnitude of the insulin response, the IUGR group displayed increased blood glucose and insulin concentrations at 120 and 180 minutes after glucose injection (Yuan et al., 2011). However, by 15 weeks, this same test resulted in increased concentrations of both, compared with controls, after only 30 minutes (Yuan et al., 2011). At 15 weeks, IUGR rats responded to insulin injection with a 40% decrease in blood glucose concentration, compared with the 50% decrease exhibited by the control group, suggesting both decreased insulin response and decreased insulin sensitivity in IUGR rats (Yuan et al., 2011). The pancreas was harvested from animals of both treatments groups, and was found to weigh less as a percentage of body weight in the IUGR group than the control group. Long et al. (2009), however, did not observe this trend in cattle. The β cells of harvested pancreas were cultured and exposed to two different concentrations of glucose (Yuan et al., 2011). It was expected that there would be a greater insulin response to the larger concentration of glucose; however the IUGR group displayed significantly

reduced insulin response to the greater glucose concentration (Yuan et al., 2011). This suggests that in IUGR animals, pancreatic β cells were less developed, or less responsive than the control animals.

In lambs from ewes nutrient restricted to 50% NRC requirements from day 28 to 78 of gestation, baseline glucose concentrations at 1 day of age were significantly increased, but basal insulin was not different (Ford et al., 2007). However, at 250 days of age, baseline glucose and insulin concentrations did not differ between IUGR lambs and control lambs (Ford et al., 2007). Growth-restricted lambs did exhibit a decreased insulin area under the curve (AUC), and a greater AUC for glucose (Ford et al., 2007). This suggests reduced insulin sensitivity (Ford et al., 2007). At one year of age, lambs energy-restricted until day 30 of gestation, and in lambs from ewes underfed from 110 days of gestation to parturition, showed similar responses to glucose tolerance tests (Gardner et al., 2005). Maximal change in glucose and insulin concentrations and time to return to baseline glucose concentrations in response to a glucose challenge were similar as well (Gardner et al., 2005). Feed restriction to 50% NRC requirements of ewes during the last 37 days of gestation results in reduced insulin sensitivity in the offspring at 12 months of age (Gardner et al., 2005), because it took longer for the insulin concentration to return to baseline after a glucose challenge in these lambs, since less overall insulin was released, and less glucose was removed from the blood.

Obesity

Growth-restricted rats allowed to feed ad libitum exhibited rapid compensatory growth and increased circulating triglyceride concentrations at birth, but decreased concentrations of glucose, insulin, and triglycerides at three weeks (Desai et al., 2007).

By 9 months, these metabolites were all increased (Desai et al., 2007). Insulin deficiency and increased cholesterol were exhibited in rats allowed delayed compensatory growth by restricting feed postpartum (Desai et al., 2007). This programmed obesity is perhaps due to leptin resistance, as lambs from nutrient-restricted ewes (day 28 to 78 of gestation) had increased leptin concentrations (Ford et al., 2007). Leptin has a characteristic peak during development that has been found to program its own axis and sensitivity (Yura et al., 2005). In sheep, this peak is from day 6 to 9 postpartum (Long et al., 2011). In lambs from overfed ewes, however, this peak was from days 0 to 4 postpartum (Long et al., 2011).

In rats, offspring of obese dams have an amplified and prolonged leptin peak when compared with offspring from control dams (Kirk et al., 2009). In rats administered leptin before the time of the leptin peak, the number of leptin receptors was reduced in the hypothalamus (Toste et al., 2006). Fewer leptin receptors results in a reduced response to leptin in these rats, and could result in leptin resistance (Yura et al., 2005). IUGR rats experienced an earlier leptin surge than control rats and exhibited increased appetite despite increased circulating leptin (Vickers et al., 2000; Yura et al., 2005). Since the role of leptin is to decrease appetite, decrease feed intake, and increase activity, this finding suggests leptin resistance (Vickers et al., 2000; Yura et al., 2005).

Lambs with an abnormal leptin peak experienced an increase in appetite, similar to the behavior expressed by rats with reduced leptin receptors, and leptin insensitivity (Yura et al., 2005; Long et al., 2010, 2011). It is hypothesized that perhaps the early leptin peak is due to increased leptin mRNA in adipose tissue (Kirk et al., 2009). It is also possible that this is related to increased cortisol in obese sheep compared with the

controls. For example Long et al. (2011) reported obese ewes lambed approximately three days earlier than control ewes, but are not sure of the reason why. This link is suggested because cortisol normally increases during late gestation and at parturition, as does plasma leptin and peri-renal mRNA expression (Magyar et al., 1980; O'Connor et al., 2007). However, this increase in leptin and leptin mRNA expression does not occur when the adrenal glands are removed (O'Connor et al., 2007). Additionally, Mustoe et al. (2012) showed that in marmoset monkeys, infants born to mothers with increased gestational cortisol for the first trimester of gestation had reduced fetal growth rates. However, these high-cortisol infants had more rapid postnatal growth rates than control infants, and experienced complete compensatory growth by 540 days of age. Thus it is possible that elevated cortisol concentrations are responsible for some sort of fetal programming.

In rats from control-fed dams, circulating leptin was negatively correlated with feed consumption, suggesting the proper leptin response (Delavaud et al., 2000). Rats from nutrient-restricted mothers, however, had circulating leptin concentrations that were positively correlated with feed consumption, suggesting leptin insensitivity or resistance (Delavaud et al., 2000). During severe nutrient restriction, in the form of a two-day fast, back, omental, and peri-renal adipose tissue leptin mRNA was decreased in ruminants, suggesting a pre-translational regulation due to nutrient uptake (Kumar et al., 1998; Tsuchiya et al., 1998). Perhaps a similar response occurs during prolonged nutrient restriction while in utero.

Organ Size

Intrauterine growth retardation is also characterized by asymmetric organ growth.

Organs such as the pancreas, liver, kidney (reduced as a percentage of body weight), and lungs (reduced as a percentage of body weight) become reduced in size so that nutrient priority is given to the brain (de Boo et al., 2008; Novitskaya et al., 2011). The brain has a reduced absolute weight, but it is increased as a percentage of body weight (Novitskaya et al., 2011). Vonnahme et al. (2003) found that when corrected for body weight, liver weight and left and right heart ventricles were larger in IUGR lambs. This may be due to an increase in liver metabolic activity (Vonnahme et al., 2003). Ruminants receive glucose primarily via gluconeogenesis, and since IUGR results in reduced blood glucose concentration, gluconeogenic enzymes in the fetal liver are increased (Lemons et al., 1986; Vonnahme et al., 2003).

Hypertension

In addition to asymmetric heart growth, IUGR lambs have larger left and right ventricles of the heart, suggesting increased ventricular afterload, which requires the ventricle to overcome more force during contraction (Vonnahme et al., 2003). This can be due to either aortic or pulmonary artery impedence, peripheral vascular resistance, or viscosity of the blood (Vonnahme et al., 2003). In this case, Vonnahme et al. (2003) suggested that it was to be due to increased placental vascular resistance, as vascularity of the placenta did not change in IUGR lambs.

Growth-restricted lambs nutrient-restricted from early to mid-gestation have a reduced number of kidney nephrons, which is associated with hypertension as well (Gopalakrishnan et al., 2005). In cattle, Long et al. (2009) reported reduced glomerular number in bovines on day 245 of gestation in IUGR calves, despite similar kidney

weight, supporting the findings of Gopalakrishnan et al. (2005) that IUGR animals have fewer nephrons.

Change in Tissue Accretion

Most fetal development involves an increase in cell number, or hyperplasia, whereas postnatal development involves an increase in cell size, or hypertrophy. IUGR animals have a physiological limit to growth, that is less than their genetic potential due to fetal programming of the somatotropic axis (Boersma and Wit, 1997; Rehfeldt and Kuhn, 2006). Consequently, bone and muscle will not reach the normal trajectory in an IUGR animal with an altered somatotropic axis, even if postnatal compensatory growth does occur. Instead, the compensatory growth experienced, will result in an increase in size of adipose tissue, due to a change from normal nutrient partitioning once GH and IGF-1 concentrations decrease (Boersma and Wit, 1997). De Blasio et al. (2010) found increased peri-renal, retroperitoneal, and visceral fat in the carcasses of placentally restricted lambs when compared to the control.

Rehfeldt and Kuhn (2006) examined the effects of birth weight on postnatal myogenesis of pigs. In pigs, not only maternal nutrition, but also position in the uterine horn, is responsible for the amount of nutrients received (Rehfeldt and Kuhn, 2006; Wu et al., 2006). Since hyperplasia of myogenic cells is most prominent in prenatal growth, whereas hypertrophy occurs predominantly postnatally, this reduced number of myogenic cells limits animal growth for life. It was also determined that as the animals grew, piglets with low birth weights experienced supramaximal growth of individual muscle fibers, resulting in giant fibers, which grew beyond the parameters of normal growth (Rehfeldt and Kuhn, 2006). Therefore, at slaughter, the low birth-weight pigs had not

only a greater percentage of organ weight to body weight, as muscle growth was restricted, but also had a greater percentage of adipose tissue, and a reduced quality of meat (Rehfeldt and Kuhn, 2006).

Lambs from underfed ewes days 28 to 78 of gestation displayed increased back fat (detected via ultrasound) at 140 days of age (Ford et al., 2007). Additionally, while LM and semitendinosus muscle weights were similar for IUGR and control lambs, the ratio of LM and semitendinosus muscle weights to hot carcass weights was less in IUGR lambs (Ford et al., 2007).

Prevention and Reversal

Thus far, scientists have been unsuccessful in attempting to prevent or reverse IUGR through hormone treatments. However, de Boo et al. (2008) attempted to reverse IUGR in sheep by supplementing maternal GH through intramuscular injections. Three groups of sheep were used: one control, and two with growth restriction induced twice daily by embolization of the uterine arteries (de Boo et al., 2008). One of the IUGR groups was treated by GH injection, and the other served as a control and was treated with saline injection (de Boo et al., 2008). The IUGR induction reduced animal body weight, and increased the mass of the liver, heart, thyroid, and peri-renal fat deposits (de Boo et al., 2008). The GH treatment was able to improve fetal weight and overcome decreased chest girth, as well as most of the decreases in organ size (de Boo et al., 2008). However, even in GH-treated animals, the brain-to-liver weight ratio remained increased, suggesting that exogenous GH treatment was not able to fully normalize organ growth (de Boo et al., 2008). Additionally, some of the GH-supplemented lambs were found to have severe brain lesions, which may be due to hyperglycemia during gestation (de Boo

et al., 2008). Even though this experiment could not fully reverse IUGR, it was the first study to suggest that, with more research, it could be possible to use hormone treatments to prevent and reverse IUGR in animals (Setia and Sridhar, 2009).

Summary

The in-utero environment has lasting effects on offspring growth, specifically through alterations in metabolic hormone concentrations as well as in receptor number, and hormone response. The mechanisms by which these alterations occur are unknown. We are trying to understand the metabolic changes that occur in offspring, both longterm and short term as a result of poor maternal nutrition during gestation. This will hopefully lead to a better understanding of the mechanisms involved, and ways to avoid negative fetal programming in the future.

Objectives and Hypotheses

My objectives in performing this experiment were three-fold. The normal model in our laboratory when conducting a fetal programming experiment is to induce nutritional insult to the mother during gestation, and then remove one of her lambs at birth. The lamb is then bottle-fed until 60 days of age. For the first days postpartum, lambs must be fed every 3 hours, which requires a significant work force to ensure sufficient nutrition. It would be much more efficient to leave the lamb with the ewe to nurse, but there has not yet been a comparison of the differences between bottle-feeding and nursing the lambs in the response to nutritional insult to the ewe during gestation. Although less expensive and less time consuming, there is the potential for more variation in the offspring with nursing, including the nutritional quality of the milk from nursing mothers. Thus, the first objective was to compare models of postnatal nutrition for fetal programming experiments: bottle-feeding milk replacer (bottle lambs) compared with leaving the lamb to nurse from the ewe (suckling lambs).

The second objective was to determine the long-term effects of poor maternal nutrition during gestation, as far as one year after birth. Most of the research focuses on short-term effects (90 days of age), but for maintaining herd health, as well as human health, knowledge about long-term effects is essential.

The last objective was to determine if the ewes experienced any long-term affects of poor diet. If poor gestational diet affects the ewes long-term, than it has the potential to negatively impact the fetus she is carrying during the time of nutritional insult, but also has the potential to affect the health of her future offspring.

Our hypotheses were that there would be no difference in results between bottle-

fed lambs and suckling lambs. Additionally, we expected that lambs born to overfed and nutrient-restricted ewes would be smallerwith a slower growth rate than lambs born to control-fed ewes. In addition, these lambs would have alterations in concentrations of circulating hormones of the somatotropic axis (GH, IGF-1, IGFBP-2, and -3), and leptin. Lastly, we hypothesized that ewes would experience long-term effects of poor gestational diet.

Materials and Methods

For this study, 40 multiparous ewes from the UConn flock were synchronized for estrus by intravaginal controlled internal drug release (CIDR) with 300 mg progresterone for 11 days (EAZI-BREED CIDR, Pfizer). Upon removal, all ewes were given a single 20mg IM injection of PGF2α (Dixon et al., 2006). Pregnancy was later confirmed by ultrasound.

Of these ewes, 36 were selected to use for the study, and were housed in individual pens. There were 25 Dorsets, 7 Shropshires, and 4 Southdowns. These animals were acclimated to a control diet for 1 week. At 31 days of gestation, each ewe was randomly assigned to one of three diets (Vonnahme et al., 2010), 100%, 60%, or 140% of NRC 1985 recommendations (n=12 per treatment). The amount fed was based on ewe body weight. Leftover feed was removed every day, and weighed to determine daily feed intake. Animals were also given ad libitum water. Ewes were fed a complete pelleted feed from the Connecticut Farmers' Co-op, in Manchester, CT. Straw was also provided in the morning. Ewes were weighed, and the body condition score (BCS) was determined weekly (Russel, 1984).

These ewes gave birth to a total of 68 lambs. The average gestation length was 143.9 days; there was no effect of treatment on length of gestation. The ewe and her lambs were housed together in pens to allow for colostrum intake. All of the lambs received colostrum from the ewe; if they had not nursed by one hour after birth, lambs were intubated and given artificial colostrum. At 24 hours postpartum, 35 lambs were removed from the ewe. If two ewes were born to the ewe, the largest ewe lamb was removed from the mother. If one ewe and one ram were born, the ram was removed, and

if two rams were born, the largest ram was removed. Eighteen of the lambs that were removed from their mothers were randomly assigned to be euthanized by Beuthanasia IV injection (sodium pentobarbital 390 mg + sodium phenytoin 50 mg/ml) at 0.22 ml/kg at this time point (6C, 6R, 6O).

The remaining 17 lambs, 6 born to control-fed ewes, 6 born to overfed ewes, and 5 born to restricted ewes, that were removed from the ewe, were bottle fed Land O'Lakes milk replacer at 1.7% of their body weight, as well as ad libitum second cutting hay, and water. At 4 weeks, the start of weaning, BT creep feed was also given ad libitum. Body weight was measured at birth, and every two days until the lambs were weaned, and then every week until slaughter. Crown-rump length and heart girth were measured weekly. From birth until the lambs were heavier than 4 kg, 10 mL of blood was collected weekly via jugular venipuncture. Once the lambs were heavier than 4 kg, 20 mL of blood was collected weekly until slaughter. These lambs were slaughtered at 13 weeks of age. At slaughter, there were 6 lambs from control-fed ewes, 4 lambs from overfed ewes, and 4 lambs from restricted ewes.

Of the lambs born, 30 returned with the ewes as part of the UConn flock. There were 9 lambs born to both control-fed and overfed ewes, and 12 lambs born to restricted-fed ewes that were monitored as part of this study. From these lambs, body weight, crown-rump length, and heart girth were measured at birth, and then monthly for one year. 10 mL blood was collected at birth via jugular venipuncture, and then 20 mL were collected monthly for one year. By the end of the year, there were 4 lambs born to control-fed ewes, 4 lambs born to overfed ewes, and 6 lambs born to restricted ewes.

After parturition, all 36 ewes were returned to the flock to be maintained for another breeding cycle. Body weight was measured at parturition, and then monthly for 12 months. At these same time points, 20 ml of blood was collected as well. By the end of the 12 months, 20 ewes remained: 5 control, 9 restricted, and 6 overfed. Ewes were removed from the study if their lamb was sent to auction before it was fully weaned.

Blood was collected into 3 different types of tubes: 14 mL was collected into uncoated blood tubes, allowed to stand at room temperature for 3 hours to clot, and then refrigerated overnight at 4°C before centrifugation. These tubes were used for serum collection. An additional 3 ml was collected into a tube coated with heparin, and the remaining 3 ml was collected into a tube coated with EDTA. Both of these tubes were immediately placed on ice, and centrifuged upon return to the laboratory. These tubes were used for plasma collection. Blood samples were centrifuged for 30 minutes at 1,200 *x g* at 4°C, and serum and plasma were isolated and frozen at -20°C until used. Serum GH (Kazmer et al., 1992), and IGF-1 (Govoni et al., 2002), concentrations were quantified (ng/ml) by radioimmunoassay (RIA). Plasma leptin concentrations were quantified (ng/ml) by Millipore Multispecies Leptin RIA Kit (Long et al., 2010). IGFBP-2 and IGFBP-3 were quantified by Western Blot (Freake et al., 2001; Govoni et al., 2002). Proteins were separated using SDS-PAGE, with serum concentration at 1 μL per well in the gel. Proteins were then transferred to a nitrocellulose membrane (BioRad Laboratories, Hercules, CA), and incubated overnight with 300,000 cpm of radioactive ^{125} I-IGF-1 (Perkin Elmer, Shelton, CT). Membranes were then washed to remove any unbound IGF-1, and the blot was imaged after membrane incubation with a multipurpose phosphor screen (Packard Instrument Company, Meriden, CT). The remaining bound

radioactivity was imaged using a Cyclone Storage Phosphor system (Packard Instrument Company), and were quantified using OptiQuant software. Digital light units were quantified, and values were expressed relative to a bovine standard included on every gel as a control.

Growth rate was determined by subtracting the body weight at birth from the body weight at 9 months of age, and dividing by the number of days of age. Lontitudinal measurements of body weight, crown-rump length, heart girth, serum GH, IGF-1, and IGFBP-2, and -3, and leptin were analyzed statistically as repeated measures, using a model that accounted for repeated samples from the same experimental unit, with the mixed model analysis of variance procedure (SAS Inst. Inc., Cary, NC). Multiple methods of analysis were used, but the final method was chosen as best fit for the data set based on the smallest AIC value. Differences were considered significant if $P < 0.05$ and were considered a trend if $0.05 < P < 0.1$.

Results

Suckling vs. Bottle-fed Lambs

Over time, all lambs increased body weight from 4.5 ± 0.21 kg at birth to 34.7 ± 0.68 kg at 3 months. At birth, the largest lamb was removed from the ewe and bottle-fed, so bottle lambs (n=17) weighed more at birth than suckling lambs (n=30); (5.0 ± 0.17 kg vs. 4.3 ± 0.13 kg; $P < 0.01$). By 1 month the suckling lambs weighed significantly more than the bottle lambs (15.4 ± 0.59 kg vs. 10.7 ± 0.75 kg; $P < 0.01$). This greater body weight was maintained (36.6 ± 0.56 kg vs. 30.8 ± 0.73 kg; $P < 0.01$) through 3 months of age (Table 1, Figure 1).

All lambs increased in size gaining approximately 39.3 ± 1.0 cm in CRL, and 38.9 ± 0.7 cm in heart girth over 3 months. Based on selection criteria, the bottle lambs had a larger CRL (48.2 ± 0.9 cm) at birth than the suckling lambs (47.4 ± 0.8 cm), although the difference was not statistically significant ($P = 0.49$). At 1 month, the suckling lambs were significantly larger than the bottle lambs (70.9 ± 0.8 cm vs. 61.3 ± 0.9 cm; $P < 0.01$). At 3 months of age, the difference in CRL was reduced to almost 5 cm but remained significant (89.1 ± 0.8 vs. 83.7 ± 1.1 cm; $P < 0.01$); (Table 2, Figure 2). Bottle lambs had a heart girth that was significantly larger than the suckling lambs (39.6 ± 0.6 cm vs. 37.6 ± 0.4 cm; $P < 0.01$) at birth, but by 1 month of age, the suckling lambs had a significantly larger heart girth (59.4 ± 0.7 vs. 50.7 ± 0.9 cm; $P < 0.01$). By 3 months, the difference in heart girth was reduced (78.7 ± 0.8 cm vs. 74.5 ± 0.9 cm; $P < 0.01$), however, the suckling lambs remained significantly larger (Table 3, Figure 3).

At birth, the suckling lambs and bottle lambs had similar concentrations of circulating IGF-1 (109 ± 14.9 ng/ml vs. 133.0 ± 19.1 ng/ml, respectively; $P = 0.33$), as

this was before treatment implementation. At 1 month, the suckling lambs had a significantly greater ($P < 0.01$) IGF-1 concentration (280.7 ± 22.9 ng/ml) than the bottle lambs (64.9 ± 28.4 ng/ml). At 2 and three months, there was no significant difference in suckling and bottle lamb IGF-1 concentration (231.5 ± 22.9 ng/ml vs. 173.3 ± 28.7 ng/ml, and 211 ± 19.9 ng/ml vs. 177.9 ± 26.5 ng/ml; $P = 0.27$ and 0.72 respectively).

All lambs experienced a decline in GH concentration with age, with suckling lambs going from 13.4 ± 3.2 ng/ml at birth to 5.5 ± 3.1 ng/ml at 3 months, and bottle lambs going from 11.1 ± 3.4 ng/ml at birth to 3.8 ± 3.4 ng/ml at 3 months. There was no difference in circulating GH concentration between bottle lambs and suckling lambs ($P = 0.698$; Table 5, Figure 5).

Bottle lambs tended to have less circulating IGFBP-3 (111.71 ± 7.3 AU) than suckling lambs (130.77 ± 6.4 AU; $P = 0.0633$). At birth, IGFBP-3 concentrations are similar, as expected, but at 1 month, suckling lambs have a significantly increased IGFBP-3 concentration ($P < 0.01$; Table 6, Figure 6). At 2 months, after the start of weaning, bottle lambs have more IGFBP-3 than suckling lambs, although not significant ($P = 0.373$).

There was no significant difference in bottle and suckling lamb leptin concentration through 3 months of age ($P = 0.58$). At birth, bottle lambs had more circulating leptin than suckling lambs (1.5 ± 0.2 ng/ml vs. 1.2 ± 0.2 ng/ml). By 3 months of age, the suckling lambs had a greater circulating leptin concentration compared with the bottle lambs (1.3 ± 0.2 ng/ml vs. 1.1 ± 0.2 ng/ml; $P = 0.23$; Table 7, Figure 7).

Suckling Lambs Long-Term

All suckling lambs increased in body weight from approximately 4.3 ± 1.5 kg at birth to 68.3 ± 2.0 kg at 9 months of age. There was no difference in suckling lamb body weight over time (CON vs. RES $P = 0.36$; CON vs. OVER $P = 0.96$), with lambs born to ewes of all 3 treatment groups gaining an average of 0.24 kg/day. At birth, lambs born to control-fed, overfed, and restricted ewes (abbreviated CON, RES, and OVER lambs) weighed 4.4 ± 1.6 kg, 4.0 ± 1.4 kg, and 4.6 ± 1.6 kg respectively (CON vs. RES, $P = 0.92$; CON vs. OVER, $P = 0.88$). At 9 months, these lambs weighed 69.2 ± 1.9 kg, 66.4 ± 1.8 kg, and 70.4 ± 2.1 kg (CON vs. RES $P = 0.66$ and CON vs. OVER $P = 0.29$) (Table 8, Figure 8).

Over the 9-month period, all suckling lambs also increased in size, gaining approximately 63.1 ± 2.8 cm in CRL, and 67.3 ± 1.5 cm in heart girth. There was no significant difference in CRL between CON, RES, and OVER lambs from birth to 9 months of age. ($P = 0.6374$, OVER vs. CON; $P = 0.9432$, for RES vs. CON); (Table 9, Figure 9). Similarly, there was no significant difference in heart girth between OVER and CON lambs ($P = 0.1622$) or between RES and CON lambs ($P = 0.4491$); (Table 10 Figure 10). However, at 9 months, Over lambs have a significantly larger ($P < 0.01$) heart girth compared with Con lambs (108.9 ± 1.8 cm vs. 102.8 ± 2.0 cm).

Additionally, over time, there was no difference in circulating IGF-1 concentrations between OVER and CON lambs (144.3 ± 15.4 ng/ml vs. 136.3 ± 14.5 ng/ml; $P = 0.71$) or between RES and CON lambs (133.4 ± 13.2 ng/ml vs. 136.3 ± 14.5 ng/ml; $P = 0.88$; Table 11, Figure 11). At 1 month, CON lambs have a reduced IGF-1 concentration compared with both OVER and RES lambs (253.2 ± 28.1 ng/ml vs. 323.2 ±

40.5 ng/ml and 270.8 ± 37.9 ng/ml), which is interesting because at birth, CON lambs had the highest IGF-1 concentration. This difference is not significant ($P = 0.21$ and 0.74). Additionally, at 2 months, OVER lambs had a greater IGF-1 concentration compared with CON lambs ($P < 0.01$). This suggests possible increased intake in OVER and RES lambs, and therefore compensatory growth.

All suckling lambs experienced an age-related decrease in circulating GH concentration. However, there was no significant difference in circulating lamb GH concentration between OVER and CON ($P = 0.9591$) or between RES and CON ($P = 0.7984$) lambs at any time point (Table 12, Figure 12).

OVER lambs had significantly more IGFBP-3 than CON lambs (167.4 ± 7.3 AU vs. 137.7 ± 6.6 AU; $P < 0.05$), but there was no difference between RES and CON lambs (122.2 ± 6.37 AU vs. 137.7 ± 6.6 AU; $P = 0.1063$). At 6 months, there was a trend for RES lambs to have less IGFBP-3 than CON lambs (128.1 ± 19.8 AU vs. 184.6 ± 22.2 AU; $P < 0.1$), and at 9 months, RES lambs had significantly less IGFBP-3 than CON lambs (116.4 ± 11.8 AU vs. 155.7 ± 13.3 AU; $P < 0.05$) (Table 13, Figure 13). Although at birth, RES lambs tended to have less IGFBP-2 than CON lambs ($P < 0.1$), there was no difference in circulating IGFBP-2 among all lambs over time (16.5 ± 1.4 AU vs. 18.9 ± 1.6 AU; $P = 0.2979$ for CON vs. RES; 17.4 ± 1.7 vs. 18.9 ± 1.6 AU; $P = 0.5364$ for CON vs. OVER; Table 14, Figure 14). All lambs experienced an age-related decrease in IGFBP-2.

There was no difference in circulating lamb leptin concentration between OVER and CON ($P = 0.6635$) or between RES and CON ($P = 0.1651$) lambs (Fig. 12). All lambs experienced a leptin peak around 5 months of age, at which point there was a trend

for RES lambs to have less circulating leptin than CON lambs (2.8 ± 0.3 ng/ml vs. 2.1 ± 0.3 ng/ml; $P < 0.1$) At 6 months, there was no difference in lamb leptin concentration (Table 15, Figure 15).

Ewes

At birth, RES ewes weighed significantly less than CON ewes (99.6 ± 3.6 kg vs. 113.8 ± 3.5 kg; $P = 0.01$). Once ewes were returned to the flock, 3 days after parturition, they were all maintained on a control diet. Even so, OVER ewes maintained a 5 kg weight increase OVER the 9 month period, and RES ewes maintained a 7 kg weight decrease. There was no significant difference between these body weight differences ($P = 0.3442$ for CON vs. OVER, and 0.1358 for CON vs. RES; Table 16, Figure 16).

Over time, OVER ewes had significantly more circulating IGF-1 than CON ewes (194.19 ± 129.9 ng/ml vs. 130.1 ± 102.5 ng/ml; $P < 0.05$), and there was a trend for RES ewes to have less circulating IGF-1 than CON ewes at birth, 1 month, and 5 months ($P < 0.1$). However, over time, there was no difference between RES and CON ewe IGF-1 concentration (122.29 ± 61.9 ng/ml vs. 130.1 ± 102.5 ng/ml; $P = 0.12$; Table 17, Figure 17).

There was no significant difference in circulating GH concentration between OVER and CON ewes (2.9 ± 0.9 ng/ml vs. 1.9 ± 0.9 ng/ml; $P = 0.4716$). However, RES ewes had significantly more circulating GH than CON ewes (averaging 4.4 ± 0.8 ng/ml vs. 1.9 ± 0.9 ng/ml; $P < 0.01$; Table 18, Figure 18).

OVER ewes maintained a significantly increased IGFBP-3 concentration as long as 9 months postpartum compared with CON ewes (408.1 ± 35.5 AU vs. 287.9 ± 43.5 AU; $P < 0.05$), but there was no difference between RES (240.1 ± 31.4 AU) and CON

(287.9 ± 43.5 AU) ewes ($P = 0.4914$). All ewe IGFBP-3 concentrations decreased after parturition, and began to increase at 6 months (Table 19, Figure 19). At parturition OVER ewes had significantly less circulating IGFBP-2 (29.35 ± 2.7 AU vs. 33.2 ± 3.3AU; $P < 0.05$), however this difference was not maintained through 9 months, and there was no difference over time between CON (24.4 ± 1.7 AU), RES (21.3 ± 1.4 AU), and OVER lambs (24.7 ± 1.2 AU) IGFBP-2 concentrations ($P = 0.8682$ for CON vs. RES; $P = 0.1687$ for CON vs. OVER) (Table 20, Figure 20.

There was a trend for OVER ewes to have more circulating leptin than CON ewes (2.6 ± 0.2 ng/ml vs. 1.8 ± 0.3 ng/ml; $P < 0.1$), but there was no difference in circulating leptin concentration between RES and CON ewes (2.0 ± 0.2 ng/ml vs. 1.8 ± 0.3 ng/ml; $P = 0.43$; Table 21, Figure 21).

Discussion

Bottle lambs were larger at birth, but suckling lambs grew at a faster rate, and by 1 month of age, were larger than bottle lambs. They maintained this larger size and heavier body weight through 3 months. Suckling lambs had an average daily gain of 0.38 kg/day compared with 0.3 kg/day for the bottle lambs. From 1 to 3 months of age, it appears that the suckling and bottle lambs were growing at the same rate, but from birth to 1 month, the suckling lambs have an increased growth rate. Emsen et al. (2004) had similar findings: lambs fed a calf milk replacer, although larger at birth, gained less weight than lambs left to nurse naturally from the ewe in an arid environment. In our study, however, the lambs were not fed calf milk replacer, the bottle lambs were weaned later, and ewes were fed significant nutrients during lactation. Emsen et al. (2004) noted that bottle lambs maintained their smaller size through 6 weeks of age, 2 weeks post weaning in their study. However, it has been shown by Heaney et al. (1982) that lambs grow better on lamb milk replacer than calf milk replacer; thus it appears as though the Emsen study was conducted to obtain results for a farmer struggling with the cost of raising his flock.A similar trend is seen for crown-rump length and heart girth, where suckling lambs are larger than bottle lambs by 1 month, and maintain this increased size through 3 months of age.

At birth, suckling and bottle lambs had similar IGF-1 concentrations, as was expected since the lambs were not yet assigned to a treatment at this point. At 1 month of age, the suckling lambs had a greater IGF-1 concentration. Increased IGF-1 is associated with increased growth rate (Yakar et al., 1999), which supports the increase in body weight and body size of the suckling lambs at 1 month of age. From birth to 1 month, the

bottle lambs experienced decreased IGF-1. Since IGF-1 is associated with nutritional intake (Baumann, 2009), this suggests restricted intake in the bottle lambs, especially since IGF-1 concentrations increased after 1 month, the time of the start of weaning. After 1 month, IGF-1 concentrations in the bottle lambs increased, and the magnitude of the difference in body size and body weight between suckling and bottle lambs decreased as well.

Associated with this reduction in IGF-1 in the bottle lambs at 1 month of age was increased GH. Although not statistically significant, bottle lamb GH concentrations were increased at this time point. As the somatotropic axis is regulated through a negative feedback loop, GH was increased in an attempt to increase IGF-1 concentrations (Giustina et al., 2008). This supports the hypothesis that bottle lamb intake was restricted.

Additionally, suckling lambs tended to have increased circulating IGFBP-3 compared with bottle lambs. A greater IGFBP-3 concentration is associated with an increased rate of growth (Govoni et al., 2005; Sabin et al., 2011). Thus the greater IGFBP-3 concentration, specifically at 1 month of age, supports our hypothesis that suckling lambs had access to more nutrition than the bottle lambs before weaning. At 2 months, bottle lambs have more circulating IGFBP-3, although not statistically significant. This suggests that at the start of weaning, bottle lambs began to experience compensatory growth.

Over time, circulating leptin concentrations in bottle lambs decreased, whereas leptin concentration in suckling lambs increased, and by 3 months of age, suckling lambs had a greater circulating leptin concentration compared with bottle lambs. Leptin concentrations are positively correlated with body weight and adiposity (Delavaud et al.,

2000), which suggests that the increase in body weight and body size in suckling lambs is at least in part due to an increase in adipose tissue, and not just increased muscle tissue. To determine if adiposity was increased, BCS was compared in the bottle lambs and suckling lambs at 2 and 3 months. The average BCS of bottle lambs for these time points was 2.9, whereas average BCS in suckling lambs was 3.1. Although only a slight difference, this difference does support the suggestion that the increased body weight in the suckling lambs is due to adipose tissue accumulation, and not muscle tissue accumulation.

Therefore, although the suckling lambs were heavier and larger than the bottle lambs, this does not necessarily mean that leaving the lamb to nurse from the ewe is a better model of postpartum nutrition for a fetal programming study. Bottle-feeding the lambs controls the nutrient amount as well as quality of the milk, and as of yet, there has not been any research into how the maternal ability to produce milk is affected after a period of nutritional stress. Thus, lambs born to underfed and overfed ewes may experience differences in milk quality if left to nurse with the ewe. This would make the postnatal diets variable amongst lambs. However, quantity and quality of ewe milk was not measured. Therefore, it is possible that for each ewe that gave birth to twins, the lamb that returned to the flock is eating enough for two, or at least more than the bottle lambs. This is supported by our data, which show that the bottle lambs grew slower before the period of weaning than suckling lambs, with reduced IGF-1 concentrations, compared with the suckling lambs. This suggests that feeding the bottle lambs milk replacer at 1.7% body weight per feeding is not enough nutrition for the bottle lambs. Our hypothesis that bottle lambs were not being fed enough pre-weaning is supported by increased growth

rates and IGF-1 concentrations after the start of weaning, when access to grain becomes available, and when the rumen begins functioning. Our laboratory will continue to study this.

There was no long-term effect of maternal diet during gestation on lamb body weight or body size. This is particularly interesting because in the bottle-fed lambs, OVER lambs were significantly larger than CON lambs in all of these linear measurements through 3 months of age.

There was no significant difference in circulating IGF-1 concentration in lambs born to ewes of all 3 treatment groups. However, at birth, CON lambs have greater concentrations of circulating IGF-1 than RES or OVER lambs. At 1 month of age, both OVER and RES lambs have more IGF-1, and at 2 months, OVER lambs have significantly more IGF-1. Although not all of these differences were statistically significant, this increase in RES and OVER IGF-1 concentration is potentially due to increased intake in an attempt to maintain a normal growth rate. As growth is, in part, mediated by IGF-1 concentration, increased IGF-1 does suggest increased growth rate (Baumann, 2009). However, there was no difference in lamb body weight to support this. There was also no difference in circulating GH concentration, which supports our finding that there was no significant difference in circulating IGF-1 concentrations, and does not support the idea of compensatory growth. The increased OVER lamb GH concentration at 6 months is due to 1 animal with a GH concentration of 19.65 ng/ml, while the average concentration of the other 3 lambs is 0.98 ± 0.49 ng/ml. Due to the size of the standard deviation, this large point was not considered an outlier, and could not be removed.

OVER lambs had significantly more IGFBP-3 than CON lambs as far as 9 months of age. At 6 months, the large difference between OVER and CON lamb IGFBP-3 concentration is due to one sample that was much larger (538.3 AU) than the others, which averaged 257.0 ± 48.8 AU. This point was not considered an outlier, and therefore could not be removed. More IGFBP-3 is associated with an increased rate of growth (Govoni et al., 2005; Sabin et al., 2011), and as there was no difference in average daily gain among CON, RES, and OVER lambs it is possible that increased IGFBP-3 concentrations in OVER lambs modulated compensatory growth in these animals. At birth, there was a trend for RES animals to have reduced IGFBP-2, however over time there was no difference in lamb IGFBP-2 concentration. IGFBP-2 is associated inversely with animal body mass index (Mattsson et al., 2008), suggesting that there is no difference in the ratio of muscle tissue to adipose tissue in these lambs. All lambs experienced an age-related decrease in IGFBP-2 concentration.

There was also no difference in circulating leptin, which was supports the similar IGFBP-2 concentrations, lack of compensatory growth, and a lack somatotropic axis dysregulation, which was unexpected. We expected that RES and OVER lambs would experience somatotropic axis dysregulation due to abnormal concentrations of IGF-1 and GH in-utero. Once born, we expected to see compensatory growth in these animals, associated with significantly greater leptin concentrations, which would mean an increase in adipose tissue accumulation rather than muscle growth. However, differences in IGF-1 concentration were non-significant, suggested similar nutrient intake among lambs, and similar rates of growth. The lack of effect of maternal diet during gestation could be due to differences in maternal milk nutrition or due to the time of nutrient stress imposed

during gestation. For example, different times of nutrient stress have different effects (Vonnahme et al., 2003; Gardner et al., 2005; Ford et al., 2007; Coupé et al., 2012).

Lastly, the ewes experienced long-term effects of gestational diet. OVER ewes maintained a 5 kg heavier body weight compared with CON ewes as far as 9 months postpartum, and RES ewes maintained a 7 kg lighter body weight over the 9 month period. Neither of these differences in body weight were statistically significant, however. We did expect some initial differences in body weight as the ewes would take some time re-alimenting to the control diet to make up for body weight differences. However, we were not expecting that body weight would remain different through 9 months of being maintained on the same diet.

We expected that the OVER ewes would display greater IGF-1 concentrations short-term, and that RES ewes would display reduced IGF-1 concentrations short-term, due to differences in gestational diet. However, since IGF-1 is impacted by nutrition, we expected that IGF-1 concentrations would quickly return to normal concentrations among the three treatment groups. However OVER ewes had significantly greater circulating IGF-1 compared with CON ewes over the 9 months, and there was a trend for RES ewes to have reduced circulating IGF-1 at birth, 1 month, and 5 months. For this reason, it is particularly interesting that RES ewes had a significantly greater circulating GH concentration than CON ewes, but that there was no difference between CON and OVER GH concentration. We expected that since OVER ewes displayed greater IGF-1 concentrations, that they would have had reduced GH, and that since there was no significant difference between RES and CON ewe IGF-1 concentrations, there would not be a difference in GH concentration. However, this was not the case, and there are times

when GH trends between GH concentration and IGF-1 concentration do not match. This is perhaps more evidence that the ewes maintain longterm effects of poor gestational diet. The increased CON ewe GH concentration at 8 months is due to 1 animal, with a GH concentration of 7.74 ng/ml. At 8 months, the remaining CON ewes have an average GH concentration of 0.81 ± 0.21 ng/ml. However, due to the magnitude of the standard errors, the value can not be considered an outlier, and remained in the dataset.

Overfed ewes maintained significantly increased IGFBP-3 concentrations as far as 9 months postpartum, possible due to increased intake after parturtion. There was no difference between RES and CON ewe IGFBP-3 concentration. All ewe IGFBP-3 concentrations declined after parturition and began to increase around 6 months, the time at which ewes were re-bred and moved from pasture grazing to the barn, where supplemental grain was added to ewe diets. IGFBP-3 concentrations continued to increase as gestation progressed. At parturition, OVER ewes had significantly less IGFBP-2 than CON ewes, which makes sense as IGFBP-2 is inversely related to body mass index (Mattsson et al., 2008). However, there was no difference between RES, CON, and OVER ewe IGFBP-2 concentration over time.

Overfed ewes maintained increased circulating leptin concentrations relative to the control ewes through 8 months postpartum. This indicates that even as late as 8 months postpartum, overfed ewes still have more adipose tissue, and are still experiencing effects of their gestational diet. After the ewes were returned to the flock postpartum, they were maintained as part of the flock; which included re-breeding. At this point, the ewes have lambed this year's offspring, which are very likely to be affected by the gestational diets of these ewes last year. Therefore, it does seem that a

period of nutritional stress during pregnancy has the potential to affect not only the offspring a gestating mother is carrying during the time of nutritional stress, but also all future offspring of that animal.

In summary, suckling lambs grew to be larger than bottle lambs, and had increased concentrations of IGF-1 and leptin, suggesting increased intake. As ewe milk quantity or quality was not measured, it is possible that there was variation in intake among suckling lambs, making it difficult to compare the merit of the two postpartum feeding models for a fetal programming experiment. There was no long-term difference in growth or metabolic hormone concentration of suckling lambs, except that OVER lambs had more circulating IGFBP-3 than CON lambs. This lack of difference is possibly due to variation in postpartum intake due to maternal milk quality and quantity, as well as the time of maternal nutrient stress. Lastly, ewes did experience longterm effects of poor gestational diet. OVER ewes maintained a non-significant increased weight, and significantly more circulating IGF-1, IGFBP-3, and leptin. RES ewes maintained significantly increased GH. This suggests that when these ewes lamb again, it is possible that these offspring will be affected by the poor nutritional conditions during the previous gestation.

Table 1: Suckling vs. Bottle Lamb Body Weight

Body Weight, kg	Birth	1 Month	2 Months	3 Months
Bottle Lambs	5.0 ± 0.17	10.7 ± 0.75	20.6 ± 0.75	30.8 ± 0.73
Suckling Lambs	4.3 ± 0.13	15.4 ± 0.59	26.4 ± 0.59	36.6 ± 0.56
P-value	0.0016	<0.0001	<0.0001	<0.0001

At birth, bottle lambs weighed significantly more than suckling lambs ($P < 0.01$), but at 1 month, suckling lambs were approximately 5 kg heavier than bottle lambs ($P < 0.01$). This significance remained through 3 months.

Tables and Figures

Table 2: Suckling vs. Bottle Lamb CRL

CRL, cm	Birth	1 Month	2 Months	3 Months
Bottle Lambs	48.2 ± 0.9	61.3 ± 0.9	71.6 ± 0.9	83.7 ± 1.1
Suckling Lambs	47.4 ± 0.8	70.9 ± 0.8	80.1 ± 0.8	89.1 ± 0.8
***P*-value**	0.488	<0.0001	<0.0001	0.0001

At birth, bottle lambs were larger in CRL than suckling lambs, though this difference was not significant ($P = 0.488$), but at 1 month, suckling lambs were approximately 10 cm larger than bottle lambs ($P < 0.01$). This significance remained through 3 months.

Table 3: Suckling vs. Bottle Lamb Heart Girth

Heart Girth, cm	Birth	1 Month	2 Months	3 Months
Bottle Lambs	39.6 ± 0.6	50.7 ± 0.9	64.2 ± 0.9	74.5 ± 0.9
Suckling Lambs	37.6 ± 0.4	59.4 ± 0.7	69.9 ± 0.8	78.7 ± 0.8
***P*-value**	0.0061	<0.0001	<0.0001	0.0011

At birth, bottle lambs were significantly larger in heart girth than suckling lambs ($P < 0.01$), but at 1 month, suckling lambs were approximately 8 cm larger than bottle lambs ($P < 0.01$). This significance remained through 3 months

Table 4: Suckling vs. Bottle Lamb IGF-1 Concentration

IGF-1, ng/ml	Birth	1 Month	2 Months	3 Months
Bottle Lambs	133.0 ± 19.1	64.9 ± 28.4	173.3 ± 28.7	177.9 ± 26.5
Suckling Lambs	109.1 ± 14.9	280.7 ± 22.9	231.5 ± 22.9	211.0 ± 19.9
***P*-value**	0.3259	<0.0001	0.1156	0.3204

At birth, bottle and suckling lambs had similar IGF-1 concentrations ($P = 0.326$), but at 1 month, suckling lambs had a significantly increased IGF-1 concentration, to 280.7 ± 22.9 ng/ml, compared with bottle lambs ($P < 0.01$). At 3 months, IGF-1 concentrations were similar again among the lambs ($P = 0.32$).

Table 5: Suckling vs. Bottle Lamb GH Concentration

GH, ng/ml	Birth	1 Month	2 Months	3 Months
Bottle Lambs	11.1 ± 3.4	11.2 ± 3.8	4.4 ± 1.3	3.8 ± 3.4
Suckling Lambs	13.4 ± 3.2	9.6 ± 3.5	6.4 ± 1.2	5.5 ± 3.1
***P*-value**	0.6154	0.7601	0.2693	0.7173

There was no significant difference in bottle and suckling lamb GH concentration from birth to 3 months of age ($P = 0.698$). All lambs experienced an age-related decrease in GH concentration.

Table 6: Suckling vs. Bottle Lamb IGFBP-3 Concentration

IGFBP-3, AU	Birth	1 Month	2 Months	3 Months
Bottle Lambs	79.8 ± 15.7	59.6 ± 16.5	200.8 ± 19.7	106.6 ± 16.3
Suckling Lambs	66.7 ± 14.5	137.9 ± 15.1	177.8 ± 16.5	140.6 ± 14.8
***P*-value**	0.5417	<0.01	0.373	0.1278

Over time, there was a trend for suckling lambs to have more IGFBP-3 than bottle lambs ($P = 0.0633$).

Table 7: Suckling vs. Bottle Lamb Leptin Concentration

Leptin, ng/ml	Birth	1 Month	2 Months	3 Months
Bottle Lambs	1.5 ± 0.2	1.4 ± 0.2	0.9 ± 0.2	1.1 ± 0.2
Suckling Lambs	1.2 ± 0.2	1.4 ± 0.2	1.1 ± 0.2	1.3 ± 0.2
***P*-value**	0.17	0.69	0.25	0.23

Although there was no significant difference in bottle and suckling lamb circulating leptin concentrations ($P = 0.58$), bottle lambs had more circulating leptin at birth compared with suckling lambs, and less circulating leptin at 3 months, suggesting suckling lambs had greater adipose tissue accumulation.

Table 8: Suckling Lamb Body Weight Over Time

BW, kg	Birth	3 Months	6 Months	9 Months
CON	4.4 ± 1.6	36.8 ± 1.6	60.1 ± 1.8	69.2 ± 1.9
OVER	4.6 ± 1.6	36.9 ± 1.7	60.0 ± 1.9	70.4 ± 2.1
RES	4.0 ± 1.4	35.9 ± 1.5	58.0 ± 1.7	66.4 ± 1.8
CON vs. OVER *P*-value	0.92	0.97	0.97	0.66
CON vs. RES *P*-value	0.88	0.68	0.34	0.29

There was no difference in suckling lamb body weight across the 3 treatment groups, with all lambs gaining approximately 0.24 kg/day over the 9 month period (P = 0.36 for CON vs. RES; P = 0.96 for CON vs. OVER).

Table 9: Suckling Lamb CRL

CRL, cm	Birth	3 Months	6 Months	9 Months
CON	47.5 ± 2.5	87.9 ± 2.4	103.7 ± 1.9	108.3 ± 3.0
OVER	46.8 ± 2.6	91.7 ± 2.5	106.2 ± 2.1	111.8 ± 3.0
RES	47.7 ± 2.3	87.9 ± 2.2	104.0 ± 1.8	111.2 ± 2.6
CON vs. OVER *P*-value	0.85	0.26	0.38	0.41
CON vs. RES *P*-value	0.96	0.97	0.64	0.55

There was no difference in suckling lamb CRL across the 3 treatment groups. (P = 0.943) for CON vs. RES; P = 0.637 for CON vs. OVER).

Table 10: Suckling Lamb Heart Girth Over Time

Girth, cm	Birth	3 Months	6 Months	9 Months
CON	37.5 ± 0.9	78.4 ± 1.6	97.4 ± 2.0	102.8 ± 2.0
OVER	38.5 ± 0.9	80.8 ± 1.7	99.1 ± 2.1	108.9 ± 2.0
RES	37.1 ± 2.3	76.9 ± 1.6	94.9 ± 1.8	103.6 ± 1.8
CON vs. OVER P-value	0.5	0.33	0.57	0.03
CON vs. RES P-value	0.71	0.52	0.35	0.79

There was no difference in suckling lamb heart girth across the 3 treatment groups. (P = 0.449 for CON vs. RES; P = 0.162 for CON vs. OVER).

Table 11: Suckling Lamb IGF-1 Concentration

IGF-1, ng/ml	Birth	3 Months	6 Months	9 Months
CON	137.7 ± 27.5	220.6 ± 24.8	89.1 ± 34.0	89.0 ± 34.0
OVER	88.0 ± 29.2	219.1 ± 27.8	85.6 ± 34.3	68.2 ± 34.3
RES	100.9 ± 24.9	191.0 ± 24.6	91 ± 28.2	87.5 ± 28.2
CON vs. OVER *P*-value	0.15	0.97	0.94	0.67
CON vs. RES *P*-value	0.23	0.39	0.97	0.97

Over time, there was no difference in circulating IGF-1 concentration in suckling lambs across the 3 treatment groups. P = 0.88 (CON vs. RES) and P = 0.71 (CON vs. OVER).

Table 12: Suckling Lamb GH Concentration

GH, ng/ml	Birth	3 Months	6 Months	9 Months
CON	10.8 ± 3.0	3.9 ± 3.2	2.4 ± 3.6	2.6 ± 3.6
OVER	10.6 ± 3.2	4.6 ± 3.5	6.2 ± 3.8	1.9 ± 3.7
RES	12.9 ± 2.7	5.5 ± 3.1	3.0 ± 3.2	4.6 ± 3.1
CON vs. OVER *P*-value	0.97	0.89	0.48	0.9
CON vs. RES *P*-value	0.61	0.72	0.91	0.68

There was no difference in circulating GH concentration in suckling lambs over the 9 month period ($P = 0.789$ for CON vs. RES; $P = 0.959$ for CON vs. OVER). All lambs experienced an age-related decrease in GH concentration.

Table 13: Suckling Lamb IGFBP-3

IGFBP-3, AU	Birth	3 Months	6 Months	9 Months
CON	66.3 ± 8.5	139.3 ± 15.8	184.5 ± 22.2	155.8 ± 13.3
OVER	54.7 ± 9.1	150.4 ± 17.9	334.0 ± 22.4	138.4 ± 13.8
RES	68.7 ± 7.7	132.8 ± 15.8	128.1 ± 19.8	116.4 ± 11.8
CON vs. OVER *P*-value	0.35	0.64	<0.01	0.36
CON vs. RES *P*-value	0.84	0.77	0.06	0.03

At birth, CON lambs had more circulating IGFBP-3 than OVER lambs but over time, OVER lambs had significantly more IGFBP-3 than CON lambs ($P < 0.05$). There was no difference in IGFBP-3 concentration between RES and CON lambs ($P = 0.1063$).

Table 14: Suckling Lamb IGFBP-2

IGFBP-2, AU	Birth	3 Months	6 Months	9 Months
CON	32.1 ± 2.3	19.7 ± 2.2	11.6 ± 3.2	11.9 ± 0.79
OVER	28.6 ± 2.4	15.3 ± 2.4	13.7 ± 3.3	12.0 ± 0.79
RES	26.9 ± 2.0	18.6 ± 2.1	10.3 ± 2.7	10.4 ±0.64
CON vs. OVER *P*-value	0.29	0.17	0.65	0.98
CON vs. RES *P*-value	0.09	0.68	0.77	0.15

There was no difference in circulating IGFBP-2 concentration among CON, RES, and OVER lambs ($P = 0.2979$ for CON vs. RES; $P = 0.5364$ for CON vs. OVER).

Table 15: Suckling Lamb Leptin Concentration

Leptin, ng/ml	Birth	3 Months	6 Months	9 Months
CON	1.4 ± 0.2	1.3 ± 0.2	2.2 ± 0.3	2.4 ± 0.3
OVER	1.1 ± 0.3	1.3 ± 0.3	2.9 ± 0.3	2.7 ± 0.3
RES	1.2 ± 0.2	1.3 ± 0.2	2.2 ± 0.3	2.1 ± 0.3
CON vs. OVER *P*-value	0.37	0.86	0.17	0.56
CON vs. RES *P*-value	0.65	0.89	0.91	0.6

There was no difference in circulating leptin concentration in suckling lambs over the 9 month period (P = 0.808 for CON vs. RES; P = 0.938 for CON vs. OVER).

Table 16: Ewe Body Weight

BW, kg	Birth	3 Months	6 Months	9 Months
CON	113.8 ± 3.5	93.4 ± 3.7	88.1 ± 3.9	94.6 ± 3.8
OVER	121.4 ± 3.5	99.3 ± 3.6	90.2 ± 3.7	97.7 ± 3.6
RES	99.6 ± 3.6	86.3 ± 3.6	82.4 ± 3.6	90.6 ± 3.6
CON vs. OVER *P*-value	0.12	0.27	0.69	0.56
CON vs. RES *P*-value	0.01	0.17	0.28	0.45

There was no significant difference in ewe body weigtht, (P = 0.34 CON vs. OVER, and 0.14 CON vs. RES) however OVER ewes did maintain a greater body weight as far as 9 months postpartum, and RES ewes maintained a reduced body weight.

Table 17: Ewe IGF-1 Concentration

IGF-1, ng/ml	Birth	3 Months	6 Months	9 Months
CON	361.4 ± 26.5	126.0 ± 31.2	56.5 ± 34.7	89.5 ± 34.7
OVER	568.3 ± 24.7	206.2 ± 26.4	98.5 ± 28.4	104.7 ± 28.4
RES	231.6 ± 23.4	176.2 ± 23.4	58.2 ± 26.3	77.4 ± 26.3
CON vs. OVER *P*-value	<0.0001	0.046	0.35	0.74
CON vs. RES *P*-value	0.0003	0.19	0.96	0.78

OVER ewes had a greater circulating IGF-1 concentration than CON ewes ($P < 0.05$) over the 9 month period. There was no difference between RES and CON ewe IGF-1 concentration (P = 0.12).

Table 18: Ewe GH Concentration

GH, ng/ml	Birth	3 Months	6 Months	9 Months
CON	7.1 ± 2.1	1.5 ± 3.0	1.3 ± 3.3	0.8 ± 3.3
OVER	5.8 ± 2.2	1.6 ± 2.8	1.1 ± 2.8	1.8 ± 2.8
RES	25.5 ± 2.1	2.1 ± 2.4	2.4 ± 2.4	1.5 ± 2.4
CON vs. OVER *P*-value	0.66	0.96	0.96	0.8
CON vs. RES *P*-value	<0.01	0.85	0.79	0.86

RES ewes had a greater circulating GH concentration than CON ewes ($P < 0.01$), but there was no difference between OVER and CON ewes ($P = 0.47$).

Table 19: Ewe IGFBP-3 Concentration

IGFBP-3, AU	Birth	3 Months	6 Months	9 Months
CON	319.8 ± 107.7	102.8 ± 44.2	225.7 ± 95.9	503.2 ± 105.7
OVER	622.1 ± 87.9	186.1 ± 36.1	294.4 ± 78.3	529.7 ± 86.4
RES	206.3 ± 81.1	127.9 ± 31.9	231.1 ± 67.8	434.8 ± 74.8
CON vs. OVER *P*-value	0.03	0.15	0.58	0.84
CON vs. RES *P*-value	0.4	0.64	0.96	0.6

OVER ewes maintained a significantly increased IGFBP-3 concentration as far as 9 months postpartum compared with CON ewes ($P < 0.05$).There was no difference between RES and CON ewes ($P = 0.4914$).

Table 20: Ewe IGFBP-2 Concentration

IGFBP-2, AU	Birth	3 Months	6 Months	9 Months
CON	33.2 ± 3.3	19.4 ± 3.3	22.8 ± 3.3	22.0 ± 3.3
OVER	29.3 ± 2.7	20.9 ± 2.7	21.1 ± 2.7	13.6 ± 2.7
RES	32.5 ± 2.3	21.1 ± 2.3	28.0 ± 2.3	17.3 ± 2.3
CON vs. OVER *P*-value	0.03	0.15	0.58	0.84
CON vs. RES *P*-value	0.4	0.64	0.96	0.6

At parturition, OVER ewes had significantly less circulating IGFBP-2 ($P < 0.05$), however there was no difference in ewe IGFBP-2 concentration maintained through 9 months postpartum ($P = 0.8682$ for CON vs. RES; $P = 0.1687$ for CON vs. OVER).

Table 21: Ewe Leptin Concentration

Leptin, ng/ml	Birth	3 Months	6 Months	9 Months
CON	1.8 ± 0.3	1.5 ± 0.5	1.4 ± 0.5	1.4 ± 0.5
OVER	2.6 ± 0.3	2.7 ± 0.4	2.0 ± 0.4	2.3 ± 0.4
RES	2.1 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.9 ± 0.3
CON vs. OVER *P*-value	0.08	0.03	0.31	0.16
CON vs. RES *P*-value	0.57	0.72	0.49	0.4

OVER ewes tended to have more leptin over time than CON ewes ($P <0.1$), but there was no difference between CON and RES ewes ($P =0.42$).

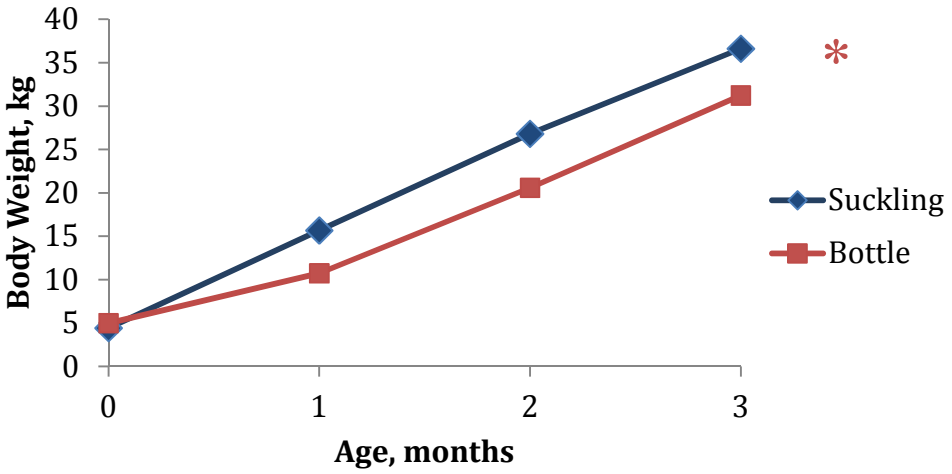

Figure 1. Suckling lambs, although slightly lighter at birth, weighed approximately 5 kg more at 1 month of age than the bottle lambs. This 5 kg weight difference was maintained through 3 months of age. * indicates $P < 0.01$

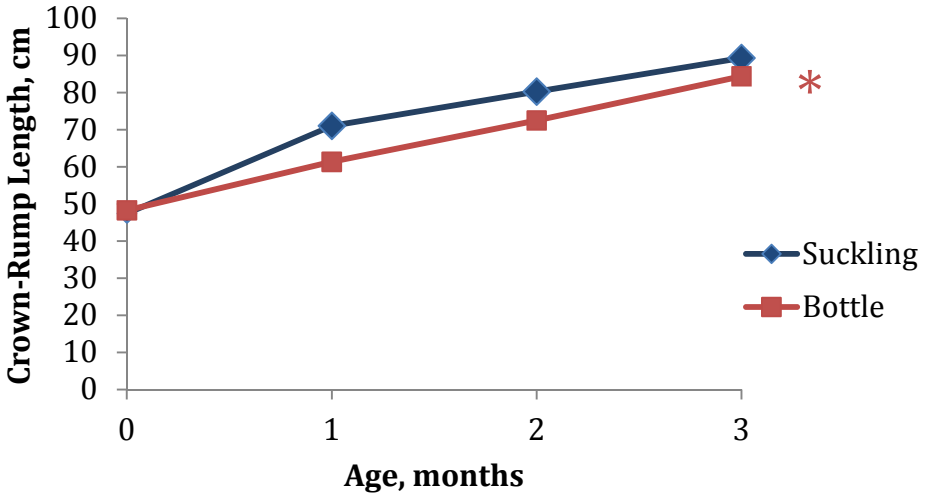

Figure 2. Suckling lambs experienced an increase in the rate of growth from birth to 1 month of age compared with the bottle lambs. Thus at 1 month of age, they were 10 cm larger than the bottle lambs. At 3 months, this difference was 5 cm, but was still present. * indicates $P < 0.01$

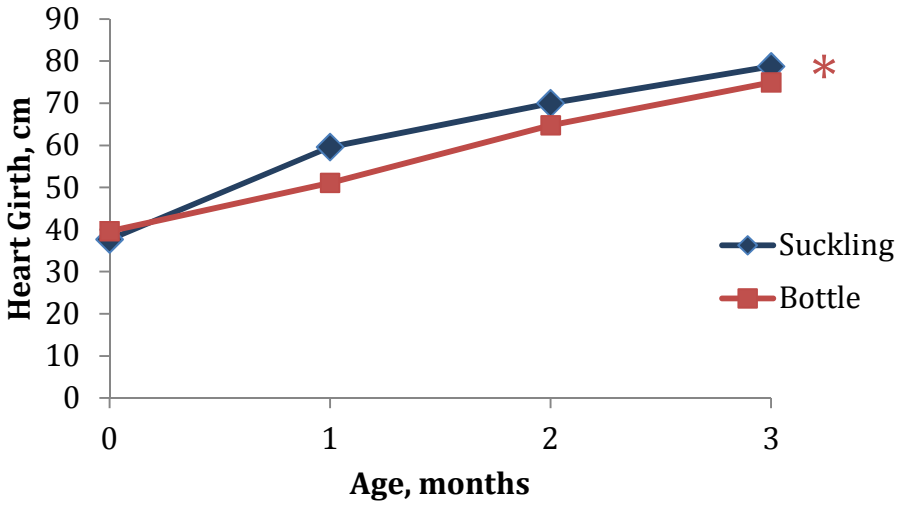

Figure 3. Suckling lambs experienced an increase in the rate of growth from birth to 1 month of age compared with the bottle lambs. At 1 month of age, they were 8 cm larger than the bottle lambs. At 3 months, this difference was reduced to 4 cm, but was still significant. * indicates $P < 0.01$

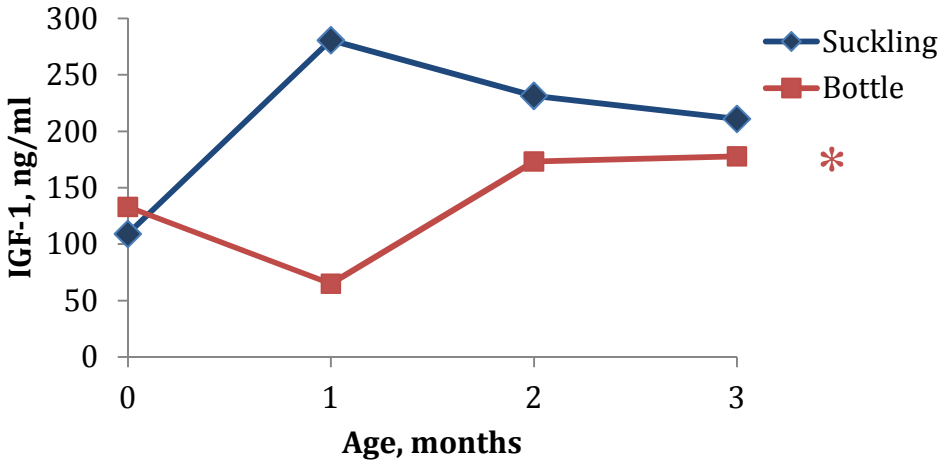

Figure 4. Suckling lambs experienced an increase in IGF-1 concentration from birth to 1 month of age compared with the bottle lambs, who experienced a decrease. At 1 month of age, suckling lambs had an IGF-1 concentration that was greater than bottle lambs by 215 ng/ml. This difference was less at 2 months of age, around the time of weaning, and was 50 ng/ml at 3 months. * indicates $P < 0.01$

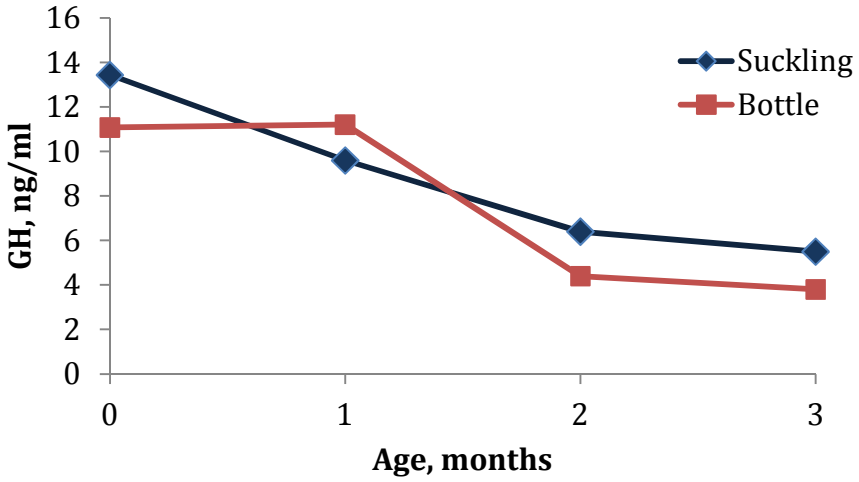

Figure 5. All lambs experienced a decrease in GH concentration with age. At 1 month, bottle lambs had an increased GH concentration compared with the suckling animals, but this difference was not significant (P = 0.69). This is possibly because of the reduced IGF-1 concentration at this time.

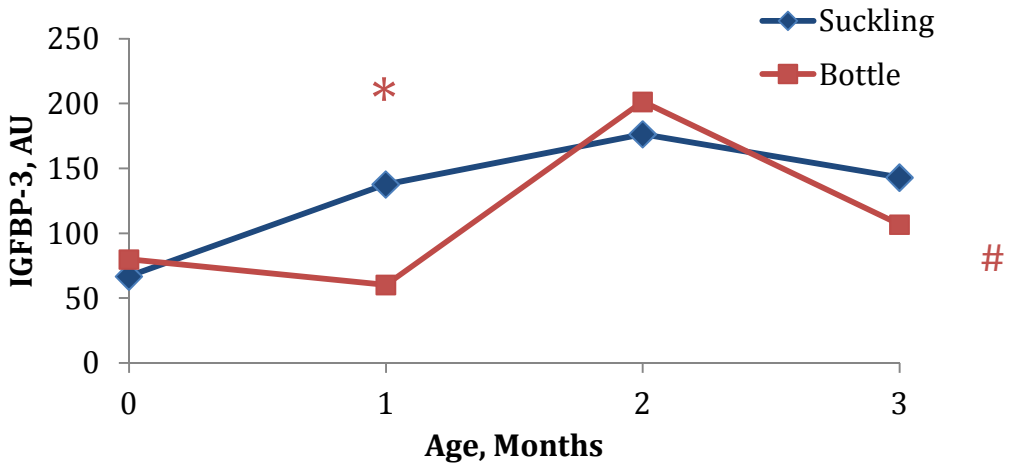

Figure 6. Over time, bottle lambs tended to have a reduced concentration of circulating IGFBP-3 compared with suckling lambs (P = 0.0633). Suckling lamb IGFBP-3 concentration appeared to increase rapidly at 1 month of age, and at 2 months, bottle lamb IGFBP-3 concentration increased, and caught up to suckling lamb IGFBP-3 concentration. * indicates $P < 0.05$; # indicates $0.05 < P < 0.1$

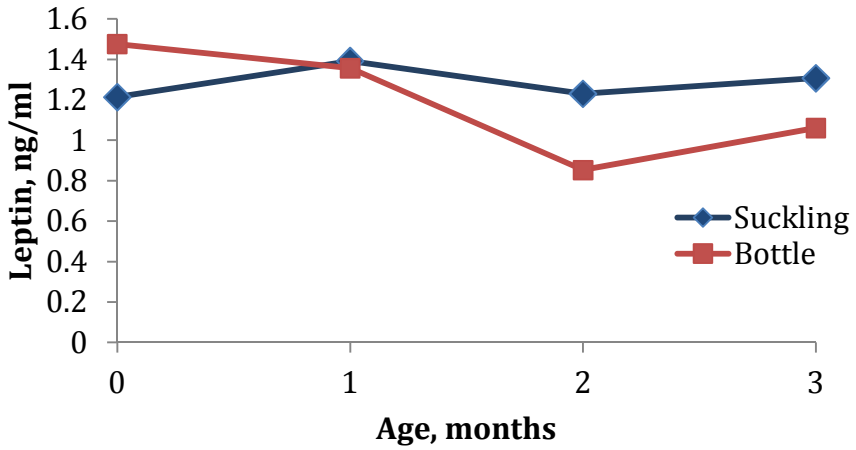

Figure 7. Suckling lambs experienced an increase in leptin concentration from birth to 1 month of age while bottle lambs experienced a decrease. At 1 month of age, suckling lambs had an increased leptin concentration, which was maintained through 3 months. This greater concentration was not statistically significant P =0.58

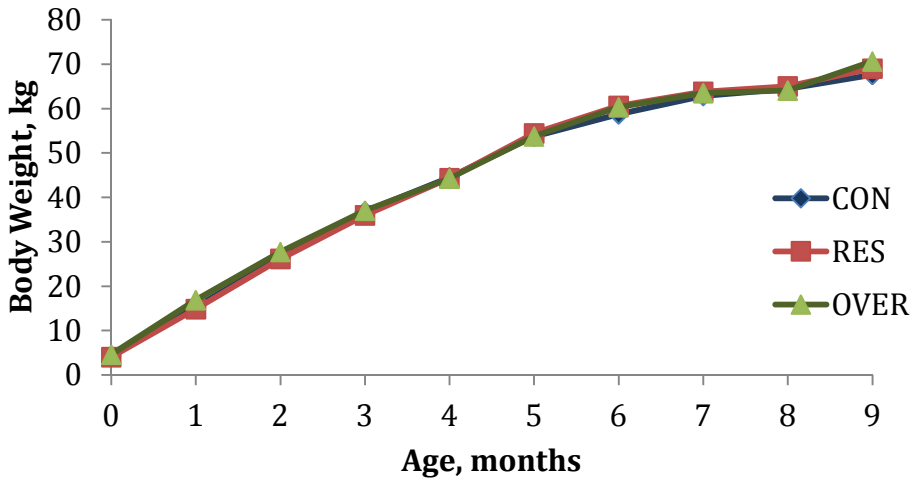

Figure 8. There was no difference in body weight between CON and RES lambs (P = 0.36) or between CON and OVER lambs (P = 0.96) over the 9 month period. Lambs from all 3 treatment groups gained an average of 0.24 kg/day.

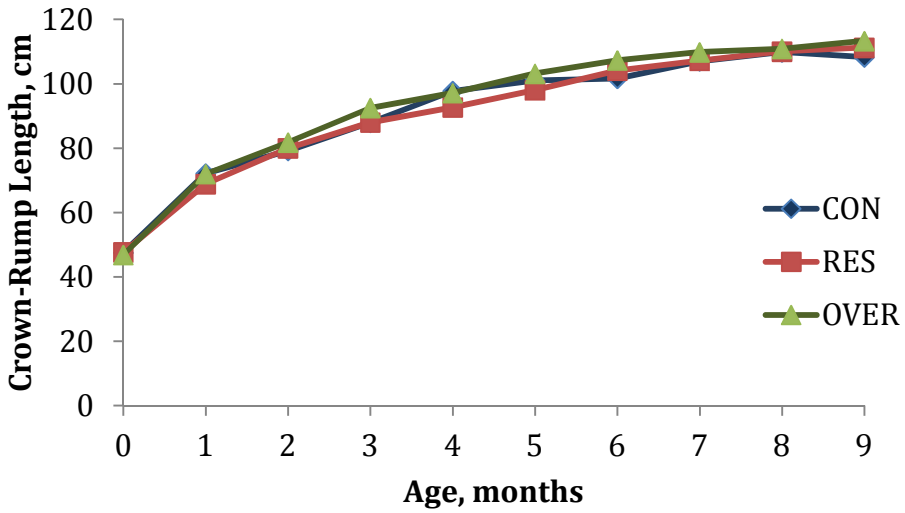

Figure 9. There was no difference in crown-rump length between CON and RES lambs (P = 0.94) or between CON and OVER lambs (P = 0.64) over the 9 month period.

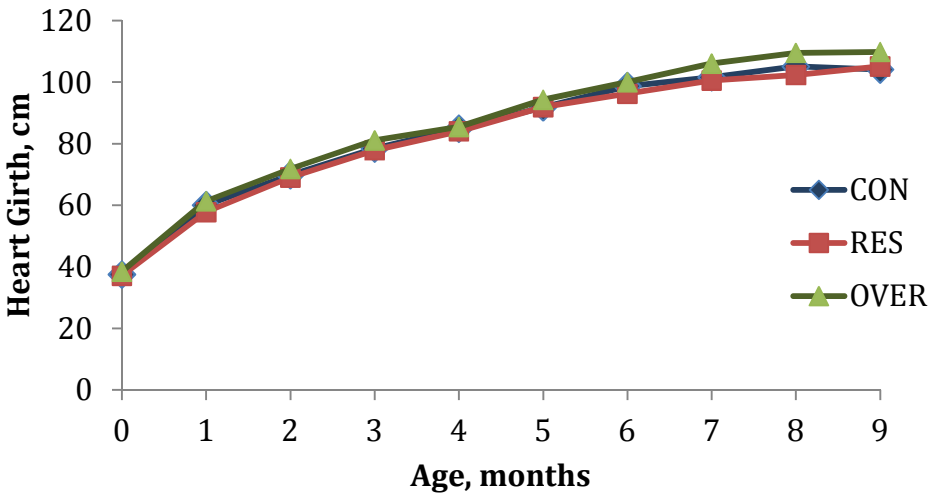

Figure 10. There was no difference in heart girth between CON and RES, lambs (P = 0.45) or between CON and OVER lambs (P = 0.16)over the 9 month period.

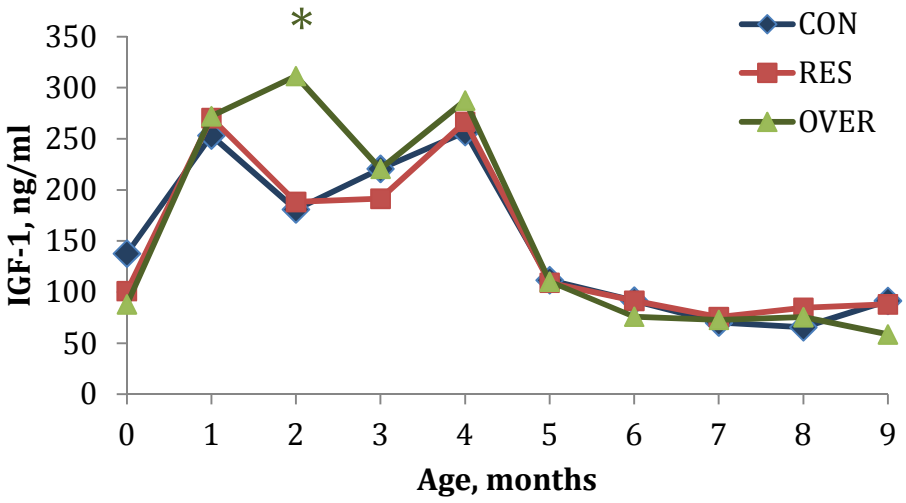

Figure 11. There was no overall difference in circulating IGF-1 between CON and RES lambs (P = 0.88) or between CON and OVER lambs (P = 0.71) over the 9 month period. However at 2 months, OVER lambs had significantly more (P < 0.05) IGF-1 than CON lambs. Average lamb IGF-1 concentration was 148.81 ± 98.1 ng/ml. * indicates P < 0.05

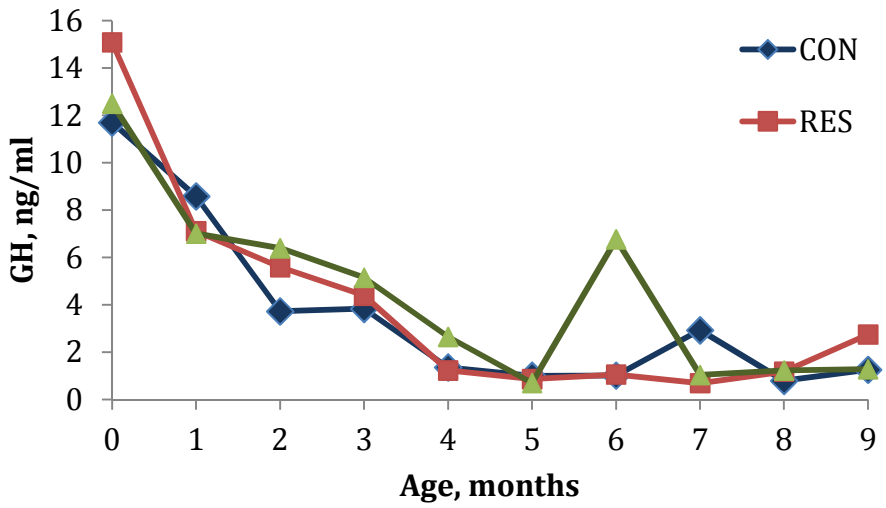

Figure 12. There was no overall difference in circulating GH between CON and RES lambs ($P = 0.80$) or between CON and OVER lambs ($P = 0.96$) over the 9 month period. Lambs from all treatment groups experienced an age-related decrease in GH concentrations. Average lamb GH concentration was 4.03 ± 3.9 ng/ml.

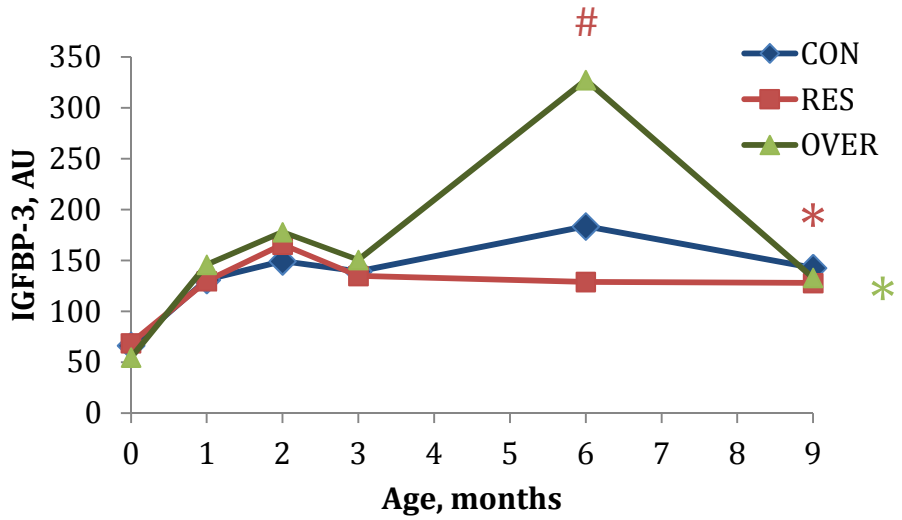

Figure 13. OVER lambs had greater circulating concentrations of IGFBP-3 than CON lambs ($P < 0.05$) over time, and at 6 ($P = 0.06$) and 9 months ($P < 0.05$), RES lambs have less IGFBP-3 than CON lambs. * indicates $P < 0.05$; # indicates $0.05 < P < 0.1$

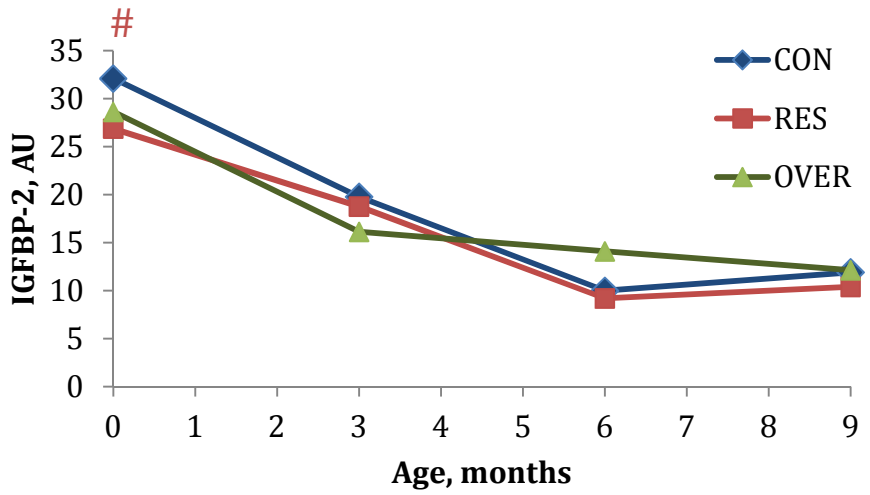

Figure 14. At birth, there was a trend for RES lambs to have less IGFBP-2 than CON lambs ($P < 0.1$). However, there was no difference in suckling lamb IGFBP-2 concentration over time ($P = 0.2979$ for CON vs. RES; $P = 0.5364$ for CON vs. OVER). # indicates $0.05 < P < 0.1$

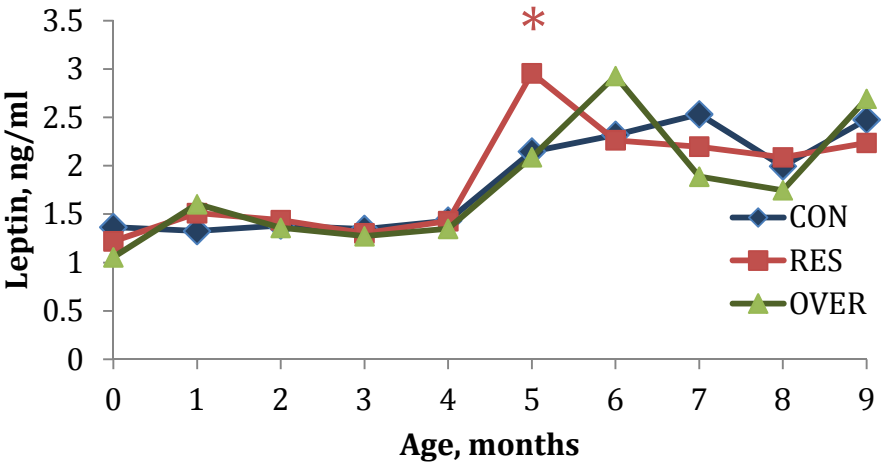

Figure 15. There was no overall difference in circulating leptin among CON, RES, and OVER lambs over the 9 month period. Lambs from all treatment groups had an average leptin concentration of 1.9 ng/ml. However, at 5 months, RES lambs had significantly more ($P < 0.05$) circulating leptin than CON lambs. * indicates *$P < 0.05$*

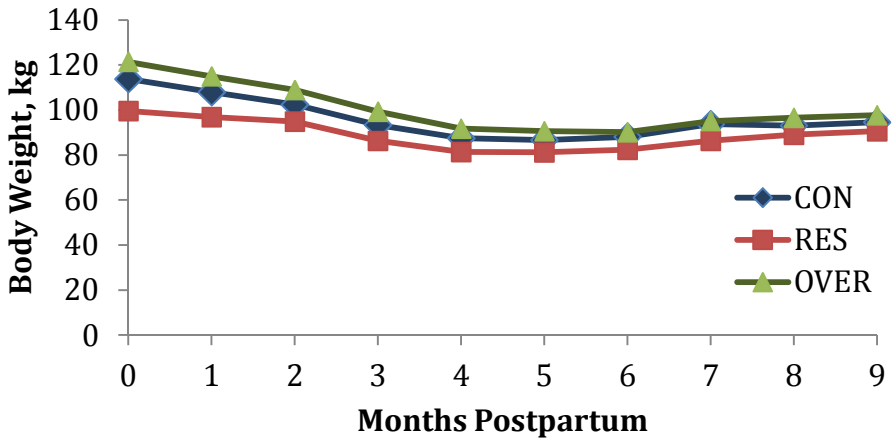

Figure 16. There was no overall significant difference in ewe body weight between birth and 9 months postpartum. However, RES ewes did maintain a 7 kg reduced body weight, and OVER ewes maintained a 5 kg body weight increase over CON ewes for the 9 months.

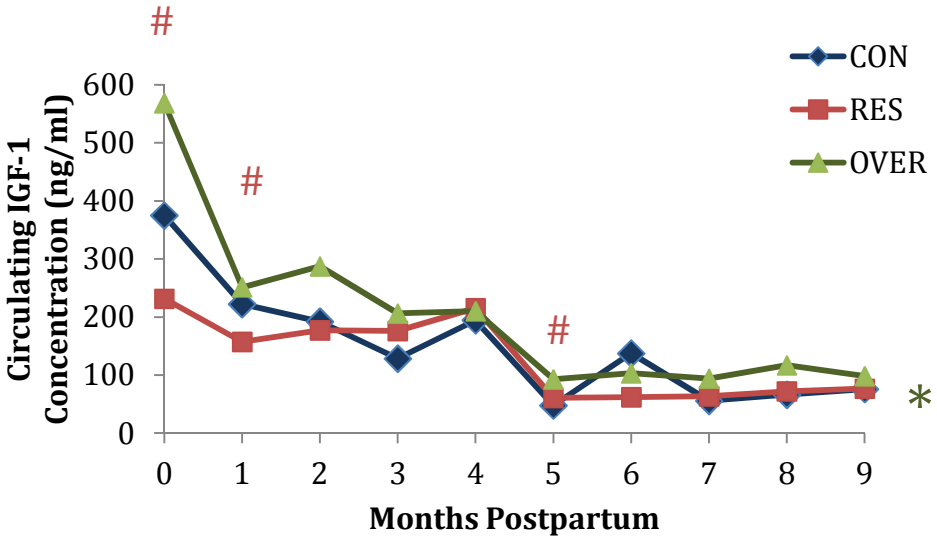

Figure 17. Over time, OVER ewes had a greater circulating IGF-1 concentration than CON ewes ($P < 0.05$), but there was no difference between RES ewes and CON ewes ($P = 0.12$) through 9 months postpartum. At birth, 1 month, and 5 months RES ewes tended to have reduced circulating IGF-1 ($P < 0.1$). CON ewes averaged an IGF-1 concentration of 151.7 ± 13.6 ng/ml, OVER ewes averaged a concentration of 192.53 ± 11.9 ng/ml, and RES ewes averaged a concentration of 123.35 ± 10.8 ng/ml. * indicates $P < 0.05$: # indicates $0.05 < P < 0.1$

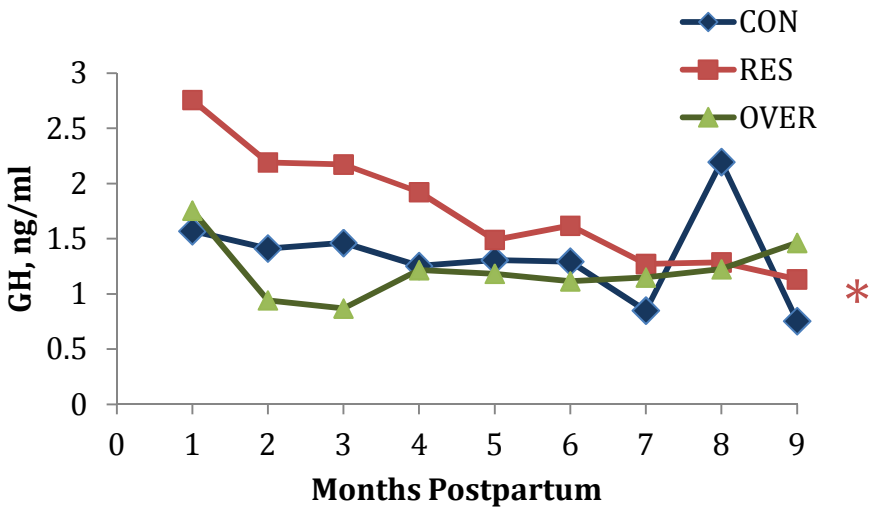

Figure 18. Over time, RES ewes had a greater circulating GH concentration than CON ewes (4.19 ± 7.72 ng/ml vs. 2.09 ± 2.39 ng/ml; $P < 0.01$), but there was no difference between OVER (1.78 ± 1.8 ng/ml)and CON ewes ($P = 0.47$). This figure does not include GH concentrations at birth because the concentration was no much larger for RES ewes, that it skewed the perception of the rest of the graph. * indicates $P < 0.05$

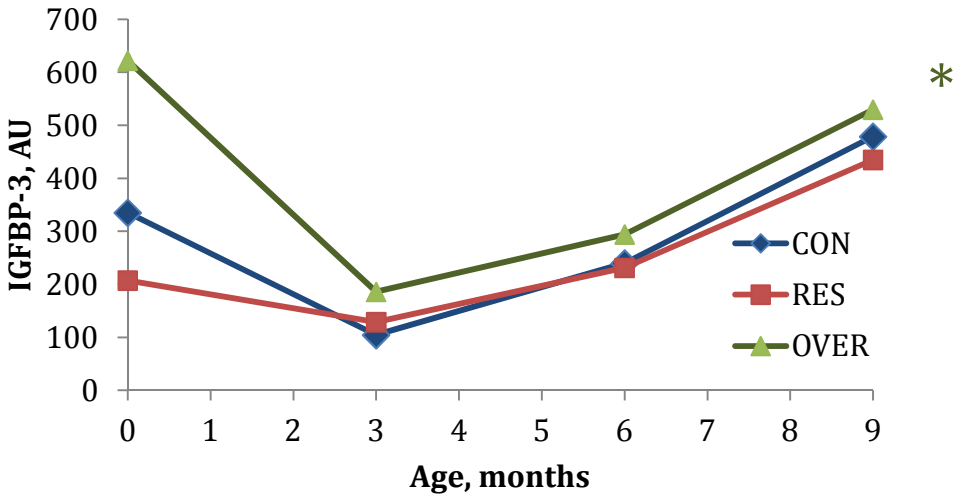

Figure 19. OVER ewes had significantly more IGFBP-3 than CON ewes as far as 9 months postpartum ($P < 0.05$). There was no difference between RES and CON ewe IGFBP-3 concentration ($P = 0.4914$). * indicates $P < 0.05$

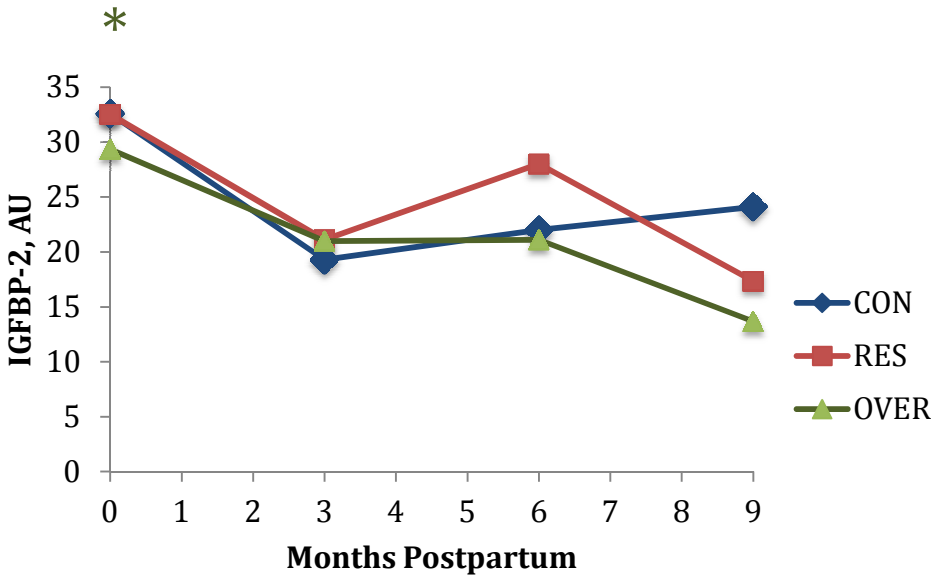

Figure 20. At parturition, OVER ewes had significantly less IGFBP-2 than CON ewes ($P < 0.05$). However there was no difference maintained over time among all ewes ($P = 0.8682$ for CON vs. RES; $P = 0.1687$ for CON vs. OVER). * indicates $P < 0.05$

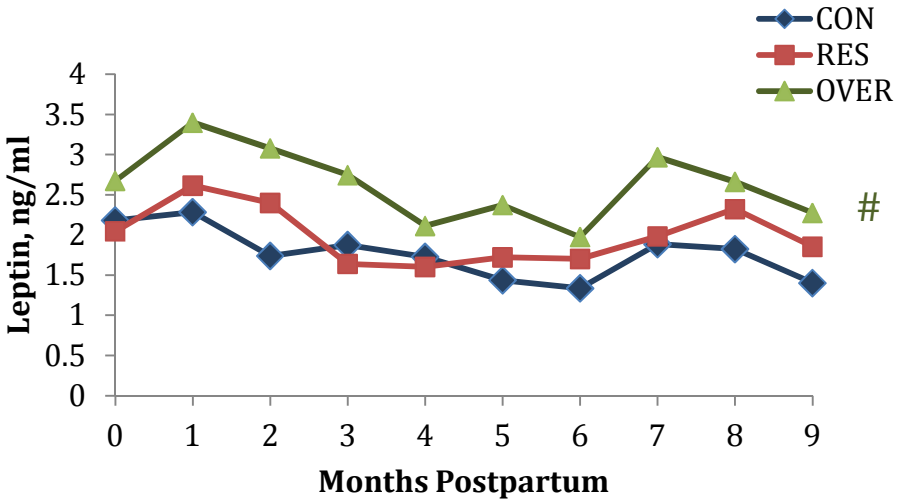

Figure 21. OVER ewes tended to have more circulating leptin over time than CON ewes ($P < 0.1$). There was no difference between CON and RES ewe leptin concentration ($P = 0.42$). Over the 9 months, CON ewes averaged leptin concentrations of 1.76 ng/ml, RES ewes averaged 1.99 ng/ml, and OVER ewes averaged 2.63 ng/ml. # indicates $0.05 < P < 0.1$.

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