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Effect of early gestational undernutrition on angiogenic factor expression and vascularity in the bovine placentome

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ABSTRACT: The effect of early gestation maternal undernutrition followed by realimentation on placentomal vascular growth and angiogenic factor expression was determined in multiparous beef cows bred to the same bull. Cows gestating only female fetuses (n = 30) were fed in equal numbers to meet the NRC requirements (control) or were fed below the NRC requirements to lose BW (nutrient restricted; NR) from d 30 to 125 of gestation. After slaughter on d 125 of gestation, 10 control and 10 NR cows were necropsied. The remaining NR cows (n = 5) were then fed to achieve a BCS equal to their control group contemporaries (n = 5) by d 220 of gestation. All cows were fed the control diet from d 220 until 250 of gestation, when the remaining control and NR cows were slaughtered and necropsied. At necropsy, placentomes were fixed via perfusion of the caruncular and cotyledonary arteries to determine capillary vascular density. Cotyledonary (fetal placental) and caruncular (maternal placental) tissues also were snap-frozen in liquid nitrogen, and mRNA concentrations of vascular endothelial growth factor and its 2 specific receptors, fms-like tyrosine kinase and kinase insert domain containing receptor, as well as placental growth factor, were determined. There was no effect of diet or day of gestation on the percentage of proliferating caruncular cells. Although diet did not impact cotyledonary cellular proliferation, there was an increase ($P < 0.05$) in the percentage of proliferating cells on d 250 compared with d 125 of gestation. Nutrient restriction from d 30 to 125 increased ($P \leq 0.10$) placental mRNA concentrations of placental growth factor and fms-like tyrosine kinase; however, there was no alteration in vascularity. By d 250 of gestation, NR cows had increased ($P < 0.05$) caruncular capillary surface density and decreased ($P < 0.05$) cotyledonary capillary area density, capillary number density, and capillary surface density compared with control cows. Although nutrient restriction had little effect on placental vascularity by d 125, upon realimentation, alterations in vascularity became apparent by d 250 of gestation, suggesting a placental programming effect.

Key words: cattle, placental efficiency, pregnancy

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INTRODUCTION

The relationship between maternal nutrient intake during pregnancy and growth of the fetus is imperative for determining pregnancy success, neonatal morbidity and mortality, as well as life-long health and productivity of offspring (Wallace et al., 1999; Godfrey and Barker, 2000). Because profitability in the livestock industry depends on efficiency of production characteristics such as growth and development after birth, it is important that the precursor of efficiency, fetal growth, be optimized.

Placental size and nutrient transfer capacity determine the fetal growth trajectory and hence directly affect birth weight (Reynolds et al., 1985; Vonnahme et al., 2001; Vonnahme and Ford, 2004b). Transplacental exchange depends on uterine and umbilical blood flows,

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which are dependent on adequate vascularization at the fetal-maternal interface (Reynolds et al., 2005a,b). Maternal nutrition impacts placental growth and capillary vascularity of the placentomes in sheep (Redmer et al., 2004). In cows, low dietary protein in the first third of gestation followed by increased protein in the second third of gestation enhanced placental development (Perry et al., 1999).

Although several angiogenic factors play a role in vascularization, the vascular endothelial growth factor (**VEGF**)-receptor system, which includes the ligands VEGF and placental growth factor (**PlGF**) and 2 receptors [fms-like tyrosine kinase (**Flt-1**) and kinase insert domain containing receptor (**KDR**)], appears to be one of the most potent and is expressed in placentas of cows (Miles et al., 2004), sheep (Cheung and Brace, 1999; Regnault et al., 2003; Borowicz et al., 2007), and pigs (Vonnahme and Ford, 2004a,b). Our hypothesis was that global undernutrition during early pregnancy in beef cows would impact placental vascular development as well as the VEGF-PlGF-receptor system needed for angiogenesis. The objective of this study was to determine effects of nutrient restriction during early pregnancy on placental vascular development and angiogenic factor expression in beef cows.

MATERIALS AND METHODS

This study was conducted at the University of Wyoming and was approved by the Institutional Animal Care and Use Committee.

A total of 116 suckled, multiparous Angus × Gelbvieh cows [initial BW, 571 ± 63 kg; initial BCS, 5.4 ± 0.7 (1 = emaciated, 9 = obese); Wagner et al., 1988] were synchronized using a progesterone insert (CIDR, Pfizer, Exton, PA) for 7 d, and when the CIDR was removed, an injection of 25 mg of $PGF_{2\alpha}$ (Lutalyse, Pharmacia & Upjohn Co., Kalamazoo, MI) was administered. Cows were bred ~12 h after the onset of estrus via AI using semen from a single bull. At breeding, the cows were sorted and blocked by initial BW, BCS, and age, and assigned to 1 of 2 diets.

Control cows were fed native grass hay (12.1% CP, 70.7% TDN, DM basis) fortified with vitamins and minerals at the NRC (2000) recommendations for a mature cow to gain 0.72 kg/d during the first 125 d of gestation. Nutrient restricted (**NR**) cows were fed minerals and vitamins at 50% of the amount provided to the control cows, and millet straw (9.9% CP. 54.5% IVDMD) to provide 68.1% of the NE_m and 86.7% of the MP requirements during the first 125 d of gestation (NRC, 2000). Cows were weighed every 14 d to adjust the rations for changes in BW throughout the experiment. On approximately d 80 of gestation, all cows were confirmed pregnant and had their fetus sexed by ultrasonography (Aloka 500 with a 5-MHz linear probe, Aloka, Wallingford, CT).

Thirty cows (15 control and 15 NR) that were gestating female fetuses were utilized for this study. After slaughter on d 125 of gestation, 10 control and 10 NR cows were necropsied. The remaining control cows (n = 5) were fed the control diet to maintain a BCS of 5.75 from d 125 to 250 of gestation, whereas the NR cows (n = 5) were realimented by feeding the NR hay and the control minerals and vitamins plus a 79.6% cracked corn, 6.1% soybean meal, 5.3% sunflower meal, 4.2% cane molasses, 2.6% safflower seed meal, and 1.6% dried skim milk (DM basis; analyzed composition = 13.2% CP and 77.6% IVDMD) supplement. The realimentation diet was formulated to provide 2.15 Mcal more NE_m/d than the control diet, so that the NR cows would achieve a BCS equal to their control contemporaries by d 220 of gestation. In a companion study, Miller et al. (2004) reported that NR cows had a BCS of 5.6, whereas control cows had a BCS of 5.7 on d 192 of gestation. On d 250 of gestation, the remaining control and NR cows were slaughtered and necropsied.

Tissue Collection

On the day of slaughter, cows were stunned with a captive-bolt gun and exsanguinated. The gravid uterus was immediately collected and weighed, and the fetus was removed and weighed. Placentomes (n = 10 to 37) in close proximity to the umbilicus of each cow were weighed, measured, and selected for perfusion-fixation and angiogenesis measurements, or were separated manually into cotyledonary and caruncular portions, weighed, and snap-frozen in liquid nitrogen for mRNA quantification via real-time, reverse transcription (**RT**)-PCR, as described below. Total placentome number was determined and recorded, and placentomes were separated into caruncular and cotyledonary portions, and each component was pooled and weighed to obtain total caruncular and total cotyledonary weights.

Capillary Vascular (Angiogenesis) Measurements

Placentomes were perfused using the method of Borowicz et al. (2007), with the following modifications. After catheterization of a main uterine arterial and a main umbilical arterial branch, caruncular and cotyledonary tissues were simultaneously perfused (using manual perfusion at low pressure) with 1) PBS; 2) Carnoy's solution; 3) PBS again, and finally, (4) a vascular casting resin (Mercox, Ladd Industries, Williston, VT). After curing of the Mercox for 1 h, 3 to 4 of the fixed and perfused placentomes were removed, further fixed by immersion in Carnoy's solution (a nonaldehyde-based fixative composed of ethanol, acetic acid, and chloroform; Borowicz et al., 2007), embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and periodic acid-Schiff's reagent, using previously reported procedures (Figure 1; Reynolds and Redmer, 1992; Borowicz et al., 2007).

After images were collected (n = 12 images·tissue^{-1}·cow^{-1}; Nikon DXM 1200 digital camera, Fryer Company Inc., Chicago, IL), measures of angiogenesis or

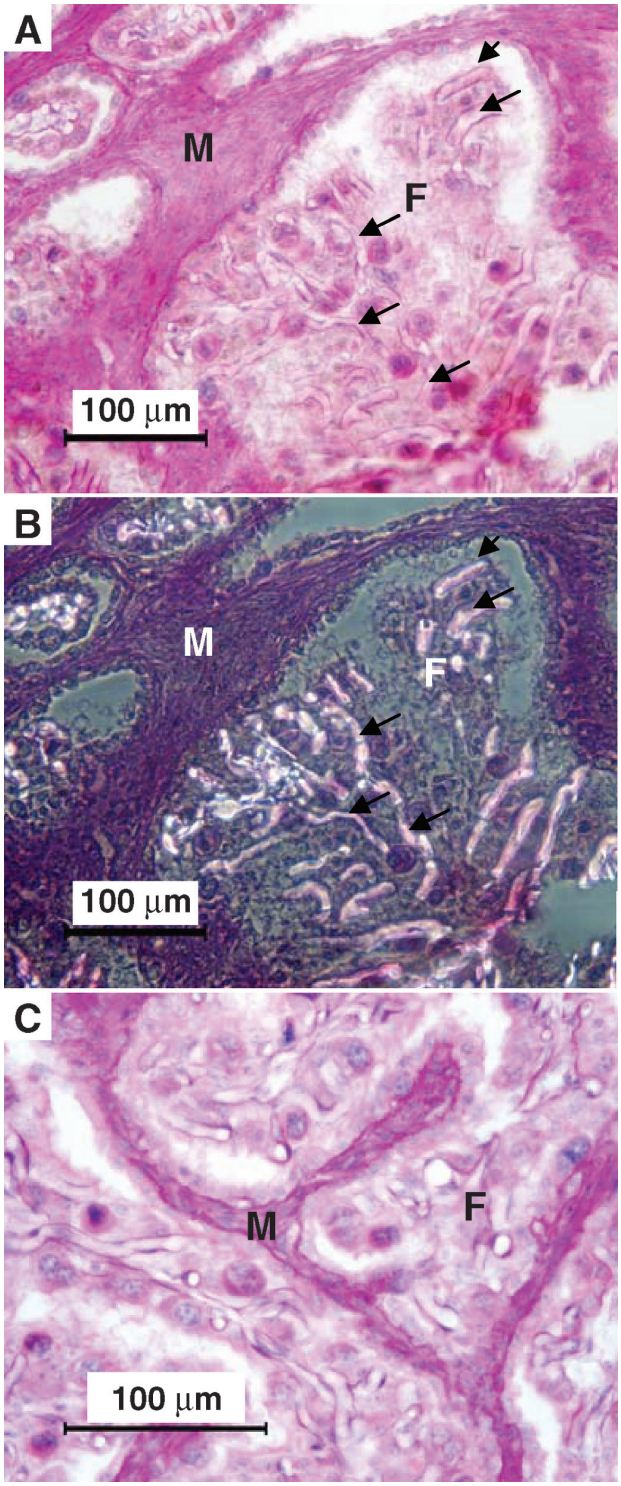

Figure 1. (A) Brightfield and (B) phase contrast photomicrographs of the same histological section of Mercox-perfused bovine cotyledon (COT) at d 250 of gestation. Arrows indicate how the luminescent plastic, which fills the capillaries in (B), corresponds with the capillary vessels seen under brightfield in (A). Staining was with hematoxylin and periodic acid Schiff's reagent. (C) A greater magnification as would have been used in the image analyses procedures described in the text; COT capillaries can readily be seen in this view. M indicates maternal tissue. F indicates fetal tissue. Size bars are shown.

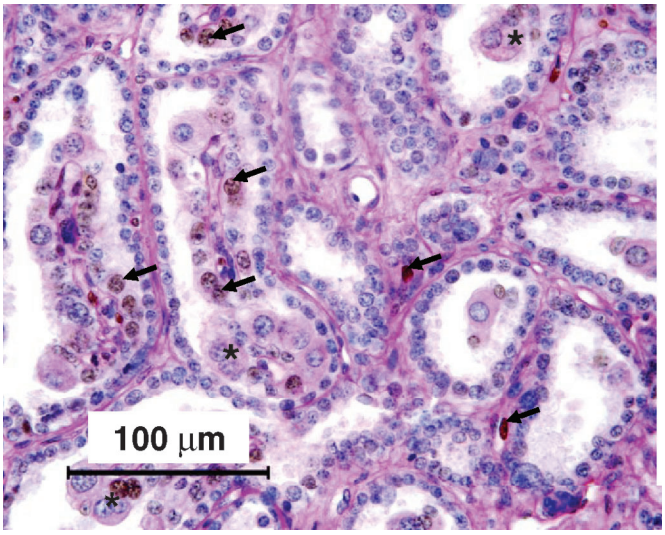

Figure 2. A representative micrograph of a placentome from d 250 of gestation showing nuclear staining (brownish; indicated by the arrows) for proliferating cell nuclear antigen (PCNA); unlabeled nuclei exhibit blue nuclear counterstaining. *Indicates several fetal binucleate cells. Size bar is shown.

vascularity were determined by image analysis (Image-Pro Plus 5.0, Media Cybernetics, Silver Spring, MD) as previously described (Borowicz et al., 2007). The following variables were determined for caruncular and cotyledonary tissue in each picture of each tissue section: capillary number density (**CND**, the number of capillaries per unit of tissue area), capillary cross-sectional area density (**CAD**, total capillary area per unit of tissue area), capillary surface density (**CSD**, capillary circumference per unit of tissue area), and average cross-sectional area per capillary (**APC**, Reynolds et al., 2005a; Borowicz et al., 2007). We have suggested, based on physical models of capillary flow and exchange, that CND is related primarily to vascular branching, CAD is related primarily to flow, and CSD is related primarily to nutrient exchange (Reynolds et al., 2005b). The data for CND, CAD, CSD, and APC were averaged across the 12 images for each cow, and the average of each was used for statistical analysis.

Cellular Proliferation

Placentomal tissues that were perfusion-fixed for capillary vascularity measurements were also used for cellular proliferation measurements. Determination of proliferating cells in the caruncular and cotyledonary tissue was similar to that reported previously (Fricke et al., 1997; Scheaffer et al., 2001; Soto-Navarro et al., 2004). Briefly, samples were sectioned at 5 μm, mounted on glass slides, and treated with a blocking buffer consisting of PBS and 1.5% (vol/vol) normal horse serum (Vector Laboratories, Burlingame, CA) for 20 min. Sections of fixed tissues were incubated with

Table 1. Bovine probe and primer sets used for reverse transcription-PCR in this study

Probe or primer[1]	Nucleotide sequence	GenBank accession No.
VEGF FP	5′-GGA TGT CTA CCA GCG CAG C-3′	X89506
VEGF RP	5′-TCT GGG TAC TCC TGG AAG ATG TC-3′	
VEGF Probe	5′(6FAM)-CTG CCG TCC CAT CGA GAC CCT G-(TAMRA)3′	
PlGF FP	5′-CCC TGG AGA CAG CCA ACG T-3′	NM_173950
PlGF RP	5′-GCT GGT CCA GAG AGC GGT ACT -3′	
PlGF Probe	5′(6FAM)-CCA TGC AGC TCA TG-(MGBNFQ)3′	
Flt-1 FP	5′-CGC CTG AAA TCT ACC AGA TCA TG-3′	AF488351
Flt-1 RP	5′-TCC ACG AAT CTT GGC CTT TCT-3′	
Flt-1 Probe	5′(6FAM) - TGG ACT GCT GGC ACA AAG ACC CAA -(TAMRA)3′	
KDR FP	5′-GCA GTG ATG GCG TCT TCT GTA A-3′	AF233076
KDR RP	5′-GCT CCA GTA TCA TTT CCA ATC ACT T-3′	
KDR Probe	5′(6FAM)-ATG CTC ACA ATT TCA-(MGBNFQ)3′	

[1]FP = forward primer; RP = reverse primer; VEGF = vascular endothelial growth factor; PlGF = placental growth factor; Flt-1 = fms-like tyrosine kinase, VEGF and PlGF receptor; KDR = kinase insert domain containing receptor, VEGF receptor.

mouse antiproliferating cell nuclear antigen monoclonal antibody (Clone PC-10, Roche Diagnostics Corp., Indianapolis, IN) at 1 μg/mL in blocking buffer. Primary antibody was detected by using a biotinylated secondary antibody (horse antimouse immunoglobulin G, Vectastain, Vector Laboratories, Burlingame, CA) and the Avidin-Biotinylated Horseradish Peroxidase Complex system (Vectastain, Vector Laboratories). Tissue sections were counterstained with Nuclear Fast Red to visualize unlabeled nuclei (Figure 2). Omission of the primary antibody served as the negative control (data not shown). After images were collected, the percentage of cells proliferating in the cotyledonary and caruncular tissues of each cow was determined (n = 12 images·tissue^{-1}·cow^{-1}) by image analysis (Image-Pro Plus 5.0, Media Cybernetics).

Real-Time RT-PCR

Messenger RNA concentration for the range of angiogenic growth factors and their receptors (Table 1) was determined using quantitative, real-time RT-PCR. Total RNA was extracted from individual caruncular and cotyledonary tissue of each cow using TriReagent (Sigma-Aldrich Co. Ltd., Dorset, UK). The quality and quantity of total RNA were determined via capillary electrophoresis using an Agilent 2100 Bioanalyzer (Agilent Technologies, Wilmington, DE). All reagents and procedures used for the real-time RT-PCR were purchased from and used as directed by Applied Biosystems (Warrington, Cheshire, UK). For each sample, approximately 30 ng of total RNA was reverse transcribed in triplicate using TaqMan Reverse Transcription Reagents and MultiScribe reverse transcription (Applied Biosystems). TaqMan probes and primers sets were designed from bovine-specific sequences of genes using Primer Express Software (Applied Biosystems). The sequences of the labeled TaqMan probes and the forward and reverse primers are detailed in Table 1. Polymerization and amplification reactions for each RT were carried out in duplicate using 96-well PCR plates in a final volume of 10 μL, with an ABI PRISM 7700 Sequence Detector (Applied Biosystems). Hybridization and polymerization were carried out at 60°C for 40 cycles for all genes of interest. Quantification was determined using a relative standard curve method, with different doses of a reference standard cDNA that was generated from RNA pooled from bovine cotyledonary and caruncular tissues from d 125 and 250 of gestation. Individual cotyledonary and caruncular mRNA for each gene of interest was expressed relative to the internal 18S RNA of each sample using 18S PDAR kit reagents (Applied Biosystems). The inter- and intraassay CV for VEGF, Flt-1, KDR, and PlGF mRNA concentrations were 2.5 and 16.6%, 3.5 and 23.7%, 3.0 and 22.9%, and 2.2 and 20.0%, respectively.

Statistics

Data were analyzed using PROC GLM (SAS Inst. Inc., Cary, NC). Class statements included day of gestation, tissue, diet, and their interactions for cotyledonary and caruncular CAD, CND, CSD, APC, cotyledonary and caruncular proliferation rates, and cotyledonary and caruncular VEGF, Flt-1, KDR, and PlGF mRNA concentrations. When the interaction was not significant ($P > 0.09$), it was removed from the model. Means were separated using the LSMEANS option of SAS. Effects were considered significant when $P < 0.05$, unless otherwise stated.

RESULTS

There was no effect of diet or day of gestation on the number of placentomes, which averaged 85.1 ± 4.2. Although fetal weight tended to be reduced ($P = 0.12$; Zhu et al., 2007), and caruncular and cotyledonary weights from NR cows were reduced ($P < 0.05$; Figure 3; Zhu et al., 2007) compared with control cows on d 125 of gestation, there was no effect of diet on fetal

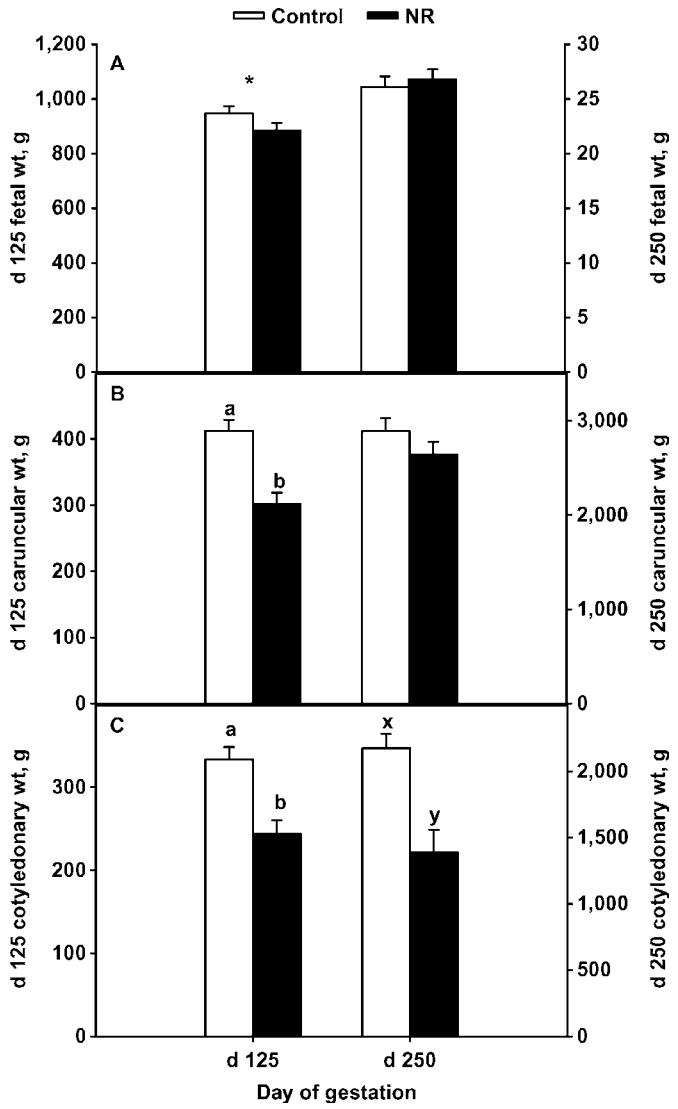

Figure 3. (A) Fetal, (B) caruncular, and (C) cotyledonary weights on d 125 and d 250 of gestation in control and nutrient restricted (NR) cows. [a,b,x,y]Means ± SEM within a day and panel differ, $P < 0.05$; *$P = 0.12$. (Adapted from Zhu et al., 2007).

and caruncular weight by d 250. However, even after realimentation, cotyledonary weight remained less ($P < 0.05$) on d 250 in NR cows compared with control cows (Figure 3).

There was no effect of diet on CND, CAD, CSD, or APC measurements in cotyledonary tissue on d 125 of gestation. By d 250, however, the cows that had been nutrient restricted from d 30 to 125 of gestation exhibited decreased ($P < 0.05$) CND, CAD, and CSD in cotyledonary tissue compared with cotyledonary tissue from control cows (Figure 4). Further, cotyledonary tissue, CND, CAD, CSD, and APC increased ($P < 0.05$) from d 125 to 250 in NR and control cows. Similarly, there was no effect of diet on any capillary vascularity measurement in the caruncle on d 125 of gestation (Figure 5). There was, however, a decrease in caruncular tissue CAD (Figure 5A) and APC (Figure 5D) from d 125 to 250 of gestation in both dietary groups. In contrast, CSD (Figure 5B) and CND (Figure 5C) increased in caruncular tissue in all cows from d 125 to 250. Capillary surface density, a measure of nutrient exchange, reached greater ($P < 0.05$) levels in NR than control cows on d 250. On d 125, caruncular tissue had increased ($P < 0.05$) CND, CAD, CSD, and APC compared with cotyledonary tissue. Although CND, CAD, and CSD remained greater ($P < 0.05$) in caruncular than cotyledonary tissue on d 250 of gestation, APC was similar across maternal and fetal tissues.

There was no effect of diet or day of gestation on the percentage of proliferating caruncular cells, which averaged 0.86 ± 0.08%. Whereas there was no effect of diet on cotyledonary proliferating cells, there was an increase ($P < 0.05$) in cotyledonary cells proliferating on d 250 compared with d 125 of gestation (1.26 ± 0.18 vs. 0.90 ± 0.07%, respectively).

There were no interactions in the RT-PCR data (Table 2). Whereas there was no effect of day on caruncular KDR and Flt-1 mRNA concentrations, caruncular VEGF and PlGF mRNA decreased ($P < 0.05$) from d 125 to 250 (VEGF: 16.52 ± 3.00 vs. 0.62 ± 0.08; PlGF: 34.56 ± 6.17 vs. 8.21 ± 1.93). Although cotyledonary VEGF and KDR were not affected by day, cotyledonary PlGF and Flt-1 mRNA decreased from ($P < 0.05$) d 125 to 250 of gestation (PlGF: 33.88 ± 5.18 vs. 8.07 ± 2.13; Flt-1: 0.24 ± 0.02 vs. 0.11 ± 0.03). On d 125 of gestation, VEGF mRNA concentrations in caruncular tissue were greater ($P < 0.01$) than in cotyledonary tissue (Table 2). Further, on d 125, PlGF mRNA concentration was increased ($P < 0.05$), and Flt-1 mRNA concentrations tended ($P = 0.10$) to be increased in NR cows compared with control cows. There were no tissue or diet effects on d 250 of gestation in any of the angiogenic factors measured.

DISCUSSION

Global nutrient restriction during early gestation followed by realimentation impacts placental angiogenesis (capillary vascularity) as well as angiogenic factor mRNA concentrations in the beef cow. In this study, mRNA concentrations of genes that are known to increase vascular permeability (i.e., PlGF and Flt-1; Peters et al., 1993; Odorisio et al., 2002) were increased in the caruncular and cotyledonary tissues at the end of the nutrient restriction period. However, capillary vascularity measurements were not altered in the cotyledon or the caruncular tissues. This is in contrast to the data of Zhu et al. (2007) who demonstrated that cotyledonary vascularity was increased by d 125 of gestation in NR vs. control cows. Although the same animals were utilized in this study and that of Zhu et al. (2007), the cotyledonary vascularity differences due to nutrient restriction between the 2 studies may be due to the types of vessels measured; this study measured capillaries, whereas Zhu et al. (2007) measured larger

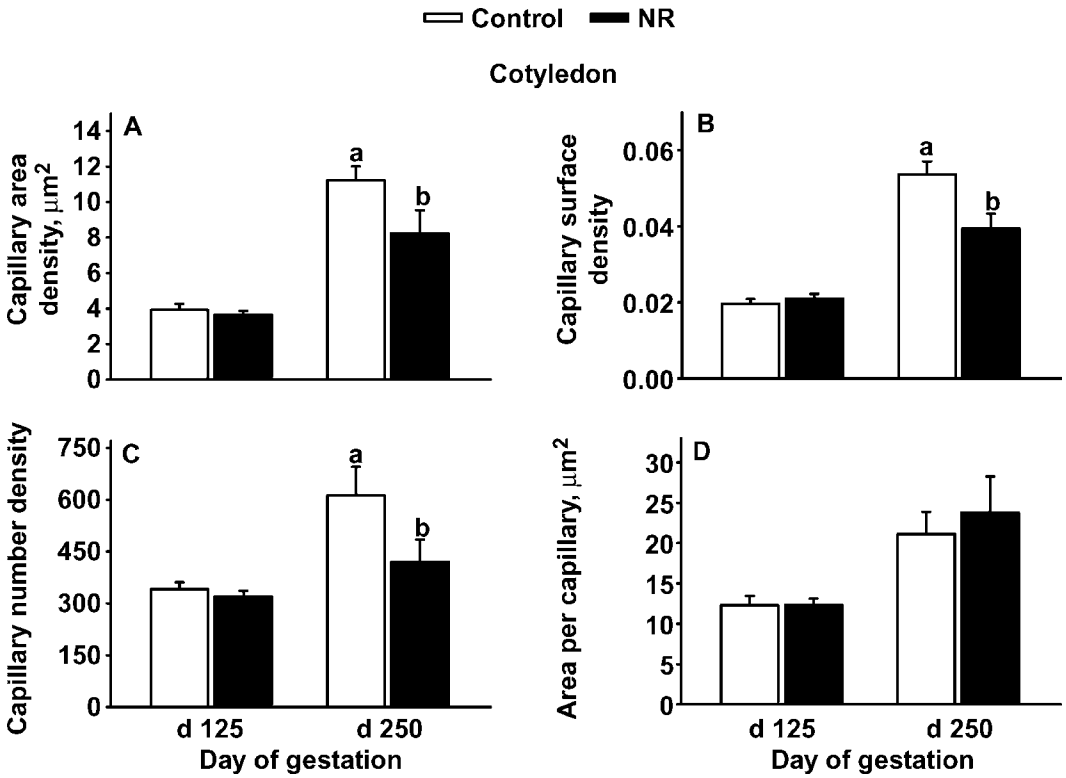

Figure 4. Cotyledonary (A) capillary area density, (B) capillary surface density, (C) capillary number density, and (D) area per capillary on d 125 and 250 of gestation in control and nutrient restricted (NR) cows. Effects of day of gestation were significant for capillary area density, capillary number density, and area per capillary. An interaction of day of gestation by diet was significant for capillary surface density. [a,b]Means ± SEM within a day and panel differ, $P < 0.05$.

caliber vessels (i.e., arterioles). Fetal weight was not significantly different on d 125 between NR and control cows. This lack of a significant fetal intrauterine growth restriction in response to maternal nutrient restriction may have resulted from the increase in cotyledonary arteriolar density reported by Zhu et al. (2007), potentially increasing blood flow through this tissue. Further, an increase in the permeability of the capillary beds may have also occurred due to an increase in PlGF and Flt-1 mRNA concentrations (Odorisio et al., 2002). This hypothesis is supported by the work of Zhu et al. (2007) who reported that placental efficiency (fetal weight/total placentomal weight) was greater on d 125 in NR than control cows.

After realimentation from d 125 to 250 of gestation, there were dramatic differences in capillary vascularity measurements. In the cotyledon, there was no difference in the tissue density of the larger caliber vessels (Zhu et al., 2007), whereas 3 of the 4 measurements (i.e., CAD, CND, and CSD) for capillary vascularity were decreased in placentomes from previous NR cows compared with control cows, demonstrating that the capillary area, numbers and surface densities had been hindered upon realimentation. It is of interest that it is only upon realimentation and not at the end of the nutrient restriction period that alterations in the capillary measurements occurred. Furthermore, even though cotyledonary capillary vascularity was reduced on d 250, caruncular tissue density of the larger caliber vessels (Zhu et al., 2007) and the capillary surface density (this study) in realimented NR cows was increased compared with cows that had adequate nutrition throughout gestation. The process of realimentation caused a change in vascular structure of the caruncular tissue in the placentome. In agreement, Zhu et al. (2007) reported an increased placental efficiency of NR vs. control cows on d 250 of pregnancy. However, detailed studies of the relationship between vascular architecture and placental function have not been conducted in this model.

Reports of changes in placental vascularity in response to realimentation of nutrient restricted ewes and cows are very limited. McMullen et al. (2005) have demonstrated that a short duration (7 d) of fasting during mid pregnancy in ewes resulted in decreased VEGF mRNA expression and placental weights on d 90. Although differences in VEGF mRNA and placental weight were not investigated from d 90 to term, placental weights were similar at lambing in NR and control ewes. In a study conducted in our laboratory with ewes (Vonnahme et al., 2003), a 50% nutrient restriction from d 28 to 78 of gestation resulted in no difference in cotyledonary tissue vascularity, as determined by arteriolar vascular density, between NR and control ewes,

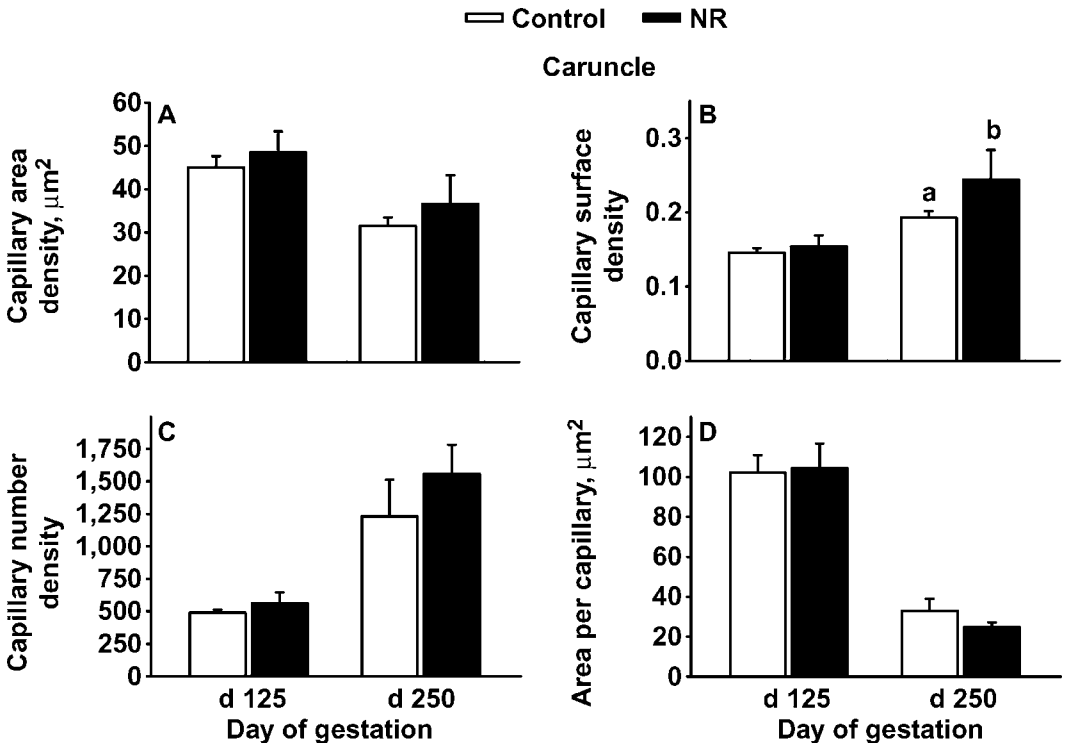

Figure 5. Caruncular (A) capillary area density, (B) capillary surface density, (C) capillary number density, and (D) area per capillary on d 125 and 250 of gestation in control and nutrient restricted (NR) cows. A day of gestation by diet interaction was significant for capillary area density, capillary surface density, and capillary number density. An effect of day of gestation was significant for area per capillary. [a,b]Means ± SEM within a day and panel differ, $P < 0.05$.

whereas NR ewes carrying twins had an increase in caruncular vascularity on d 78. However, no data have been generated describing changes in placentomal vascularity following realimentation in that model.

In this study, there was a decrease in total placentome weight on d 125 in NR vs. control cows that remained suppressed even after realimentation on d 250 (Zhu et al., 2007). Although the cotyledonary and carun-

Table 2. Expression of angiogenic factors and their receptors[1] in caruncular and cotyledonary tissues from control and NR[2] cows on d 125 and 250 of gestation, expressed as a ratio 18S RNA in the same tissue

	Caruncular tissue		Cotyledonary tissue		Tissue		Diet	
Item	Control	NR	Control	NR	Pooled SEM	*P*-value	Pooled SEM	*P*-value
D 125								
VEGF	20.85	12.64	0.88	0.78	6.37	$P < 0.001$	2.85	NS
PlGF	21.27	46.52	27.85	39.90	16.62	NS	7.43	$P < 0.05$
KDR	0.07	0.24	0.16	0.16	0.12	NS	0.05	NS
Flt-1	0.13	0.29	0.23	0.25	0.12	NS	0.05	$P = 0.10$
D 250								
VEGF	0.62	0.62	0.99	0.64	0.29	NS	0.13	NS
PlGF	7.64	8.78	7.88	8.26	4.67	NS	2.09	NS
KDR	0.27	0.34	0.26	0.22	0.14	NS	0.06	NS
Flt-1	0.11	0.14	0.13	0.08	0.06	NS	0.03	NS

[1]VEGF = vascular endothelial growth factor; PlGF = placental growth factor; Flt-1 = fms-like tyrosine kinase, which is a VEGF and PlGF receptor; and KDR = kinase insert domain containing receptor, which is a VEGF receptor.

[2]Control cows were fed native grass hay (12.1% CP, 70.7% TDN, DM basis) fortified with vitamins and minerals at NRC (2000) recommendations for a mature cow to gain 0.72 kg/d during the first 125 d of gestation. Nutrient restricted (NR) cows were fed minerals and vitamins at 50% of the amount provided to the control cows, and millet straw (9.9% CP. 54.5% IVDMD) to provide 68.1% of the NE_m and 86.7% of the MP requirements (NRC, 2000) during the first 125 d of gestation and were realimented thereafter.

cular portions were decreased in NR vs. control cows at the end of the nutrient restriction, only the weight of the cotyledonary tissue remained suppressed at d 250. In contrast, in several sheep studies where nutrient restriction was imposed from early to mid pregnancy followed by realimentation, significant compensatory growth of the placenta was found to occur (Foote et al., 1958; Robinson et al., 1995; Heasman et al., 1998). The differences in the impacts of nutrient restriction and realimentation in the current study and the sheep models may result from inherent species differences in placental development between sheep and cattle or may result from the type of diet (i.e., high energy) that these cows received upon realimentation. In the ewe, the growth of the cotyledonary mass is exponential during the first 10 to 11 wk of pregnancy, thereafter slowing markedly until term (Stegeman, 1974). In the cow, the cotyledonary growth progressively increases throughout gestation (this study; Reynolds et al., 1990). Using the same technique as utilized in this experiment, proliferating cells in the maternal and fetal portions of the placenta increase from mid to late gestation in the sheep (Reynolds et al., 2005b). We found only increases in proliferation in the cotyledon of the bovine placentome, and not the caruncle. Furthermore, in the sheep caruncular portion of the placenta, CAD, CND, CSD, and APC increase 3.3-, 1.5-, 1.7-, 2.2-fold from d 50 to 140 in normal pregnancy (Reynolds et al., 2005b; Borowicz et al., 2007). In the ovine cotyledon, CAD, CND, CSD increases 6.2-, 12.3-, 6.0-fold, and capillary size decreases 1.9-fold from d 50 to 140 in normal pregnancy.

Using the same methods to calculate capillary vascularity in the current study, in control cows caruncular CAD decreased by 1.3-fold and capillary size decreased by 3.1-fold, whereas CND and CSD increased 2.5-fold and 1.3-fold from d 125 to 250 of gestation. Furthermore, cotyledonary CAD, CND, CSD, and capillary size increased 2.9-, 1.8-, 2.7-, and 1.7-fold from d 125 to 250 of gestation, respectively. Similarly, larger diameter placental blood vessels from control cows exhibited increased caruncular vessel numbers, cotyledonary vascular density, and cotyledonary vessel numbers from d 125 to 250 of gestation in the study of Zhu et al. (2007). Thus, the pattern of placental angiogenesis appear to differ between the cow and sheep, and therefore caution must be used when comparing the responses to altered nutrition during pregnancy between species.

Although maternal nutrient delivery during pregnancy has been shown to program the growth and development of the fetus, during pregnancy and later into adult life, it appears that maternal nutrition also programs the development of the placenta. Development of the placental vascular bed is imperative to support the growth and development of the fetus. Although nutrient restriction from d 30 to 125 did not alter the vascular architecture of the bovine placenta, placental function must have been altered as fetal weight was reduced. It appears that realimentation after ~90 d of nutrient restriction is the stimulus not only for altering placental vascularity and development but also placental function in the cow. Therefore, future studies relating changes in vasculature architecture with placental function and nutrient transfer transport capacity in this model are warranted.

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