Theriogenology 81 (2014) 1248–1256

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

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

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ARTICLE INFO

*Article history:*
Received 10 December 2013
Received in revised form 5 February 2014
Accepted 7 February 2014

*Keywords:*
Beef cow
Nutrient restriction
Pregnancy
Uterine blood flow

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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http://dx.doi.org/10.1016/j.theriogenology.2014.02.006

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 |