THE LONG-LASTING IMPACT OF NUTRITION: DEVELOPMENTAL PROGRAMMING

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Introduction

Livestock producers are interested in utilizing nutrients in the most efficient way to optimize growth. Often, one tends to focus on the growth that an animal achieves after birth, however, the majority of mammalian livestock (i.e. swine, sheep, and cattle) spend 35-40% of their life (i.e. from conception to consumption) within the uterus, being nourished solely by the placenta. The maternal system can be influenced by many different extrinsic factors, including nutritional status, which ultimately can program nutrient partitioning and ultimately growth, development and function of the major fetal organ systems (Wallace, 1948; Wallace et al., 1999; Godfrey and Barker, 2000; Wu et al., 2006). The trajectory of prenatal growth is sensitive to direct and indirect effects of maternal environment, particularly during early stages of embryonic life (Robinson et al., 1995), the time when placental growth is exponential. Moreover, pre-term delivery and fetal growth restriction are associated with greater risk of neonatal mortality and morbidity in livestock and humans. Offspring born at an above average weight have an increased chance of survival compared with those born at a below average weight in all domestic livestock species, including the cow, ewe, and sow. Just as growth-restricted human infants are at risk of immediate postnatal complications and diseases later in life (Godfrey and Barker, 2000), there is increasing evidence that production characteristics in our domestic livestock may also be impacted by maternal diet (Wu et al., 2006). Some of the complications reported in livestock include increased neonatal morbidities and mortalities (Hammer et al., 2011), intestinal and respiratory dysfunctions, slow postnatal growth, increased fat deposition, differing muscle fiber diameters and reduced meat quality (reviewed in Wu et al., 2006).

The objective of this proceedings paper is to highlight some of our laboratory's investigations on how maternal environment can impact fetal and placental development, impacts on uterine and/or umbilical blood flow in cattle and sheep, and potential timing of intervention, or potential therapeutics, which may increase uteroplacental blood flow.

Placental Development and Uteroplacental Blood Flow

The placenta plays a major role in the regulation of fetal growth. In ruminants, the fetal placenta attaches to discrete sites on the uterine wall called caruncles. These caruncles are aglandular sites which appear as knobs along the uterine luminal surface of non-pregnant animals, and are arranged in two dorsal and two ventral rows throughout the length of the uterine horns (Ford, 1999). The placental membranes attach at these sites via chorionic villi in areas termed cotyledons. The caruncular-cotyledonary unit is called a placentome and is the primary functional area of physiological exchanges between mother and fetus.

Placental nutrient transport efficiency is directly related to uteroplacental blood flow (Reynolds and Redmer, 1995). All of the respiratory gases, nutrients, and wastes that are exchanged between the maternal and fetal systems are transported via the uterus-placenta (Reynolds and Redmer, 1995, 2001). Thus, it is not surprising that fetal growth restriction in a number of experimental paradigms is highly correlated with reduced uteroplacental growth and development (Reynolds and Redmer, 1995, 2001). Establishment of functional fetal and uteroplacental circulations is one of the earliest events during embryonic/placental development (Patten, 1964; Ramsey, 1982). It has been shown that the large increase in transplacental exchange, which supports the exponential increase in fetal growth during the last half of gestation, depends primarily on the dramatic growth of the uteroplacental vascular beds during the first half of pregnancy (Meschia, 1983; Reynolds and Redmer, 1995). Therefore, an understanding of factors that impact uteroplacental blood flow will directly impact placental function and thus fetal growth.

Adequate uteroplacental blood flow is critical for normal fetal growth, and therefore, not surprisingly, experimental conditions designed to investigate fetal growth retardation and placental insufficiency, be it over-nutrition, nutrient restriction, hyperthermia, or high altitude, commonly share reduced uterine and umbilical blood flows (for review see Reynolds et al., 2006). Therefore, modifying uterine blood flow and nutrient transfer capacity in the placenta allows for increased delivery of oxygen and nutrients to the exponentially growing fetus. Fowden et al. (2006) reviewed key factors affecting placental nutrient transfer capacity, which were size, nutrient transporter abundance, nutrient synthesis and metabolism, and hormone synthesis and metabolism. Discovery of novel therapeutic agents that improve placental function would decrease the incidence of morbidity and mortality as well as suboptimal offspring growth performance in livestock species.

Therapeutic agents targeting placental blood flow increased fetal growth in compromised pregnancies (Reynolds et al., 2006). There is an ever-increasing wealth of data that are demonstrating how realimentation, or other therapeutic agents, may be used to rescue at-risk pregnancies. In our laboratory, we have investigated the role that realimentation, protein supplementation, and melatonin supplementation has on uteroplacental blood flow and/or vascular reactivity of the placental arteries. In order to perform the former, we have employed the use of Doppler ultrasonography. Other methods of determining blood flow are effective, but require surgery and increased numbers of animals to determine blood flow at different time points during pregnancy because of the growth of the uterine vasculature as gestation advances. Uterine and umbilical artery cardiac cycle waveforms were plotted in Doppler mode by velocity (cm/s; y-axis) and time (s; x-axis). Fetal or maternal heart rate (beats/min), pulsatility index (PI), resistance index (RI), and blood flow (BF) were calculated using preset functions on the ultrasound instrument. Abbreviations for the various instrument-generated functions are as follows: peak systolic velocity (PSV), end diastolic velocity (EDV), mean velocity (MnV), and cross sectional area of the vessel (CSA). Equations are as followed: PI = [PSV (cm/s) – EDV (cm/s)] / MnV (cm/s); RI = [PSV (cm/s) – EDV (cm/s)] / PSV (cm/s); blood flow (BF, mL/min) = MnV (cm/s) × cross sectional area of the vessel (cm2) × 60 s. By continuously monitoring the same animal, which has not undergone surgical manipulation, we feel that we can effectively determine how different interventions may regulate uteroplacental blood flow. Our current animal models are outlined below.

Nutrient Restriction

In normal pregnancies, resistance of the uteroplacental arteries have been documented to decrease as gestation advances. Our laboratory has reported that when pregnant ewe lambs are nutrient-restricted, lamb birth weight is reduced compared to control fed ewes (Swanson et al., 2008; Meyer et al., 2010). While placental weights are not different, we have demonstrated that when ewes are restricted, there is ~33% decrease in endothelial nitric oxide synthase mRNA expression on d 130 of gestation in the maternal portion of the placenta compared to control-fed animals (Lekatz et al., 2010a). We hypothesized that this reduction in birth weight was due to a greater placental vascular resistance, and decreased uteroplacental blood flow in restricted ewes compared to control ewes. In order to evaluate the effects of maternal nutrient restriction on the umbilical hemodynamics, we have a model of global restriction that begins on day 50 of gestation until term (~145 days). Restricted ewes had increased ($P = 0.01$) PI and RI compared to control ewes (Lekatz et al., 2010a; Lemley et al., 2012). Moreover, we have demonstrated that umbilical blood flow is reduced when a nutrient restriction is applied (Lemley et al., 2012).

Therapeutic supplements thought to target placental blood flow and nutrient delivery to the fetus have been shown to increase fetal growth in animal models of intrauterine growth restriction (Vosatka et al., 1998; Richter et al., 2009; Satterfield et al., 2010); however, few studies have addressed uteroplacental hemodynamics in models of improved fetal growth. For instance, melatonin supplementation was shown to negate the decreased birth weight in nutrient- restricted rats (Richter et al., 2009), which was attributed to increased placental antioxidant enzyme expression in nutrient-restricted rats supplemented with melatonin. Our hypothesis was that dietary melatonin treatment during a compromised pregnancy would improve fetal growth and placental nutrient transfer capacity by increasing uterine and umbilical blood flow. The uteroplacental hemodynamics and fetal growth were determined in ewes that received a dietary supplementation with or without melatonin (5 mg) in adequately fed (100% of NRC recommendations) or nutrient-restricted (60% of control) ewes. Dietary treatments were initiated on d 50 of gestation and umbilical blood flow, as well as fetal growth (measured by abdominal and biparietal distances) were determined every 10 d from d 50 to d 110 of gestation. By d 110 of gestation, fetuses from restricted ewes had a 9% reduction ($P = 0.01$) in abdominal diameter compared to fetuses from adequately nourished ewes, whereas fetuses from melatonin supplemented ewes tended to have ($P = 0.08$) a 9% increase in biparietal diameter (Lemley et al., 2012).

We did observe a significant melatonin treatment by day interaction ($P < 0.001$) for umbilical artery blood flow which was increased in melatonin supplemented ewes from day 60 through 110 of gestation compared to control (no melatonin supplementation). Moreover, at day 110 of gestation melatonin supplemented ewes had a 20% increase in umbilical artery blood flow compared to control ewes. In addition, a significant nutritional plane by day interaction ($P < 0.0001$) was observed for umbilical artery blood flow, which was decreased in restricted ewes from day 80 through 110 of gestation compared to adequately fed ewes. Moreover, at day 110 of gestation, restricted ewes had a 23% decrease in umbilical artery blood flow compared to adequately fed ewes (Lemley et al., 2012). While we are continuing our investigations into the impacts of melatonin supplementation in at-risk pregnancies, we feel that melatonin treatment may be useful in negating the consequences of intrauterine growth restriction that occur due to specific abnormalities in umbilical blood flow.

In cattle, we have recently demonstrated that nutrient restriction from early to mid-pregnancy (i.e. day 30-140) does not alter uterine blood flow (Camacho et al., 2014). However, upon realimentation, the uterine artery blood flow increases in those cows that were previously restricted, but only to the horn in which the calf is housed (Camacho et al., 2014). Interestingly, it appears that realimentation alters the growth trajectory of the bovine placenta (Vonnahme et la., 2007), something that has not been investigated in the ewe. Recent data in our laboratory demonstrates that the placental artery reactivity to vasoactive agents in vitro are more responsive to vasodilators (Reyaz and Vonnahme, unpublished data), and there is an increase in capillary numbers (Mordhorst and Vonnahme, unpublished data), perhaps to allow for more nutrient uptake. The ability of the uterus-placenta to compensate upon realimentation is quite intriguing and we are continuing our studies to determine which portions of the placenta (i.e. maternal or fetal) may contribute to compensatory prenatal growth of the fetus.

Protein Supplementation

While the literature is now booming with increasing evidence of how nutrient restriction impairs several physiological parameters, few concentrate on enhancing postnatal growth in livestock species. In a recent series of papers in cattle, cows gestated on range (where crude protein of forage is $< 6\%$) that were protein supplemented during late gestation had calves similar in birth weight, but had calves with increased weaning weight compared to protein unsupplemented cows (Stalker et al., 2006, Martin et al., 2007; Larson et al., 2009). It is valuable to note that the protein supplementation enhanced growth after birth. Furthermore, the pregnancy rates in heifer calves born from protein supplemented cows were enhanced compared to control cows (93 vs 80%; Martin et al., 2007). It was our hypothesis that the increased fertility and growth rate of the calves from supplemented dams may be due to enhanced uterine blood flow and/or placental nutrient transfer. Ongoing studies in our laboratory are investigating how protein supplementation during late gestation can impact uterine blood flow. For the past 2 years we have investigated how protein supplementation (in the form of DDGS) can impact uterine blood flow. When we use DDGS with a low quality forage source, uterine blood flow is reduced compared to control cows (Mordhorst and Vonnahme, unpublished observations). Just recently, we demonstrated that when DDGS is given with a corn-stalk forage base, we increase uterine blood flow (Kennedy and Vonnahme, unpublished observations). We are investigating how specific nutrients differed between these 2 studies in order to tease apart the mechanism that may be impacting how protein influences uterine blood flow in the beef cow.

In order to more fully understand the impacts of maternal protein on uteroplacental blood flow and placental vascular development, we also have used an ovine model where the diets are isocaloric, with differing levels of protein in the diet. Singleton fetuses from ewes consuming the high protein diet are heavier on d 130 of gestation compared to fetuses from ewes consuming the low protein diet, with no differences in placental weight apparent (Camacho et al., 2010). When uterine blood flow was obtained from a single time point (d 130 of gestation), ewes consuming the high protein diet had a decrease in uterine blood flow compared to the low group, with the control being intermediate (Camacho et al., 2010). This is similar to our first year protein supplementation work with beef cattle. Moreover, when investigating the ability of the fetal placental arteries to vasodilate to increasing concentrations of bradykinin, placental arteries from high protein ewes had a decreased responsiveness compared to control and low protein ewes (Lekatz et al., 2010b). Understanding if additional calories (i.e. cow study), or a greater proportion of total calories coming from protein (i.e. sheep study), needs to be elucidated, and further work is underway in our laboratory.

Summary and Conclusions

We hope to improve approaches to management of livestock during pregnancy which may impact not only that dam's reproductive success, but her offspring's growth potential and performance later in life. Future applications of this research may be used to develop therapeutics for at-risk pregnancies in our domestic livestock. If these therapeutics can be used on-farm, producers would have the ability to increase animal health while also reducing costs of animal production. While each species is unique in its placental development and vascularity, comparative studies may ultimately assist researchers in understanding how the maternal environmental impacts placental, and thus fetal, development.

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