

Effects of maternal undernutrition and selenium intake on fetal weight, placental weight, and placental endothelial nitric oxide synthase mRNA expression in ewes

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Maternal nutrition during pregnancy is important for several reasons including fetal growth. Producers should be particularly interested in maternal nutrition since optimal fetal growth will ultimately lead to production efficiency after birth. The placenta transfers nutrients through the blood stream from the dam to the fetus; therefore, proper placental function is necessary to insure the fetus is receiving adequate nutrition for growth and development. Endothelial nitric oxide synthase (eNOS) is a factor necessary for proper blood flow. This report focuses on two studies in our laboratory that investigate how maternal intake during pregnancy impacts fetal weight and placental weight. In addition, eNOS gene expression in the placenta was measured to better understand how blood flow in the placenta may be altered by dietary treatments. In our model, nutrient restriction during late-gestation reduces fetal weight; however, placental weight was not altered, whereas eNOS was reduced by restriction and high selenium treatments. Further research is needed to determine proper timing of realimentation necessary for adequate placental function.

Introduction

Maternal Nutrition and Developmental Programming

The relationship between maternal nutrition and fetal growth is important for determining pregnancy success, neonatal morbidity and mortality, and ultimately life long health and productivity. Since profitability in the livestock industry is dependent upon efficiency of production characteristics, such as growth and development after birth, the precursor of efficiency, i.e. fetal growth, must be optimal.

“Developmental programming” is the concept that a stimulus or insult to the mother during pregnancy can have long-term effects on the developing fetus (Barker et al., 1993). Maternal nutrition has been shown to alter birth weight and may influence the health and productivity of the offspring later in life (Barker et al., 1993). Our laboratory has been working to better understand how maternal nutrition can affect the offspring of our livestock species.

Importance and development of the placenta in sheep

The placenta is the organ that transfers nutrients from the dam to the fetus during pregnancy. For this reason, the placenta is not only important for the maintenance of the pregnancy, but also for the health of the fetus. Because of its important role in nutrient transport, it is not surprising that research has demonstrated fetal growth restriction is highly correlated with reduced placental growth and development (Reynolds and Redmer, 1995). If the placenta is not properly

transporting nutrients, the fetus may suffer a nutrient deficiency due to placental inefficiency.

In the sheep, the placenta attaches to caruncles, which are discrete sites on the uterine wall. These caruncles make up the maternal portion of the placenta. The fetal portion of the placenta is known as the cotyledon, and this tissue interdigitates with the maternal tissues of the placenta. Together, the maternal and fetal tissues form a button-like structure known as a placentome. The placentome is highly vascular and is the site of nutrient exchange between the dam and the fetus. There are several factors that regulate blood flow, including nitric oxide, which is released by endothelial nitric oxide synthase (eNOS) activity. It stands to reason, that the greater the level of eNOS in the placenta, the greater the blood flow and, therefore, nutrient exchange between the dam and fetus. Research has shown that about 90% of placental growth in the sheep occurs by day 90 of gestation; however, at this time, only 10% of fetal growth has occurred (Redmer, et al., 2005). It is likely that maternal nutrition may impact the development of the placenta before day 90, only later to affect the function of the placenta when the fetus is exponentially growing. Therefore, improper placental development could lead to poor fetal growth.

Selenium

Selenium is an essential trace mineral for normal growth and development of livestock. Over the past decade, the view on selenium has changed drastically. It was once thought that too

much selenium was toxic, and strict guidelines on the recommended levels of selenium were put in place. Now, selenium is recognized as an important trace mineral that may be anticarcinogenic and provide other health benefits when taken at supranutritional levels (Clark et al., 1996). The USDA reports that much of the Midwest, including pockets of North and South Dakota, has high levels of selenium in the soil, making it likely that forages will have a high selenium content. Little data exist demonstrating the effects of supranutritional levels of maternal selenium intake during pregnancy, and our laboratory has been working to better understand what role selenium may play in developmental programming.

Our laboratory has recently focused on how both maternal undernutrition and selenium intake during pregnancy affect the placenta and the offspring. This report will cover some of our findings from these studies.

Materials and Methods

Experiment One

All studies were approved by the North Dakota State University Animal Care and Use Committee. In experiment one, 36 Targhee-cross ewe lambs were used. Twenty-one days prior to breeding all ewes were assigned to either a high-selenium group (HSe) that received a pelleted selenium supplement (~43 ppm Se) or to an adequate selenium treatment group (ASe) that received a pelleted supplement containing no added selenium (~0.32 ppm Se). Selenium supplementation continued until the end of the study at day 135 of gestation. All ewe lambs received a similar diet consisting of chopped alfalfa hay top dressed with corn. On day 64 of gestation, ewe lambs were either assigned to a control-diet group that received 100% of NRC requirements (CON) for a gestating ewe lamb, or to a restricted-diet group that received 60% of this amount (RES). Dietary treatments also continued until the end of the study at day 135 (Figure 1). On day 135 of gestation, fetuses and placentomes were collected and weighed. Further, a portion of caruncular and

cotyledonary tissue was frozen for eNOS mRNA expression.

Experiment Two

For the second study, 64 Targhee-cross ewe lambs were assigned to a treatment. Treatments were similar to experiment one with the following exceptions. This study focused on the affects of maternal undernutrition and selenium intake, and it also took into account what role the stage of pregnancy played on the affects of these treatments. At breeding (day 0), ewe lambs were assigned to an adequate selenium group (ASe) or a high selenium group (HSe). Selenium treatments were given throughout the study until slaughter at day 130 of gestation. All ewes received 100% NRC requirements from breeding until day 50 of gestation at which time the plane of nutrition treatments were assigned. During mid-gestation, from days 50 to 90, ewes received 100% NRC requirements (CON) or 60% of this amount (RES). During late-gestation, from days 90 to 130, ewes received 100% NRC requirements (100) or 60% of this amount (60; Figure 2). On day 130 of gestation, fetuses and placentomes were collected and weighed, and caruncular and cotyledonary tissue was frozen for eNOS mRNA expression.

Results

In experiment one, fetal weight on day 135 of gestation from RES ewe lambs were reduced compared to the CON ewe lambs ($P = 0.02$; 11.58 vs. 13.16 \pm 0.42 lbs). However, placentome weights did not differ between groups. Placental efficiency, which is a measure of total fetal weight divided by total placentome weight, was reduced in the RES group compared to the CON group ($P = 0.06$; 10.42 vs. 11.53 \pm 0.40). Selenium levels did not have any effect on either fetal or placentome weight. Placental expression of eNOS was not different in any of the groups.

In experiment two, fetal weight on day 130 of gestation from the RES ewe lambs that were restricted during late-gestation were reduced compared to

the control ewe lambs ($P = 0.01$; 9.02 vs. 9.96 \pm 0.02 lbs). As in experiment one, placentome weights did not differ between groups. In this study, placental efficiency was not different. Selenium did not have an effect on fetal or placentome weights. The levels of eNOS expression in the maternal placental tissues (caruncle) at day 130 was higher ($P = 0.04$) in the group fed both ASe and the CON diet during late-gestation compared to all other groups (ASe-RES, HSe-CON, and HSe-RES) (Figure 3). During mid-gestation (Figure 4) and late-gestation (Figure 5), HSe-CON ewe lambs had reduced ($P = 0.04$) cotyledonary eNOS expression compared to the ASe ewes (Figure 4). RES ewes fed ASe or did not differ in cotyledonary eNOS expression.

Discussion

In both experiments, fetal weight was reduced in the restricted group, demonstrating that maternal nutrient restriction from day 64, or day 90, is important in determining fetal weight. It is interesting to note that in experiment two, when ewe lambs were restricted during mid-gestation, and then realimented, fetal weight was not altered, indicating that nutrient restriction from day 90 to 130 alone is enough time to reduce fetal weight by day 130.

Despite the fact that fetal weights were reduced, it appears that placental growth was spared, regardless of nutritional levels. Furthermore, even though placental weight was not impacted, the reduction in fetal weight could have been caused by one of two events: the amount of nutrients alone was not enough to attain proper fetal growth regardless of placental function; or if an excess of nutrients were available, placental function was altered such that adequate nutrient delivery was reduced. When nutrient restriction occurs earlier in pregnancy (~day 30 to 80), placental weight is reduced (Heasman et al., 1999) and reductions in vascularity of the placenta are observed in restricted ewes (Vonnahme et al., 2003). Furthermore, maternal nutrient restriction can result in reduced fetal weights during early

to mid pregnancy. These data, taken together with our data, suggest that placental weight may be dictated by nutritional levels earlier in pregnancy, and nutrient levels after day 50 are not restrictive of placental growth near term.

Even though maternal nutritional levels from mid to late pregnancy did not impact placental weight, we may have influenced placental function. We examined expression of eNOS in both the maternal and fetal tissues of the placenta. It appears that nutritional levels imposed after day 64 do not impact the expression of eNOS. However, in experiment two where nutrient restriction began 14 days earlier, placental eNOS expression was reduced in all late-gestation groups except for the overall control group (ASe-CON). This indicates that nutrient restriction, selenium intake, or the combination of the two, are altering eNOS expression. In the caruncular tissue, restricting intake and/or high selenium reduced eNOS expression. In the fetal placental tissues, eNOS expression in ASe-

CON and ASe-RES ewes did not differ, whereas eNOS expression in HSe-CON ewes was reduced compared to both, indicating that selenium may play more of a role in regulating eNOS expression in the cotyledon than nutrition. Selenoproteins have been shown to reduce reactive oxidative species, which includes nitric oxide; therefore, selenium may influence eNOS activity. Our laboratory is currently conducting further studies to investigate this hypothesis.

Our laboratory is also currently studying the offspring from ewe lambs used in a study similar to the ones described above to determine what long-term effects nutrient restriction and selenium may have on the health and productivity of the offspring. These studies have shown that post-natal physiology appears to be affected by both nutrient-restriction and selenium levels during pregnancy. Future studies involving the timing of restriction and realimentation in pregnancy are necessary in order to determine critical periods of adequate nutrition for proper fetal growth.

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