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Impacts of maternal nutritional plane on umbilical artery hemodynamics, fetal and placentome growth in sheep

L.A. Lekatz[1], J.S. Luther[1,2], J.S. Caton[1], K.A. Vonnahme[1,3]

[1]Center for Nutrition and Pregnancy, Department of Animal Sciences, North Dakota State University, Fargo, ND, USA.
[2]Department of Animal and Food Science, University of Wisconsin, River Falls, WI, USA.

Abstract

The present study aimed to examine the impact of maternal nutritional plane on umbilical hemodynamics. Ewes (n = 15) were assigned to 1 of 3 dietary treatments [control (CON; 100% of NRC requirements), restricted (RES; 60% of CON) or overfed (OVR; 140% of CON)] beginning on day 40 of gestation. Umbilical artery hemodynamics, fetal growth and placentome growth were measured on days 40, 45, 52, 80, 94 and 108 of gestation by Doppler ultrasonography. The percentage change in umbilical artery pulsatility and resistance indices remained steady through day 80 of gestation, and then decreased (P < 0.03) by day 108 of gestation. Moreover, plane of nutrition affected (P < 0.03) the percentage change in pulsatility index from day 40, with RES ewes having a greater (P < 0.03) change compared to CON (16.7 ± 7.5 *vs*. -15.6 ± 7.5%), with OVR being intermediate (3.2 ± 7.5%). Fetal biparietal and abdominal diameters increased (P < 0.01) throughout gestation, and fetal heart rate decreased (P < 0.01) from day 52 to 108 of gestation. Placentome diameter increased (P < 0.01) through day 80 of gestation, was similar (P > 0.05) on days 80 and 94 of gestation, and then decreased (P < 0.01) by day 108 of gestation. Maternal plane of nutrition can impact umbilical resistance indices, and ultimately may impact blood flow to the fetus.

Keywords: maternal nutrition, pulsatility index, resistance index, sheep, umbilical artery.

Introduction

Adequate blood flow to the fetus is critical for normal growth and development during gestation. In normal pregnancies, uterine and umbilical blood flows increase exponentially throughout gestation (Reynolds *et al*., 1995); however, in models of intrauterine growth restriction (IUGR) caused by maternal undernutrition or overnutrition, blood flow to the fetus is reduced (Chandler *et al*., 1985; Leury *et al*., 1990; Wallace *et al*., 2001; Carr *et al*., 2012; Lemley *et al*., 2012).

The pulsatility index (PI) and resistance index (RI) are pulsed-wave Doppler measurements of downstream resistance in arteries. These indices normally decline as gestation progresses and correlate positively with measured umbilical vascular resistance and negatively with umbilical blood flow (Newnham *et al*., 1987; Wallace *et al*., 2001; Acharya *et al*., 2004). Few studies have looked at umbilical artery PI and RI in sheep IUGR models, but Galan *et al*. (1998, 2005) and Carr *et al*. (2012) concluded that these indices are increased in the umbilical artery of compromised pregnancies.

We have demonstrated that first parity ewes fed 40% more, or 40% less, than adequately fed ewes have lambs that are growth restricted at or near term (Lekatz *et al*., 2010a, b; Meyer *et al*., 2010, Lemley *et al*., 2012). We hypothesized that the plane of nutrition will alter the umbilical artery PI and RI, which could have severe implications on blood flow to the fetus.

Materials and Methods

The NDSU Animal Care and Use Committee approved all animal procedures for this study (#A0617). Nulliparous, Rambouillet ewes with similar (P > 0.05) body weights (52.6 ± 1.7 kg) carrying singleton fetuses (n = 15) were individually housed and on day 40 of gestation, ewes were assigned randomly to 1 of 3 nutritional plane treatments supplying 60% (RES, n = 5), 100% (CON, n = 5) or 140% (OVR, n = 5) of global nutritional requirements (National Research Council - NRC, 1985; Meyer *et al*., 2010). Ewes had free access to water and a trace mineralized salt block (Roto Salt Company, Penn Yan, NY, USA). Diets were fed once daily at 8 h in a complete pelleted ration. Ewes were weighed on day 40 of gestation and then every 7 or 14 days until day 130 at which time ewes were weighed for a final ewe weight. Ewe body condition score was assigned (1 to 5 scale; 1 = emaciated, 5 = obese) by two trained technicians on days 40, 68, 96, 124 and 130, and the two body condition scores were averaged for each day. Diets were adjusted for body weight every 14 days.

Umbilical artery hemodynamics were assessed using a duplex B-mode (brightness mode) and D-mode (Doppler spectrum) program of a color Doppler ultrasound instrument (model SSD-3500; Aloka America, Wallingford, CT, USA) fitted with a 7.5-MHz finger transducer (Aloka UST-672) as previously described (Lemley *et al*., 2012). Ultrasonography

[3]Corresponding author: Kim.Vonnahme@ndsu.edu
Phone: +1(701)231-5883; Fax: +1(701)231-7590
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evaluations took place on days 40, 45, 52, 80, 94 and 108 of gestation. All dams were scanned transabdominally. Briefly, for each ultrasound examination dams were placed into an elevated crate, wool from the abdomen and rear flanks was removed, skin cleaned with soapy water and sufficient Aquasonic transmission gel (Parker Laboratories, Fairfield, NJ, USA) was applied to the probe. Three similar cardiac cycle waveforms were obtained and averaged per ewe within a gestation day. Cardiac cycle waveforms were plotted in D-mode by velocity (cm/s; y-axis) and time (s; x-axis). Fetal heart rate (beats/min), pulsatility index (PI) and resistance index (RI) were calculated using preset functions on the instrument (Fig. 1). Abbreviations for equations are: peak systolic velocity (PSV), end-diastolic velocity (EDV) and mean velocity (MnV). Equations are as follows: PI = [PSV (cm/s) – EDV (cm/s)] / MnV (cm/s); RI = [PSV (cm/s) – EDV (cm/s)] / PSV (cm/s). Percentage change in PI and RI were calculated as: [(value on any day – day 40 value) / day 40 value] × 100.

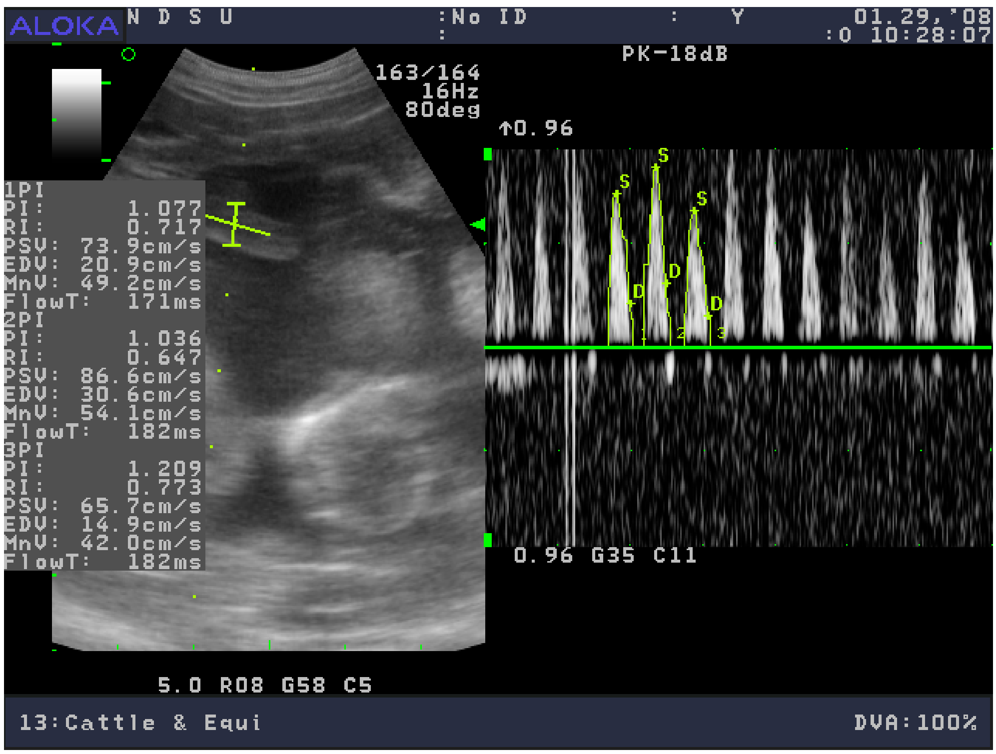

Figure 1. An image of umbilical cord hemodynamics with pulsatility index (PI) and resistance index (RI) measurements present on the left hand side. The umbilical cord was located, the angle of insonation was determined, and wave forms of the cardiac cycle were recorded. Measurements for at least 3 waveforms were averaged to determine PI and RI.

Placental and fetal growth parameters were assessed using B-mode. Fetal abdominal diameter was recorded at the base of the rib cage and above the entry point of the umbilicus (Fig. 2). Fetal biparietal distance was also determined (Fig. 3). Average placentome diameter was determined by randomly selecting ten placentomes and recording the diameter at the largest position.

The effects of nutritional plane and day of gestation on ewe body weight, percentage change from day 40 in ewe body weight, ewe body condition score percentage change from day 40 in ewe body condition score, percentage change from day 40 in umbilical artery PI and RI, fetal abdominal diameter, biparietal diameter, heart rate and average placentome diameter were analyzed with the MIXED procedure of SAS (SAS software version 9.2; SAS Institute, Cary, NC, USA) using repeated-measures ANOVA (day 40 to 108 of gestation), and if the F test was significant, means were separated using the PDIFF option of the LSMEANS statement. The model statement included nutritional plane, day of gestation and their interaction. Least square means and SEM are reported. Statistical significance was declared at $P < 0.05$.

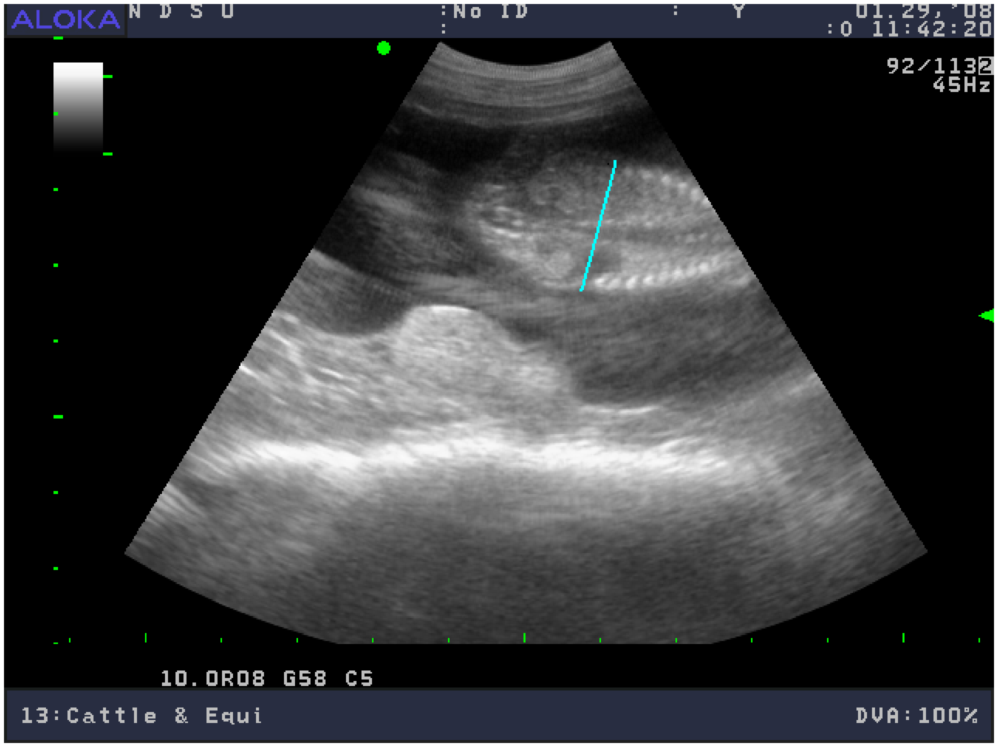

Figure 2. An image of a day 70 fetus. The blue line indicates the fetal abdominal width that was obtained by measuring across the body at the umbilicus. Left of the blue line, the outline of the kidney is apparent.

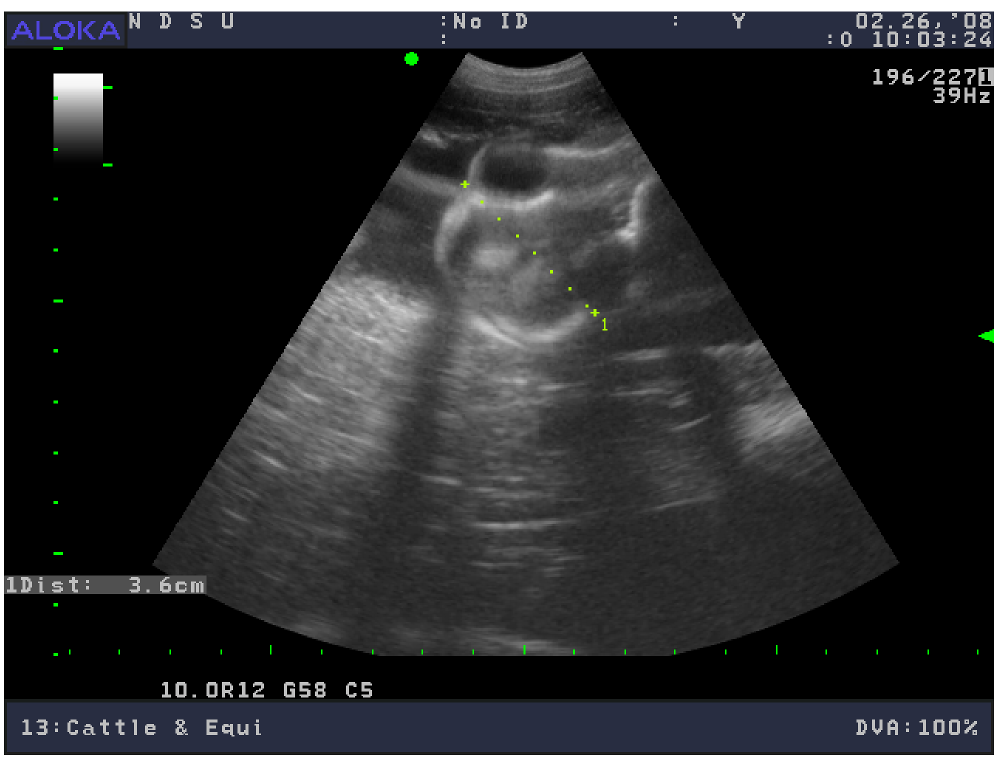

Figure 3. An image of a day 90 fetus where fetal biparietal diameter was obtained by measuring the width of the frontal bone immediately dorsal to the orbital sinus.

Results

There was a nutritional plane by day of gestation interaction (P < 0.01) for ewe body weight (Fig. 4A), percentage change from day 40 in ewe body weight (Fig. 4B), ewe body condition score (Fig. 4C) and percentage change from day 40 in ewe body condition score (Fig. 4D). Final ewe body weight was lower (P < 0.01) in the RES ewes compared to the CON and OVR ewes, which did not differ (50.0 ± 2.8 *vs*. 59.7 ± 2.8 and 63.8 ± 2.8 kg for RES, CON, and OVR, respectively). The percentage change from day 40 to 130 in ewe body weight was different (P < 0.01) among all three groups (-3.7 ± 2.2, 9.6 ± 2.2, and 25.0 ± 2.2% for RES, CON, and OVR, respectively). Ewe body condition score on day 130 and percentage change from day 40 to 130 of gestation in ewe body condition score were different among all three groups (body condition score: 2.4 ± 0.2, 3.7 ± 0.2, and 4.3 ± 0.2 for RES, CON, and OVR, respectively and percentage change in body condition score: -30.3 ± 5.3, 4.7 ± 5.3, 26.5 ± 5.3% for RES, CON, and OVR, respectively).

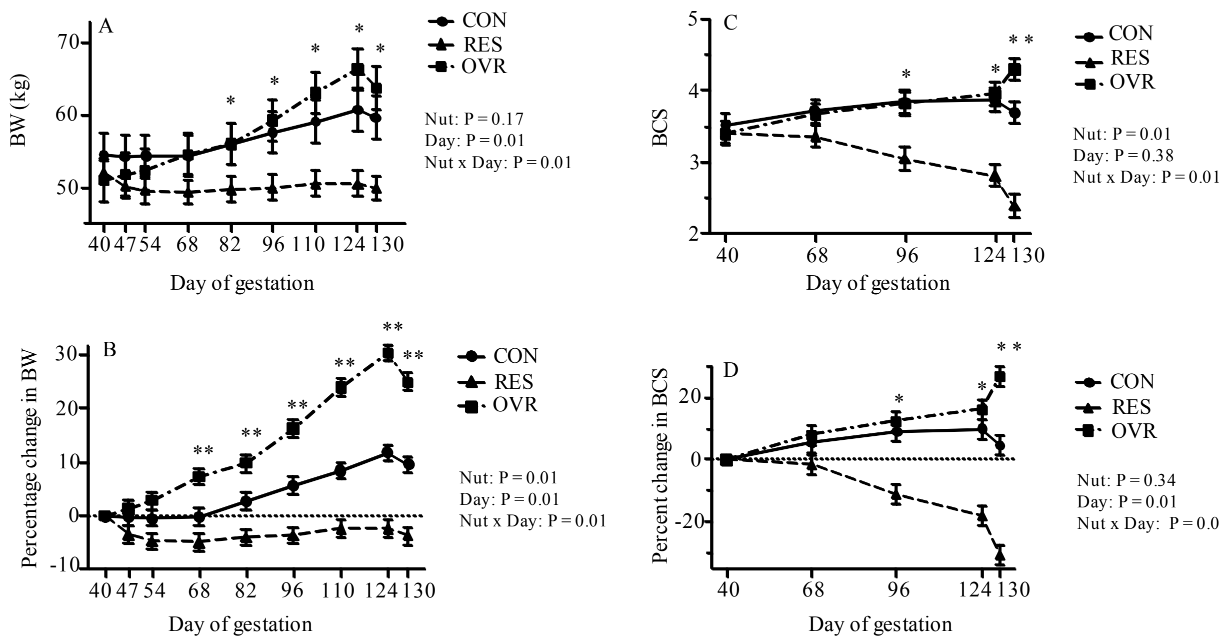

Figure 4. The interaction of maternal nutritional plane by day of gestation [Control (CON) = 100% of energy requirements; restricted (RES) = 60% of CON; overfed (OVR) = 140% of CON] on ewe body weight (A; BW), percentage change from day 40 in ewe body weight (B), ewe body condition score (C; BCS) and percentage change from day 40 in ewe body condition score (D). Percentage change in ewe body weight and ewe body condition score were calculated as [(value on any day - day 40 value) / day 40 value] × 100. *Means between RES and CON and between RES and OVR were different (P < 0.05) on that particular day, with CON and OVR being similar (P > 0.05). **Means between RES and CON, RES and OVR, and CON and OVR were different (P < 0.05) on that particular day.

The nutritional plane by day of gestation interaction was not significant for either the percentage change in umbilical artery PI (P > 0.05; Fig. 5A) or RI (P > 0.05; Fig. 5D). Both the percentage change in PI and RI were affected by day of gestation (P < 0.03; Fig. 5B and 5E). Similar patterns were observed for percentage change in PI and RI with values remaining steady through day 80 of gestation when values numerically peaked and decreased thereafter (Fig. 5B and 5E). Plane of nutrition also affected the percentage change in umbilical artery PI (P < 0.03; Fig. 5C) as CON ewes decreased and RES ewes increased PI (16.7 ± 7.5 *vs*. -15.6 ± 7.5%) with OVR being intermediate (3.2 ± 7.5%). The effect of plane of nutrition on the percentage change in RI followed a similar pattern to that of percentage change in PI, but was not different (P > 0.05; Fig. 5F).

There was no plane of nutrition by day of gestation interaction (P > 0.05) or main effects (P > 0.05) for fetal biparietal diameter, abdominal diameter, heart rate measurements or placentome diameter. As gestation advanced, fetal biparietal and abdominal diameters increased (P < 0.01) and fetal heart rate decreased (P < 0.01) from day 52 to 108 (Table 1). Placentome diameter increased (P < 0.01) through day 80 of gestation, was similar (P > 0.05) on days 80 and 94 of gestation and then decreased (P < 0.01) by day 108 of gestation (Table 1).

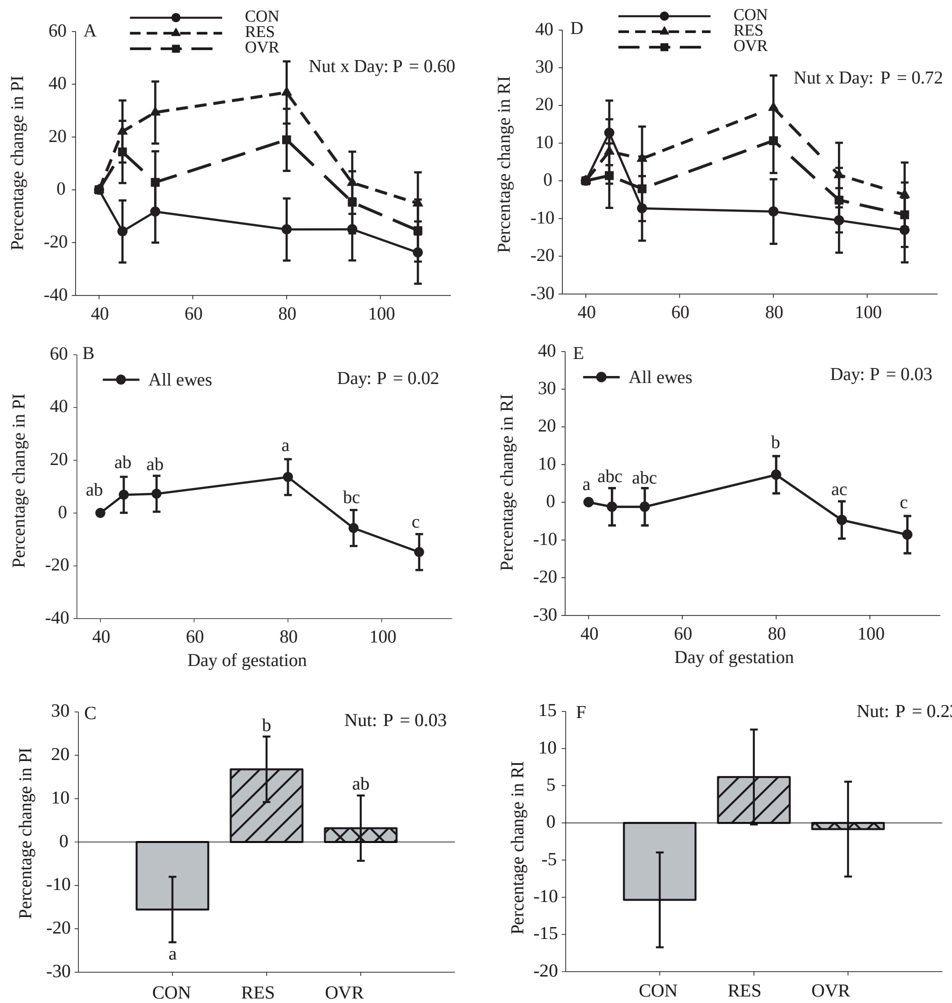

Figure 5. The impacts of maternal nutritional plane [Control (CON) = 100% of energy requirements; restricted (RES) = 60% of CON; overfed (OVR) = 140% of CON; C and F] and day (40, 45, 52, 80, 94 and 108 of gestation; B and E) and their interaction (A and D) on percentage change from day 40 in pulsatility index (PI; A, B, and C) and resistance index (RI; D, E, and F). Percentage change in PI and RI were calculated as [(value on any day – day 40 value) / day 40 value] × 100. Plane of nutrition dietary treatments were initiated on day 40. [abc]LSMeans with different subscripts differ by P < 0.05.

Table 1. Fetal biparietal diameter, abdominal diameter, heart rate, and placentome diameter on days 40, 45, 52, 80, 94, and 108 of gestation.

	Measurement							
Day of gestation	Biparietal diameter (cm)	SEM	Abdominal diameter (cm)	SEM	Heart rate (beats per minute)	SEM	Placentome diameter[1] (cm)	SEM
40	1.22[a]	0.03	1.27[a]	0.03	219[a]	1.06	1.12[a]	0.060
45	1.74[b]	0.04	1.74[b]	0.02	222[a]	2.62	1.73[b]	0.058
52	2.02[c]	0.03	2.37[c]	0.04	221[a]	1.89	2.17[c]	0.058
80	3.73[d]	0.09	4.58[d]	0.08	199[b]	3.14	2.70[d]	0.058
94	4.31[e]	0.11	6.71[e]	0.12	183[c]	3.00	2.68[d]	0.058
108	5.20[f]	0.11	7.33[f]	0.17	178[c]	2.63	2.42[e]	0.058

[abcdef]LSMeans with different superscripts differ by P < 0.05. Biparietal diameter P-values: Nutrition by day interaction P > 0.05; Nutrition P > 0.05; Day P < 0.01. Abdominal diameter P-values: Nutrition by day interaction P > 0.05; Nutrition P > 0.05; Day P < 0.01. Heart rate P-values: Nutrition by day interaction P > 0.05; Nutrition P > 0.05; Day P < 0.01. Placentome diameter P-values: Nutrition by day interaction P > 0.05; Nutrition P > 0.05; Day P < 0.01. Average placentome diameter was obtained by measuring the widest diameter of ten different placentomes and calculating the average.

Discussion

Relatively few IUGR models in sheep have used Doppler ultrasonography to measure fetal and placental growth and umbilical artery indices throughout gestation. The limited data available, including the present study, are in agreement that fetal growth measurements increase with advancing gestation, placentome size peaks around day 80 of gestation and umbilical artery PI and RI decrease throughout gestation in normal pregnancies (Galan *et al*., 1998, 2005; Carr *et al*., 2012; Lemley *et al*., 2012).

The ewes in this study were part of a larger study (Meyer *et al*., 2010) utilizing 84 ewes. The observed patterns for ewe body weight, percentage change from day 40 in ewe body weight, ewe body condition score and percentage change from day 40 in ewe body condition score were expected due to the design of the study (Meyer *et al*., 2010).

In the present study, fetal biparietal and abdominal diameters, measured via Doppler ultrasonography, increased with advancing gestation. This is in agreement with Carr *et al*. (2012) and Lemley *et al*. (2012) who also used Doppler ultrasonography to measure fetal growth throughout gestation. Using an established model of overfeeding adolescent ewes to result in IUGR, Carr *et al*. (2012) observed IUGR fetuses had smaller abdominal circumferences by day 98 of gestation and smaller biparietal diameters by day 110 of gestation compared to fetuses from control ewes, and these reductions in fetal growth remained through the end of ultrasound measurements on day 126 of gestation. When ewes were nutrient restricted beginning on day 50 of gestation, reductions in fetal abdominal girth were not observed until day 110 of gestation (Lemley *et al*., 2012). In the present study, maternal plane of nutrition did not alter fetal biparietal and abdominal diameters; however, ultrasound measurements ended on day 108 of gestation. Because maternal nutrition did not influence fetal growth measurements until later in gestation (Carr *et al*., 2012; Lemley *et al*., 2012), it is likely that differences in fetal growth measurements would have been observed in the present study if ultrasound measurements continued past day 108 of gestation.

Placentome diameter increased from day 40 to 80, and then decreased by day 108 of gestation. Similar patterns of placental growth have been reported in studies measuring placentome diameter with ultrasound technology (Doizé *et al*., 1997; Carr *et al*., 2012; Lemley *et al*., 2012). The percentage change in PI and RI also peaked on day 80 of gestation before decreasing through day 108 of gestation. This is similar to Lemley *et al*. (2012), where RI peaked at the time when placentome size peaked. After attainment of maximal size, ovine placentomes enhance their vascular development (Borowicz *et al*., 2007). Perhaps this increase in vascularity allows for the decrease in resistance indices as gestation advances (Newnham *et al*., 1987; Wallace *et al*., 2001; Acharya *et al*., 2004).

Resistance indices (i.e., PI and RI) decreased ~10-15% in CON ewes, whereas a similar increase was observed in RES ewes. Because Doppler-derived measurements of PI and RI correlate negatively to blood flow (Acharya *et al*., 2004), these results could indicate reduced umbilical blood flow (Lemley *et al*., 2012) or reduced placentome vascularity (Luther *et al*., 2007) in the RES ewes compared to CON. Because differences in PI and RI are observed before differences in fetal size (Carr *et al*., 2012; Lemley *et al*., 2012), the use of these Doppler-derived indices may serve as an easy and noninvasive method to recognize compromised pregnancies earlier in gestation.

In conclusion, maternal nutritional plane appears to impact resistance indices of the umbilical artery in sheep. More studies are needed to determine how monitoring umbilical resistance could help improve fetal outcomes.

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