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Effects of maternal nutrient restriction followed by realimentation during midgestation on uterine blood flow in beef cows

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ABSTRACT

The objective was to examine the effect of maternal nutrient restriction followed by realimentation during midgestation on uterine blood flow (BF). On Day 30 of pregnancy, lactating, multiparous Simmental beef cows were assigned randomly to treatments: control (CON; 100% National Research Council; $n = 6$) and nutrient restriction (RES; 60% of CON; $n = 4$) from Day 30 to 140 (period 1), and thereafter, realimented to CON until Day 198 of gestation (period 2). Uterine BF, pulsatility index (PI), and resistance index (RI) were obtained from both the ipsilateral and contralateral uterine arteries via Doppler ultrasonography. Generalized least square analysis was performed. Ipsilateral uterine BF in both groups increased quadratically ($P < 0.01$) during period 1 and linearly ($P < 0.01$) during period 2. There was a treatment ($P = 0.05$) effect during period 2; where RES cows had greater ipsilateral BF versus CON. Ipsilateral uterine PI and RI decreased linearly ($P \leq 0.01$) during period 1 across treatments. Contralateral uterine BF in CON cows tended ($P < 0.09$) to be greater versus RES in both periods. Contralateral PI in both groups increased linearly ($P \leq 0.01$) during period 1. Contralateral uterine RI was increased ($P \leq 0.05$) in RES cows versus CON in both periods. There was no interaction or treatment effect ($P \geq 0.24$) for total BF during either period. Nutrient restriction does not alter total uterine BF, but it may increase vascular resistance. However, up on realimentation, local conceptus-derived vasoactive factors appear to influence ipsilateral uterine BF.

1. Introduction

Beef cows are commonly managed in grazing systems where the quality of forage varies according to the regional conditions, and this can negatively impact the nutritional and physiological status of the dam and the development of their offspring [1]. Intrauterine growth restriction is associated with altered fetal organ development and subsequent performance of offspring [2,3]. The most common and easiest therapeutic to administer is to realiment the undernourished dam; however, there is a scarcity of information on how realimentation impacts placental and fetal development. From a clinical perspective, if at-risk pregnancies could be detected early, therapeutics, which could simply be offering more feed, could be applied. Vonnahme, et al. [4] demonstrated that placental vascularity was augmented when previously restricted beef cows were realimented to nutritional planes similar to controls, but data in other mammals are largely lacking.

Placental nutrient transport efficiency is directly related to uteroplacental blood flow (BF; [5]). Increases in transplacental

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exchange, which support the rapid increase in fetal growth during the last half of gestation, depend primarily on growth of the placenta during early pregnancy followed by dramatic development and reorganization of the uteroplacental vasculature during the last half of gestation [5,6].

Color Doppler ultrasonography is a noninvasive technique, which has been used to measure uterine BF and arterial indices of resistance in cattle [7–9]. However, to our knowledge, uterine BF in models of nutrient restriction has not been measured up on realimentation in any species. We hypothesized that uterine BF in nutrient restricted cows would be reduced during the restriction period, but up on realimentation, uterine BF would surpass that of adequately fed control cows. The objective of this study was to examine the effect of maternal nutrient restriction followed by realimentation during midgestation on uterine BF and other hemodynamics.

2. Materials and methods

All animal procedures were approved by the North Dakota State University (NDSU) Animal Care and Use Committee (#A12046).

2.1. Animals and management

A total of 18 lactating, multiparous Simmental beef cows were transported from the NDSU Beef Research and Teaching Unit (Fargo, ND, USA) to the NDSU Beef Cattle Research Complex within 3 days of artificial insemination. All cows were artificially inseminated the same day (April 13, 2012) by two different sires. On arrival, radio frequency identification tags were placed in the right ear of cows, and body weight (BW) was measured. Cows were placed in a pen equipped with eight individual Insentec roughage intake control system feeders (Insentec B.V., Marknesse, Netherlands). Cows were trained to use the Insentec system, and fed a common diet until Day 30 of gestation. If cows did not train to the system, they were removed from project (n = 6). Cows were limit fed using the Insentec feeding system to provide the desired net energy (NE) intake. Dietary NE of grass hay was estimated using approaches described by Weiss, et al. [10] and National Research Council [11]. Limestone was added to the total mixed diet to maintain a Ca-to-P ratio of approximately 1.3:1. Cows were fed once daily at 8 AM, and had free access to water and traced mineralized salt blocks (American Stockman, North American Salt Company, Overland Park, KS, USA; 95.5%–98.5% NaCl, 3500 mg/kg Zn, 2000 mg/kg Fe, 1800 mg/kg Mn, 280–420 mg/kg Cu, 100 mg/kg I, 60 mg/kg Co).

On Day 27 and 28 postinsemination, pregnancy was confirmed via transrectal ultrasonography (500-SSV; Aloka, Tokyo, Japan) using a linear transducer probe (5 MHz). Moreover, the CL was identified, and the gravid uterine horn was determined so that the ipsilateral uterine artery could be identified. On Day 30 of pregnancy, 12 lactating (714.8 ± 23.4 kg of BW), multiparous (parity 4.7 ± 3.3) beef cows were assigned randomly to dietary treatments: control (CON; n = 6) and nutrient restriction (RES; n = 6) from Day 30 to 140 (period 1), and thereafter, realimented to control until Day 198 (period 2) of gestation. Cows were fed the same diet (Table 1) at either 100% or 60% of National Research Council recommendations for NE for maintenance, lactation (until weaned at Day 90), and fetal growth [11], and to meet or exceed the recommendations for metabolizable protein. Feed intake was adjusted relative to predicted NE requirements for the following periods (Days 30–85, Days 86–140, and Days 141–198 of gestation). Per experimental design, dry matter (DM) intake in period one was reduced ($P = 0.05$) in RES cows compared with CON (6.01 vs. 12.02 ± 0.45 kg DM). This resulted in RES cows consuming less ($P < 0.01$) as a percentage of BW compared with CON (1.00% vs. 1.75 ± 0.02% of DM per kg BW). During period 2, formerly RES cows continued to have less ($P = 0.05$) DM intake than CON (8.22 vs. 10.13 ± 0.65 kg DM); however as a percentage of DM per BW, they were similar ($P = 0.22$; 1.54% vs. 1.63 ± 0.05%, RES vs. CON cows, respectively). On Day 90 of gestation, all calves were weaned, and diets were adjusted to meet their nutrient requirements according to their stage of gestation.

Body condition score (BCS) was estimated monthly using a 1 to 9 scale (with 1 = emaciated and 9 = obese; [12]) from Day 30 to 198 of gestation. Cows were weighed every 2 weeks at approximately 7 AM throughout the experiment and dietary intake adjusted relative to BW. Percentage of BW change was calculated by BW difference (final BW–initial BW) divided by initial BW times 100, where initial BW was BW at Day 30 of gestation. At Day 198, all cows were fed a common diet until calving.

2.2. Feed analysis

Diet samples were collected weekly and dried in a 55 °C oven, ground to pass a 1-mm screen, and analyzed for DM, ash, and crude protein (Kjeldahl) by standard procedures [13]. Neutral detergent fiber and acid detergent fiber concentration was determined by the method of Robertson and Van Soest [14] using an Ankom fiber analyzer (Ankom Technology Corp., Fairport, NY, USA).

2.3. Ultrasonography evaluation

Hemodynamic measurements of the uterine artery ipsilateral and contralateral to the conceptus were obtained via a color Doppler ultrasonography (model SSD-3500; Aloka America, Wallingford, CT, USA) fitted with a 7.5 MHz finger transducer (Aloka UST-995) on Days 30, 58, 86, 114, 140, 152, 159, 166, and 198 of gestation. Ultrasonic evaluations were taken at the same time of day between 8 AM and 12 PM, and lasted approximately 30 minutes per cow.

Table 1
Diet composition and nutrient analysis.

Ingredient	% of dietary dry matter
Grass hay	92.5
Corn condensed distiller's solubles	7.0
Limestone	0.5
Analyses	
Ash, %	11.5
Crude protein, %	9.3
Neutral detergent fiber, %	67.3
Acid detergent fiber, %	40.1

Cows were examined via ultrasonography within 2 days of every reported sampling time. Briefly, the probe was inserted through the rectum, and the aorta was located. In B mode using the finger probe, the origin of the external iliac, ipsilateral to the gravid uterine horn, was located, and the transducer was moved caudally to locate the internal iliac artery. The umbilical artery begins as a major branch of the internal iliac, and gives rise to the uterine artery [7]. After the uterine artery was identified as a movable and pulsating artery, a longitudinal section was visualized by manually turning the transducer of the probe. The probe was aligned to the uterine artery at an average angle of insonation of $79.0 \pm 0.2^\circ$, and uterine artery hemodynamic measurements were collected.

Three similar cardiac cycle waveforms from three separate ultrasonography evaluations from each side (ipsilateral and contralateral uterine artery) were obtained with spectral Doppler, and averaged per cow within a gestational day (nine measurements per side per sampling day). Maternal heart rate (HR), pulsatility index (PI), resistance index (RI), and uterine artery BF were calculated by preprogrammed Doppler software where PI = (peak systolic velocity − end diastolic velocity)/mean velocity; RI = (peak systolic velocity − end diastolic velocity)/peak systolic velocity; and BF (mL/min) = mean velocity (cm/s) × cross-sectional area (CSA; cm^2) × 60 seconds. Total BF was calculated as the sum of ipsilateral and contralateral uterine artery BF.

2.4. Statistical analysis

One RES cow was removed from the project because she was carrying twins, and one RES cow was removed from the project because of early embryonic loss. At completion of the project, there were six CON cows and four RES cows. All data were analyzed as a completely randomized design using generalized least squares (MIXED procedure; SAS Inst. Inc., Cary, NC, USA). Effects of maternal nutrition on dependent variables were examined by repeated measures analysis within periods (period 1 = nutrient restriction and period 2 = realimentation). Factors included in the model were treatment (i.e., CON and RES), day, sire, and treatment by day, where day was the repeated variable, and cow nested within treatment was the subject. Fetal sire had no influence ($P > 0.25$) on the variables tested, and therefore was removed from all model statements. Appropriate (minimize information criterion) covariance structures were used. Linear and quadratic coefficients for day effects were constructed for unequally spaced orthogonal polynomial contrasts for dependent variables. Regression coefficient solutions for significant polynomials were generated using day as a continuous variable (removing day as a classification variable), and is presented in figures. P values were considered significant if it was less than 0.05 and a tendency when P was greater than 0.05 and less than 0.10.

3. Results

3.1. Cow BW and BCS

During period 1 (restriction), there was no treatment by day interaction ($P = 0.68$; Fig. 1A) for maternal BW. However, there was a treatment effect ($P = 0.04$), where RES cows were lighter than CON cows during the restriction period. Also, there was a linear day ($P < 0.01$) response, where BW decreased linearly regardless of nutritional treatment during period 1. During period 2, there was no treatment by day interaction ($P = 0.79$; Fig. 1A) for maternal BW. Similar to period 1 there was a treatment effect ($P = 0.05$), where RES cows were lighter compared with CON cows during period 2. There was a linear day ($P < 0.01$) response, where both treatment groups had a linear increase in BW during period 2. When BW was expressed as percentage BW change, there was a treatment by linear day ($P = 0.04$; Fig. 1B) response, where both treatment groups had a linear decrease in percentage BW change with RES cows having a greater rate of change compared with CON cows. During period 2, there was no treatment by day interaction ($P = 0.93$) or treatment effect ($P = 0.13$). But, there was a linear day ($P = 0.02$) response, where both treatment groups had a linear increase in percentage BW change during period 2. Maternal BCS during period one showed a treatment by linear day ($P < 0.01$) response. Cows from RES group had a greater linear decrease in BCS compared with CON cows during period 1. During period 2, there was no treatment by day interaction ($P = 0.69$) or day effect ($P = 0.20$). However, there was a treatment effect ($P = 0.02$), where RES cows continued to have lower BCS compared with RES cows during period 2.

3.2. Blood flow and resistance indices

There was no treatment by day interaction ($P = 0.98$; Fig. 2A) or treatment effect ($P = 0.81$) during period 1 for ipsilateral uterine artery BF. However, there was a linear and quadratic day response ($P \leq 0.01$), where ipsilateral uterine BF increased during the restriction period for both treatment groups. During period 2, although there was no treatment by day interaction ($P = 0.86$), there was a treatment effect ($P = 0.05$), where RES cows had greater ipsilateral uterine BF compared with CON after being realimented. There was also a linear day response ($P < 0.01$) for ipsilateral uterine artery BF. Both treatment groups had a linear increase in BF during period 2. There was no treatment by day interaction ($P = 0.60$; Fig. 2B) but there was a treatment effect ($P = 0.04$) for ipsilateral uterine artery CSA during period 1. The RES cows had bigger CSA compared with CON cows. In addition, there was a linear and quadratic response ($P \leq 0.01$) for ipsilateral uterine artery CSA during the restriction period, where CSA increased quadratically, regardless of treatment. Similar to period 1, there was no treatment by day interaction ($P = 0.98$), but there was a treatment effect ($P = 0.01$) for ipsilateral uterine artery CSA during period 2. Cows from RES group continued to have greater CSA compared with CON after being realimented. Ipsilateral uterine artery CSA increased linearly ($P < 0.01$) during period 2 in both treatments. Although ipsilateral uterine artery PI during period 1 did not show a treatment by day interaction ($P = 0.83$; Fig. 2C) or treatment effect ($P = 0.38$), there was a linear day response ($P < 0.01$) for ipsilateral uterine artery PI. Both treatment groups decreased linearly during the

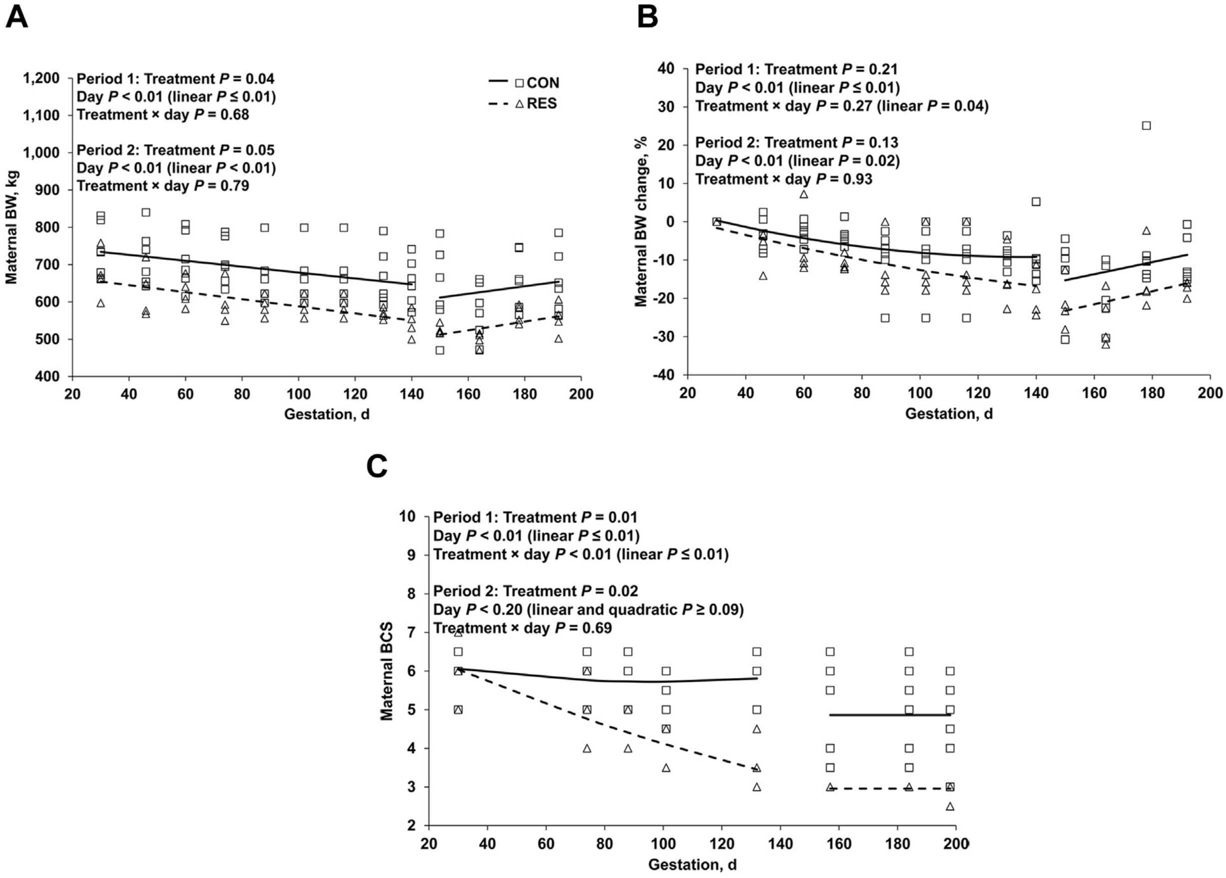

Fig. 1. Maternal BW (panel A), percentage BW change (panel B), and BCS (panel C) from Days 30 to 198 of gestation. Individual cows received either control (100% NRC; CON □) or nutrient restricted diet (60% of NRC from CON; RES Δ) from Day 30 until 140 (period 1) and thereafter being realimented until Day 198 of gestation (period 2). Data were analyzed within period. BCS, body condition score; BW, body weight; CON, control; NRC, National Research Council; RES, nutrient restriction.

restriction period. During period 2, there was no interaction or main effect of treatment and day ($P \geq 0.35$) for ipsilateral uterine artery PI. Ipsilateral uterine RI responded similar to PI. There was no treatment by day interaction ($P = 0.74$) or treatment effect ($P = 0.13$) during period 2. But there was a linear day ($P < 0.01$) response, where both treatment groups RI decreased linearly during the restriction period. In period 2, there was no treatment by day, treatment, or day effect ($P \geq 0.34$) for ipsilateral uterine artery RI.

Contralateral uterine BF is illustrated in Figure 3A. There was no treatment by day interaction ($P = 0.10$), but there was a tendency ($P = 0.09$) for a treatment effect during period 1. Cows from CON group tended to have greater contralateral uterine BF compared with RES cows. There were treatment by linear and quadratic day responses ($P \leq 0.02$), where contralateral uterine BF from CON cows increased, whereas RES cows did not change during period 1. During period 2, although there was no treatment by day interaction ($P = 0.42$), there was a tendency ($P = 0.07$) for a treatment effect, where CON cows continued to have greater contralateral uterine BF compared with RES cows after realimentation. There was also a linear day response ($P = 0.04$) for contralateral uterine BF. Cows from CON and RES groups had a linear increase in contralateral uterine BF during period 2. There was no treatment by day interaction ($P = 0.60$; Fig. 3B), but there was a treatment effect tendency ($P = 0.06$) for contralateral uterine artery CSA during period 1. The CON cows had bigger CSA compared with RES cows. There was no day effect ($P = 0.72$) for contralateral uterine artery CSA during the restriction period. Similarly, during period 2, there was no treatment by day interaction ($P = 0.86$), but there was a treatment effect ($P < 0.01$) for contralateral uterine artery CSA. Cows from CON group continued to have greater CSA compared with RES cows during period 2. There was no day effect ($P = 0.72$) for contralateral uterine artery CSA. Contralateral uterine artery PI is illustrated in Figure 3C. During period 1, contralateral uterine PI did not show a treatment by day interaction ($P = 0.25$) or treatment effect ($P = 0.19$). However, there was a linear day response ($P < 0.01$) for contralateral uterine PI. Both treatment groups decreased linearly during the restriction period. During period 2, there was no interaction or main effect of treatment and day ($P = 0.15$) for contralateral uterine PI. Contralateral uterine RI during period 1 did not show a treatment by day interaction ($P = 0.78$). However, there was a treatment effect ($P = 0.05$) for contralateral uterine RI during period 2, where RES cows had increased RI compared with CON cows. Also, there was a linear and quadratic day ($P \leq 0.01$) response, where RI decreased quadratically during period 1

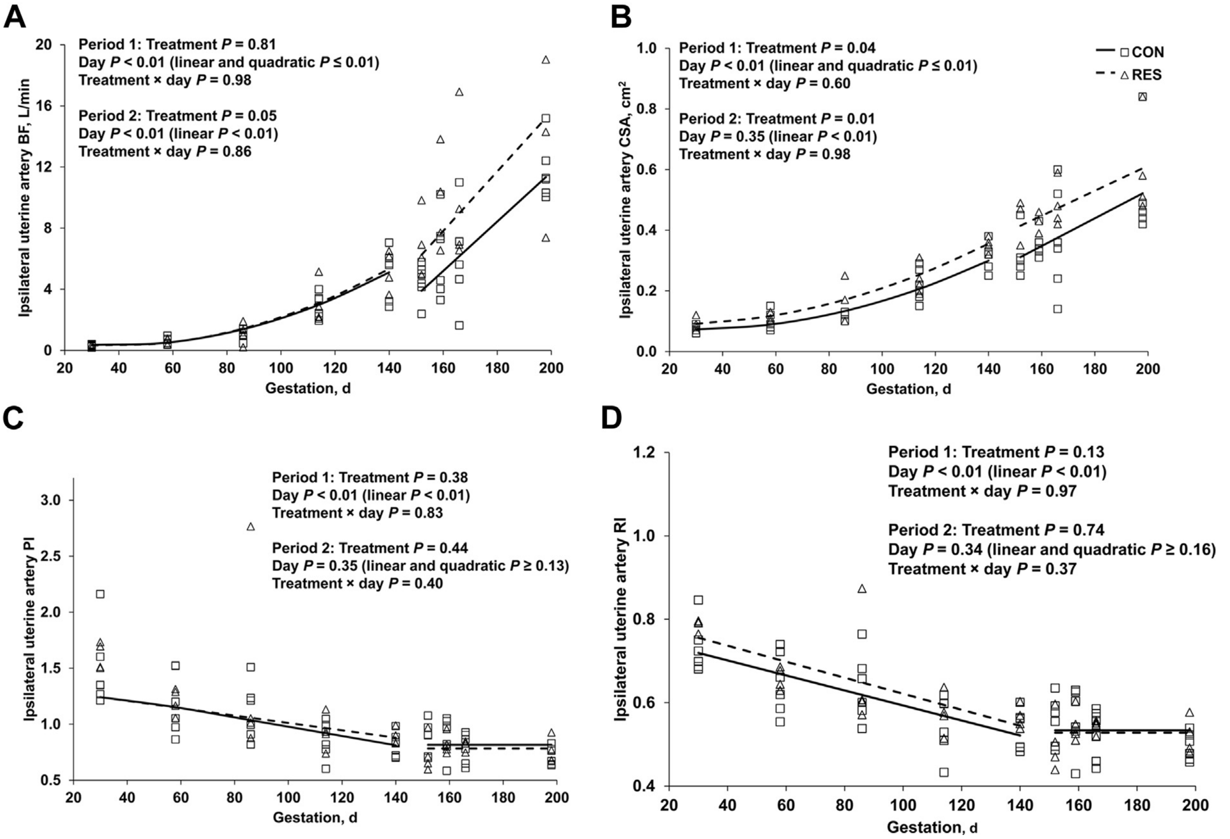

Fig. 2. Ipsilateral uterine artery blood flow (BF; panel A), cross-sectional area (CSA; panel B), pulsatility index (PI; panel C), and resistance index (RI; panel D) from Day 30 until 198 of gestation. Individual cows received either control (100% NRC; CON □) or nutrient restricted diet (60% of NRC from CON; RES Δ) from Day 30 until 140 (period 1) and thereafter being realimented until Day 198 of gestation (period 2). Data were analyzed within period. CON, control; NRC, National Research Council; RES, nutrient restriction.

in both groups. During period 2, there was no treatment by day interaction ($P = 0.87$) or day effect ($P = 0.75$) for contralateral uterine RI. However, there was a treatment effect ($P = 0.04$) for contralateral uterine RI where RES cows continued to have increased RI compared with CON cows.

Total uterine BF is illustrated in Figure 4A. During period 1, there was no treatment by day interaction ($P = 0.80$) or treatment effect ($P = 0.60$) for total BF. However, there was a linear and quadratic day ($P \leq 0.01$) response for total uterine BF. In both treatment groups, total BF increases quadratically during period 1. Also, there was no treatment by day interaction ($P = 0.96$) or treatment effect ($P = 0.24$) for total uterine artery BF during period 2. But there was a linear day ($P < 0.01$) response for total BF, where total BF increased linearly during period 2 regardless of treatment. Maternal HR showed a treatment by quadratic day ($P = 0.02$; Fig. 4B) response during period 1. In addition, there was a linear and quadratic day ($P \leq 0.03$) response for maternal HR. During period 2, although there was no treatment ($P = 0.40$) or day effect ($P = 0.20$) for maternal HR, there was a treatment by quadratic day ($P = 0.03$) response.

4. Discussion

The hypothesis that during nutrient restriction total uterine BF would be reduced was rejected. Moreover, although total uterine BF was similar after realimentation, ipsilateral uterine BF was enhanced in cows that were previously restricted. In many sheep models investigated to date [15,16], nutrient restriction results in reduced uterine and/or umbilical BF. This could be innate species differences, or also because of parity or age of the dam. Regardless, until more beef cattle work is performed to confirm our results, caution should be used when comparing data acquired in sheep as it may not be directly applicable to beef cattle. Inanition in swine during mid-pregnancy (Days 50–90; gestation length = 114 days) resulted in no change to uterine BF, similar to our findings in the beef cow, and resulted in no change in weight of the total uterine mass [17]. Despite this lack of change in uterine BF, Hard and Anderson [17] further demonstrated that blood volume was reduced during inanition, but increased by 24% within 20 days of realimentation. Unfortunately, how realimentation influenced uterine blood flow was not measured in the Hard and Anderson study. In women experiencing hyperemesis gravidarum, uterine BF per 100 g of fetus is increased compared with control women [18], but the authors were unable to locate information on how normal intakes may have impacted uterine BF in those pregnancies. Although there is a paucity of information on how restriction and/or realimentation impacts uterine BF, it appears most pregnant females,

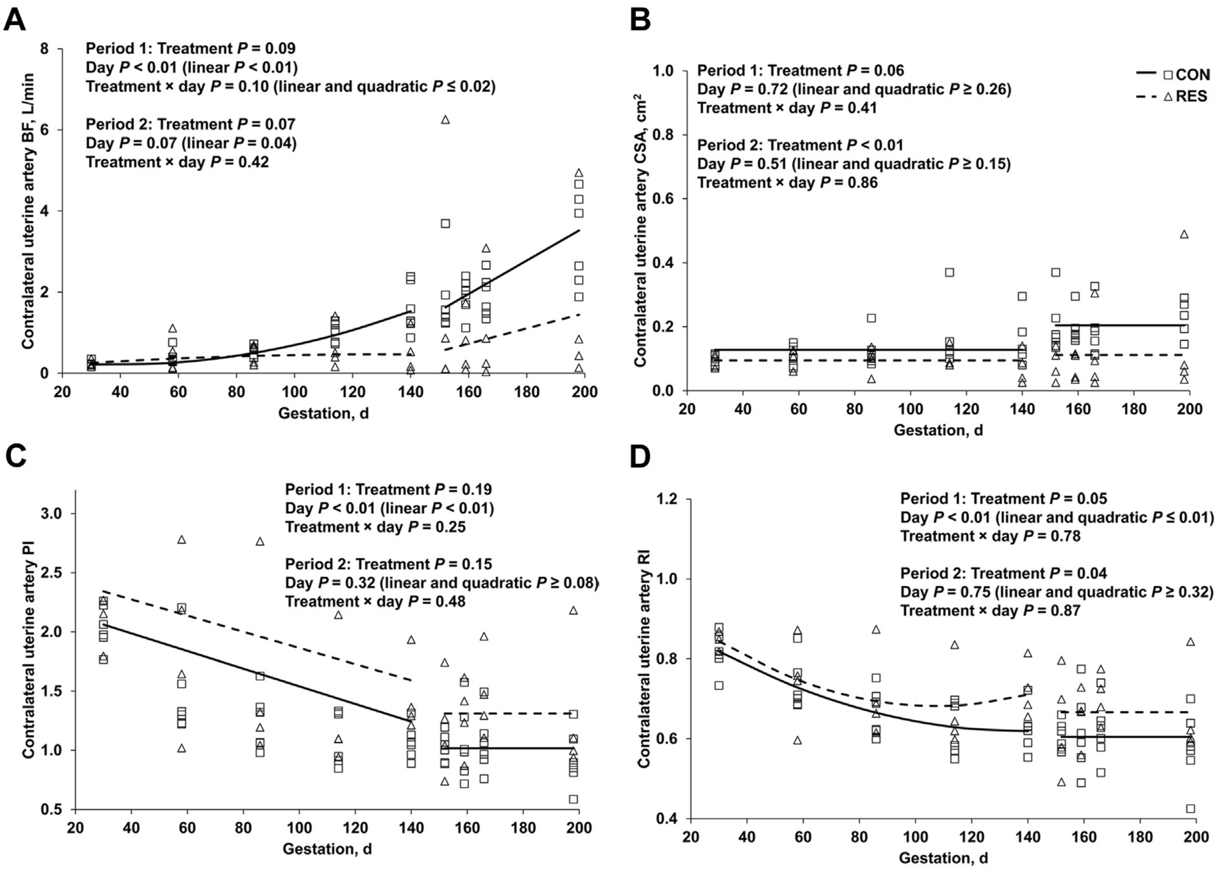

Fig. 3. Contralateral uterine blood flow (BF; panel A), cross-sectional area (CSA; panel B), pulsatility index (PI; panel C), and resistance index (RI; panel D) from Day 30 until 198 of gestation. Individual cows received either control (100% NRC; CON □) or nutrient restricted diet (60% of NRC from CON; RES Δ) from Day 30 until 140 (period 1) and thereafter being realimented until Day 198 of gestation (period 2). Data were analyzed within period. CON, control; NRC, National Research Council; RES, nutrient restriction.

excluding the sheep, alter their body reserves to allow continuation of adequate blood flow for the developing conceptus.

To our knowledge, this is the first experiment to examine uterine BF during early to midgestation in nutrient-restricted pregnant cows followed by realimentation during late gestation using color Doppler ultrasonography. Foundational uterine BF work in beef cattle has been conducted using more invasive techniques such as electromagnetic blood flow transducers [19] or infusion of deuterium water [20]. By using color Doppler ultrasonography to assess uterine BF and vascular resistance throughout gestation in pregnant beef cows, we were able to examine the same animal continuously throughout gestation with no surgical preparation and with minimal interference to dam. Similarly, Bollwein, et al. [7] measured uterine BF in cows during the estrous cycle. They suggested that Doppler ultrasonography was a reliable method to determine uterine BF, and did not require use of blood flow probes and/or chronically catheterized animals. Moreover, findings from this study and others suggest that the use of Doppler ultrasonography as the technique to investigate uterine BF during pregnancy may also constitute a reliable method. In several breeds of cattle, when Doppler ultrasonography assessed uterine hemodynamics throughout gestation (i.e., Days 30–270), RI decreased and uterine BF increased exponentially with increased BF in ipsilateral versus contralateral horns [8,21]. Moreover, RI was negatively correlated to uterine BF [21].

The present study suggests that nutrient restriction during early to midgestation followed by realimentation impacts uterine BF in pregnant beef cows. Restriction did not alter uterine BF in the horn that carried the fetus (i.e., ipsilateral side); however, on realimentation, those cows that were previously restricted had increased BF compared with CON cows on the side that houses the calf. Interestingly, resistance indices (RI and PI) were not affected by dietary treatment. The contralateral side responded differently to restriction and realimentation, with CON cows having increased uterine BF compared with RES. Similar to the ipsilateral uterine artery, contralateral PI was not affected by dietary treatment; however, contralateral RI was increased in RES cows compared with CON. Moreover, we observed a concomitant increase in uterine BF with decreasing resistance indices as gestation

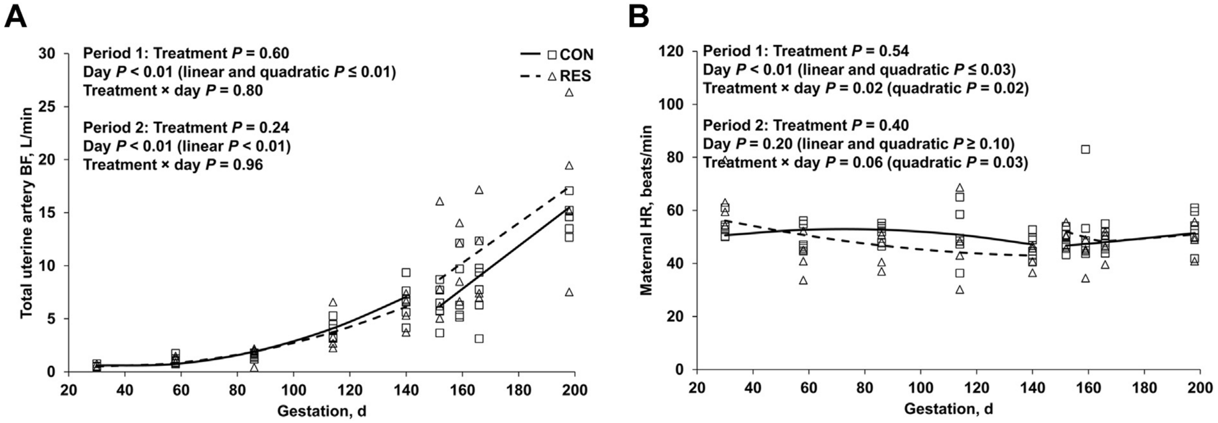

Fig. 4. Total uterine artery blood flow (BF; panel A) and maternal heart rate (HR; panel B) from Day 30 until 198 of gestation. Individual cows received either control (100% NRC; CON □) or nutrient restricted diet (60% of NRC from CON; RES Δ) from Day 30 until 140 (period 1) and thereafter being realimented until Day 198 of gestation (period 2). Data was analyzed within period. CON, control; NRC, National Research Council; RES, nutrient restriction.

progressed in both uterine arteries. Interestingly, resistance indices were not affected by treatment although uterine BF was increased. The CSA of the ipsilateral uterine artery increased in size as gestation progressed, and this increase was more dramatic (sevenfold increase) than the contralateral (numerical twofold increase) side. According to Poiseulle law, the major determinant impacting flow is the diameter of the artery as diameter is elevated to the fourth power, suggesting that small changes in vessel diameter will have big changes in BF. So, although the changes in velocities (i.e., peak systolic, end diastolic, or mean; data not shown) were not major contributors (i.e., there were not major differences of treatment in our RI and PI measurements), BF was still impacted. It is interesting to think why the ipsilateral and contralateral BF responded so differently to nutrient availability. We hypothesize that nutrient restriction may have altered the growth trajectory of the placenta. Although the majority of the placenta is surely housed in the side where the fetus is located, we cannot predict the placental occupation in the contralateral side, which may impact BF to the contralateral uterine horn. Perhaps, the variation in CSA and BF observed during later gestation in the contralateral horn is because of the variation in placental size. Although only a tendency for increased contralateral BF was observed, the authors predict that placental occupation within the contralateral horn may have been greater for the CON cows compared with the RES during period 2. Future studies are necessary to determine how nutrient restriction and realimentation impact the bovine placenta.

Regardless of dietary treatment total uterine BF increased 30-fold from Days 30 to 198 of gestation. In addition, both resistance indices decreased. Research using Doppler technology to assess changes during pregnancy in women suggests that to have better placentation and a greater birth weight, uterine artery resistance needs to decrease early for better fetal and maternal health [22,23]. For example, in human pregnancies, when uterine artery resistance remains high or does not decrease during the last third of gestation, there is an association with deficiency in nutrient supply. This also has been related with preeclampsia, intrauterine growth restriction, or, in more severe cases, fetal death [24]. Results from the present study in general followed similar uterine BF patterns compared with those reported by Bollwein, et al. [21] and Panarace, et al. [8] when using Doppler ultrasonography in cows. In addition, Reynolds and Ferrell [20] measured uterine BF at different stages of pregnancy in cows using a steady-state diffusion method. This study showed an exponential increase in uterine BF from Days 137 to 250 of gestation, whereas we calculate a linear increase in the present study during period 2. The differences between Reynolds and Ferrell's study and the present study can be attributed to differences in timing for data collection and techniques used. Regardless if there is an exponential or linear increase in uterine BF, pregnancy is associated with increases in cardiac output and uterine BF and a reduction in systemic vascular resistance. This increase in uterine BF supports fetal growth during the last trimester of gestation, and provides adequate oxygen and nutrient delivery. In sheep, during early gestation, the caruncular vascular bed only receives 27% of the total uterine BF. However, of the overall increase in uterine BF during late gestation, more than 85% is directed toward the caruncular vascular beds, which transfer oxygen and nutrients to the placenta and fetus through the adjacent cotyledonary vasculature [25].

The placenta plays an important role in providing physiological exchange between the maternal and fetal systems [5]. During placentation, angiogenesis and vascularization at the fetal–maternal interface is extensive and, subsequently, a rapid increase in uterine and umbilical BF results [5]. To support the growth of the developing fetus during late gestation, although the placenta is not growing as much as early gestation, placental function increases dramatically after midgestation [26]. Vonnahme, et al. [4] demonstrated that nutrient restriction during early to midgestation (from Day 30 to 125 of gestation) did not affect vasculature of the bovine placenta. However, after nutrient restricted cows were realimented, placental vasculature was altered near term, indicating the placenta compensated after restriction. In the current experiment, ipsilateral uterine BF was increased in RES compared with CON cows in late pregnancy, but total uterine BF was not affected by dietary treatment suggesting that the conceptus might be driving the local effect observed in BF. Perhaps, placentomes in closer proximity to the fetus function to either produce more vasodilatory or less vasoconstrictive factors that enhance local BF. The ability for the bovine placenta to adapt to nutritional changes warrants further investigation into placentome vascularity and vascular function.

When nutrient availability during pregnancy is reduced, the dam might go through a series of metabolic and physiological adaptations to protect her body stores from depletion by the conceptus [27]. In the present study, cow BCS was decreased through gestation in RES compared with CON cows even after realimentation. However, BW was not affected by nutrient restriction and all cows, regardless of dietary treatment, lost BW from the beginning of the experiment and started gaining BW after realimentation. Although control diets were designed to meet or exceed NE requirements, CON cows lost BW during early gestation, suggesting that cows had greater nutrient requirements than those estimated or feed energy values were overestimated. Nutrient requirements could be greater than estimated because of several factors, which could include differences in environmental conditions, genetics, lactation, or fetal growth [11]. Predicting energy values of feeds is difficult and using approaches to predict total digestible nutrients [10] from diet analysis along with converting total digestible nutrients values to NE values [11] may overvalue the energy value of lower quality forage [28] and, therefore, provide less NE than predicted. Previous research has shown that when beef cows are nutrient restricted during pregnancy, realimentation results in increased BW and similar BW to CON are achieved by Day 245 of gestation [29]. However, Meyer, et al. [29] restricted cows to 68% of NE recommendations and realimented cows above 100% of nutrient requirements to achieve a similar BW to CON animals by Day 220 of gestation.

4.1. Conclusions

In summary, nutrient restriction from early to midgestation does not alter total uterine BF. Interestingly, on realimentation, there is enhanced uterine BF, but only to the horn where the majority of the conceptus is housed, although it was unknown how much of the conceptus was present in the contralateral horn. It also appears that cattle respond differently to inadequate nutrition during early to midgestation compared with sheep. In addition, results from this experiment suggest that the bovine placenta is programmed to function differently after a period of nutrient restriction. Perhaps, timely management strategies applied during gestation might enhance conceptus development. Although more research is necessary, opportunities may be available to intervene during times of poor nutrition. Moreover, further research needs to be done to determine how realimentation at different critical time points impacts uterine BF and conceptus development, during late gestation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.theriogenology.2014.02.006.

References

[1] Funston RN, Larson DM, Vonnahme KA. Effects of maternal nutrition on conceptus growth and offspring performance: implications for beef cattle production. J Anim Sci 2010;88:E205–15.

[2] Godfrey KM, Barker DJB. Fetal nutrition and adult disease. Am J Clin Nutr 2000;7:1344S–52S.

[3] Wu G, Bazer FW, Wallace JM, Spencer TE. Board-invited review: intrauterine growth retardation: implications for the animal sciences. J Anim Sci 2006;84:2316–37.

[4] Vonnahme KA, Zhu MJ, Borowicz PP, Geary TW, Hess BW, Reynolds LP, et al. Effects of early gestational undernutrition on angiogenic factor expression and vascularity in the bovine placentome. J Anim Sci 2007;85:2464–72.

[5] Reynolds LP, Redmer DA. Utero-placental vascular development and placental function. J Anim Sci 1995;73:1839–51.

[6] Meschia G. Circulation to female reproductive organs. In Shepherd T, Abboud FM, editors. Handbook of physiology: American Physiological Society, Bethesda, MD: p. 241–269

[7] Bollwein H, Meyer HH, Maierl J, Weber F, Baumgartner U, Stolla R. Transrectal Doppler sonography of uterine blood flow. Theriogenology 2000;53:1541–52.

[8] Panarace M, Garnil C, Marfil M, Jauregui G, Lagioia J, Luther E, et al. Transrectal Doppler sonography for evaluation of uterine blood flow throughout pregnancy in 13 cows. Theriogenology 2006;66:2113–9.

[9] Herzog K, Bollwein H. Application of Doppler ultrasonography in cattle reproduction. Reprod Domest Anim 2007;42(Suppl.2):51–8.

[10] Weiss WP, Conrad HR, St. Pierre NR. A theoretically-based model for predicting total digestible nutrient values of forages and concentrates. Anim Feed Sci Technol 1992;39:95–110.

[11] NRC. Nutritional requirements of beef cattle. Seventh edition rev. Washington, DC: National Academy Press; 2000.

[12] Wagner JJ, Lusby KS, Oltjen JW, Rakestraw J, Wettemann RP, Walters LP. Carcass composition in mature Hereford cows: estimation and effect on daily metabolizable energy required during winter. J Anim Sci 1988;66:603–12.

[13] AOAC. Official methods of analysis. Fifteenth edition Arlington, VA: 1990.

[14] Robertson JB, Van Soest PJ. The detergent system of analysis and its application to human foods. In: James PT, Theander O, editors. The analysis of dietary fiber. New York: Marcell Dekker; 1981123–158W.

[15] Reynolds LP, Caton JS, Redmer DA, Grazul-Bilska AT, Vonnahme KA, Borowicz PP, et al. Evidence for altered placental blood flow and vascularity in compromised pregnancies. J Physiol 2006:51–8.

[16] Lemley CO, Meyer AM, Camacho LE, Neville TL, Newman DJ, Caton JS, et al. Melatonin supplementation alters uteroplacental hemodynamics and fetal development in an ovine model of intrauterine growth restriction (IUGR). Am J Physiol 2012;302: R454–67.

[17] Hard DL, Anderson LL. Interaction of maternal blood volume and uterine blood flow with porcine fetal development. Biol Reprod 1982;27:79–90.

[18] Zubek L, Monos E, Csepli J. Significance of blood flow measurement in the cervix uteri on the 1st trimester of pregnancy. Zentralbl Gynakol 1986;108:900–5.

[19] Ford SP, Christensen RK. Blood flow to uteri of sows during the estrous cycle and early pregnancy: local effect of the conceptus on the uterine blood supply. Biol Reprod 1979;21:617–24.

[20] Reynolds LP, Ferrell CL. Transplacental clearance and blood flows of bovine gravid uterus at several stages of gestation. Am J Physiol 1987;253:735–9.

[21] Bollwein H, Baumgartner U, Stolla R. Transrectal Doppler sonography of uterine blood flow in cows during pregnancy. Theriogenology 2002;57:2053–61.

[22] Carbillon L, Uzan M, Largilliere C, Perrot N, Tigaizin A, Paries J, et al. Prospective evaluation of uterine artery flow velocity waveforms at 12-14 and 22-24 weeks of gestation in relation to pregnancy outcome and birth weight. Fetal Diagn Ther 2004;19:381–4.

[23] Gomez O, Figueras F, Martinez JM, Del Rio M, Palacio M, Eixarch E, et al. Sequential changes in uterine artery blood flow pattern between the first and second trimesters of gestation in relation to pregnancy outcome. Ultrasound Obstet Gynecol 2008;28:802–8.

[24] Tamura H, Miwa I, Taniguchi K, Maekawa R, Asada H, Taketani T, et al. Different changes in resistance index between uterine artery and uterine radial artery during early pregnancy. Hum Reprod 2008;23:285–9.

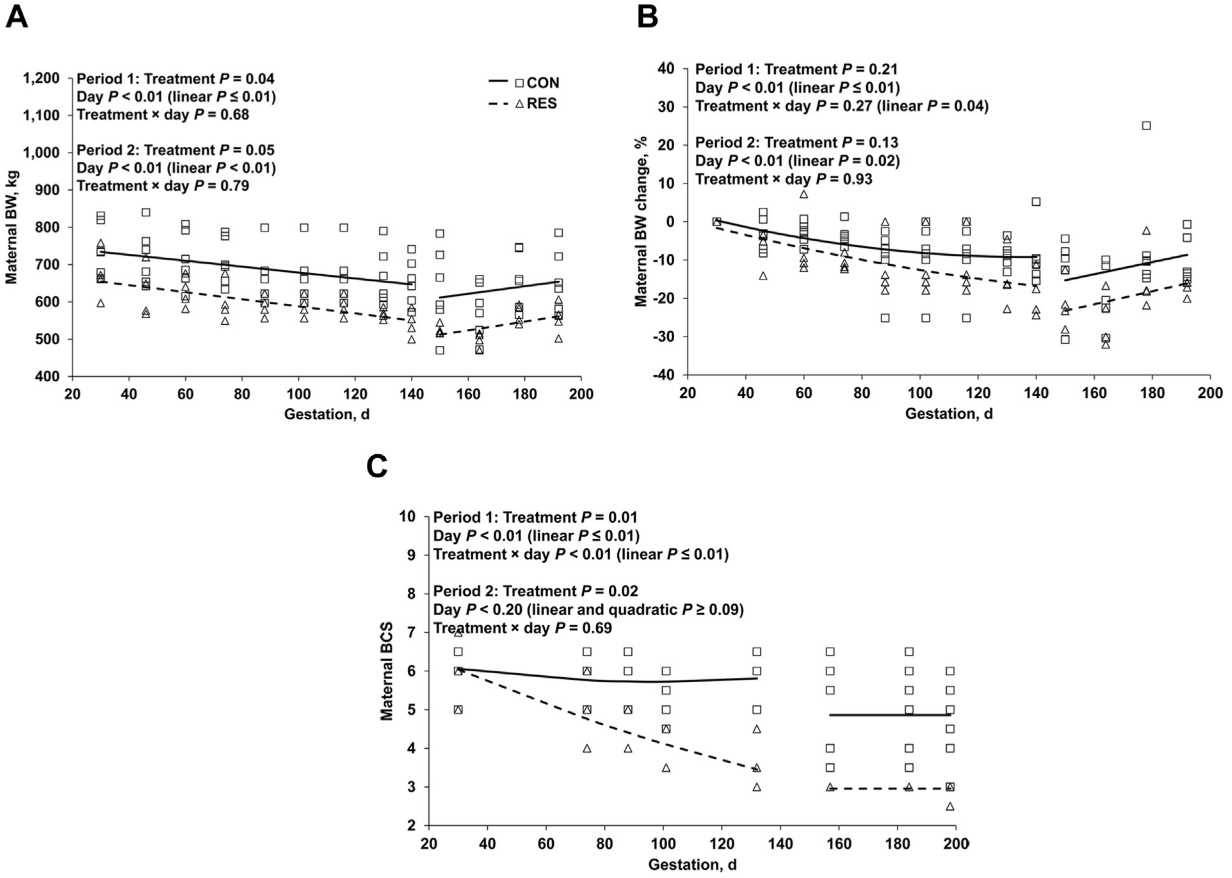
[25] Rosenfeld CR, Fixler DE. Cardiovascular changes and heart disease in pregnancy. In: Willerson JT, Saunders CA, editors. Clinical cardiology, the science and practice of clinical medicine, vol. 3. New York, NY: Grune and Stratton; 1977. p. 477–82.

[26] Reynolds LP, Ferrell CL, Robertson DA, Ford SP. Metabolism of the gravid uterus, fetus and utero-placenta at several stages of gestation in cows. J Agric Sci Camb 1986;106:437–44.

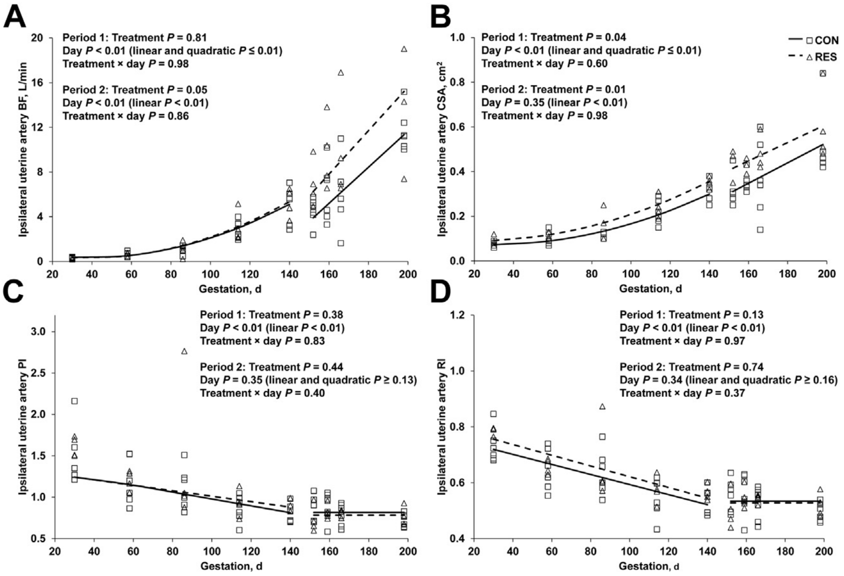
[27] Rosso P, Streeter MR. Effects of food or protein restriction on plasma volume expansion in pregnant rats. J Nutr 1979;109:1887–92.

[28] Weiss WP. Estimating the available energy content of feeds for dairy cattle. J Dairy Sci 1998;81:830–9.

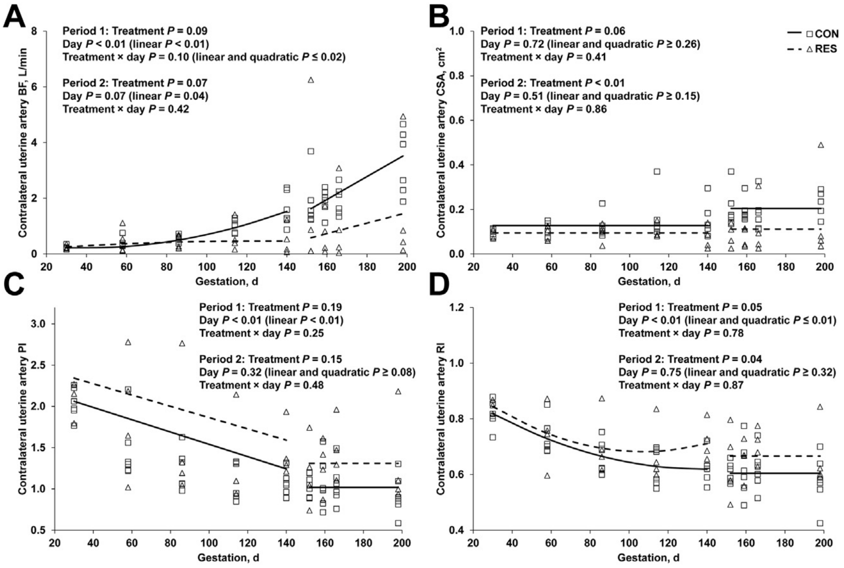
[29] Meyer AM, Reed JJ, Vonnahme KA, Soto-Navarro SA, Reynolds LP, Ford SP, et al. Effects of stage of gestation and nutrient restriction during early to mid-gestation on maternal and fetal visceral organ mass and indices of jejunal growth and vascularity in beef cows. J Anim Sci 2010;88:2410–24.



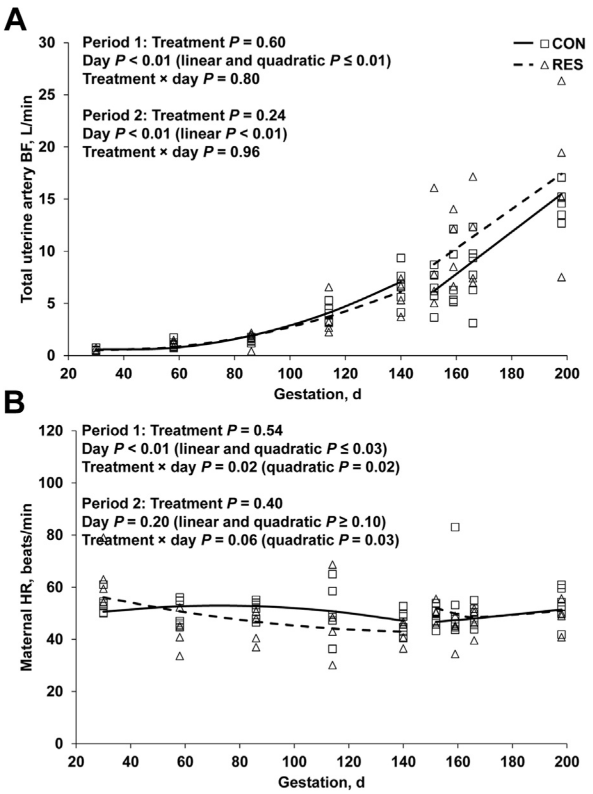
Supplementary Fig. 1. Linear and quadratic coefficients for day effects were constructed for unequally spaced orthogonal polynomial contrasts for dependent variables. Regression coefficient solutions for significant polynomials were generated using day as a continuous variable. During period 1, best-fit line for CON (–) was BW = 0.76 (±0.02) − 0.0008 (±0.0001) × day, and for RES (–) was BW = 0.68 (±0.03) − 0.0009 (±0.0001) × day. CON percentage BW change = −0.006 (±0.004) − 0.00023 (±0.00009) × day + 0.0000008 (±0.0000005) × day^2, and RES percentage BW change = −0.004 (±0.005) − 0.00022 (±0.00012) × day + 0.0000005 (±0.0000006) × day^2. CON BCS = 0.0064 (±0.0004) − 0.000014 (±0.000009) × day + 0.00000007 (±0.00000005) × day^2, and RES BCS = 0.0071 (±0.0005) − 0.000036 (±0.0000011) × day + 0.00000006 (±0.00000007) × day^2. During period 2, CON BW = 0.46 (±0.10) − 0.0010 (±0.0006) × day, and RES BW = 0.34 (±0.12) − 0.0012 (±0.0007) × day. CON percentage BW change = −0.04 (±0.02) + 0.00016 (±0.00010) × day, and RES percentage BW change = −0.05 (±0.02) + 0.00017 (±0.00012) × day. CON BCS = 0.0049 (±0.0004), and RES BCS = 0.0030 (±0.0005). BCS, body condition score; BW, body weight; CON, control; RES, nutrient restriction.



Supplementary Fig. 2. Linear and quadratic coefficients for day effects were constructed for unequally spaced orthogonal polynomial contrasts for dependent variables. Regression coefficient solutions for significant polynomials were generated using day as a continuous variable. During period 1, best-fit line for CON (—) was BF = 0.98 (±0.18) − 0.035 (±0.007) × day + 0.00046 (±0.00006) × day^2, and RES (–) was BF = 0.96 (±0.23) − 0.035 (±0.008) × day + 0.00048 (±0.00007) × day^2. CON CSA = 0.086 (±0.024) − 0.0010 (±0.0008) × day − 0.000018 (±0.000004) × day^2, and RES CSA = 0.094 (±0.029) − 0.0006 (±0.0009) × day + 0.000018 (±0.000005) × day^2. PI = 1.39 (±0.13) − 0.0041 (±0.0010) × day, and RES PI = 1.33 (±0.16) − 0.0033 (±0.0012) × day. CON RI = 0.773 (±0.030) − 0.00180 (±0.00032) × day, and RES RI = 0.813 (±0.037) − 0.00192 (±0.00039) × day. During period 2, CON BF = −20.7 (±4.7) + 0.162 (±0.032) × day, and RES BF = −23.3 (±5.8) + 0.195 (±0.039) × day. CON CSA = −0.38 (±0.21) + 0.0046 (±0.0013) × day, and RES CSA = −0.21 (±0.26) + 0.0041 (±0.0015) × day. CON PI = 0.815 (±0.027), and RES PI = 0.782 (±0.033). CON RI = 0.534 (±0.011), and RES RI = 0.528 (±0.014). BF, blood flow; CON, control; CSA, cross-sectional area; PI, pulsatility index; RES, nutrient restriction; RI, resistance index.



Supplementary Fig. 3. Linear and quadratic coefficients for day effects were constructed for unequally spaced orthogonal polynomial contrasts for dependent variables. Regression coefficient solutions for significant polynomials were generated using day as a continuous variable. During period 1, best-fit line for CON (—) was BF = 0.39 (±0.11) − 0.0096 (±0.0045) × day + 0.000127 (±0.000034) × day^2, and RES (–) was BF = 0.11 (±0.14) + 0.0055 (±0.0055) × day − 0.000021 (±0.000042) × day^2 CON CSA = 0.128 (±0.010), and RES CSA = 0.095 (±0.013). CON PI = 2.285 (±0.056) − 0.00745 (±0.00030) × day, and RES PI = 2.547 (±0.077) − 0.00683 (±0.00042) × day. CON RI = 0.944 (±0.067) − 0.0047 (±0.0018) × day + 0.000017 (±0.000010) × day^2, and RES RI = 0.993 (±0.091) − 0.0058 (±0.0023) × day + 0.000027 (±0.000013) × day^2. During period 2, CON BF = −4.64 (±1.61) + 0.041 (±0.011) × day, and RES BF = −2.27 (±1.97) + 0.019 (±0.014) × day. CON CSA = 0.204 (±0.19), and RES CSA = 0.112 (±0.023). CON PI = 1.02 (±0.12), and RES PI = 1.31 (±0.14). CON RI = 0.605 (±0.017), and RES RI = 0.666 (±0.021). BF, blood flow; CON, control; CSA, cross-sectional area; PI, pulsatility index; RES, nutrient restriction; RI, resistance index.



Supplementary Fig. 4. Linear and quadratic coefficients for day effects were constructed for unequally spaced orthogonal polynomial contrasts for dependent variables. Regression coefficient solutions for significant polynomials were generated using day as a continuous variable. During period 1, best-fit line for CON (–) was BF = 1.56 (±0.30) – 0.052 (±0.011) × day + 0.00065 (±0.00009) × day^2, and RES (–) was BF = 1.01 (±0.37) – 0.031 (±0.014) × day + 0.00048 (±0.00011) × day^2. CON HR = 46.2 (±5.8) + 0.19 (±0.13) × day – 0.00128 (±0.00070) × day^2, and RES HR = 63.3 (±0.71) – 0.27 (±0.16) × day + 0.00087 (±0.00085) × day^2. During period 2, CON BF = –24.50 (±5.02) + 0.202 (±0.032) × day, and RES BF = –19.86 (±6.15) + 0.188 (±0.039) × day. CON HR = 42.3 (±67.1) – 0.03 (±0.78) × day + 0.0004 (±0.0022) × day^2, and RES HR = 275.1 (±82.2) – 2.58 (±0.95) × day + 0.0073 (±0.0027) × day^2. BF, blood flow; CON, control; HR, heart rate.