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NUTRITIONAL CHARACTERISTICS OF THE DAIRY COW DURING EARLY GESTATION AND SUBSEQUENT GROWTH AND CARDIAC MEASUREMENTS OF HER OFFSPRING

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ABSTRACT

The objective was to determine whether maternal nutritional status and milk production during early gestation influenced or were correlated with parameters of the calf at birth and at 1 month of age. From parturition until 90 days pregnant, blood samples were collected every 14 days in dairy cows and plasma was assayed for concentrations of glucose and β-Hydroxybutyrate (BHBA). Calves (n = 39) born from these cows were measured for blood pressure and size characteristics as well as carotid artery hemodynamics measured via Doppler ultrasonography Pulsatility Index (PI) and Resistance Index (RI). Several values were then calculated to assess the cardiovascular health of the calf. The GLM and CORR procedures of SAS were used to analyze data and significance was determined when $p \leq 0.05$ and tendencies were discussed when $p > 0.05$ and ≤ 0.10. In calves at birth, mean milk production of dams during early gestation was positively correlated with heart girth. Length of gestation was positively correlated with heart girth of calves at birth and at 1 month of age. Mean concentration of glucose in dams was positively correlated with wither height in calves at 1 month of age. Length of gestation was negatively correlated with RI in calves at 1 month of age. Milk production in the dam was positively correlated with hip and wither height and PI but negatively correlated with mean arterial pressure in 1 month old calves. At birth, twins weighed less than singletons and females had an increased heart rate compared to males. At 1 month of age, size parameters and mean blood flow differed between singletons and twins. Males had lesser blood velocity but greater area of the carotid artery compared to females. These data lead to speculation that early gestational environment may impact growth and hemodynamic parameters in calves.

Keywords: Bovine, Developmental Programming, Doppler Blood Flow

1. INTRODUCTION

Growth characteristics and cardiac function in a young calf may affect health and production characteristics later in life. Previous research indicates the importance of maternal diet and nutrient availability to the health and viability of her offspring. The energy density of the diet pre-partum can impact neonate weight, size and immune function (Gao *et al*., 2012). Maternal nutritional and endocrine characteristics influence the growth of the placenta and fetus, from as early as a few days after fertilization. Negative influences on the placenta impact fetal development and subsequent offspring development (Robinson *et al*., 1995). Maternal nutrition can alter blood flow to the uterus, changing nutrient availability to the fetus via the

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placenta (Vonnahme and Lemley, 2012). Blood flow is an important indicator of cardiac health and nutrient availability to particular tissues.

Undernutrition in early pregnancy followed by re-alimentation to adequate nutrition in later pregnancy alters placenta vascularity and blood flow to the uterus (Vonnahme *et al*., 2007; 2013) which may impact fetal growth and development. The compensation of the placenta to insults may improve some outcomes in offspring. However, these mechanisms cannot yet be fully explained. Further, the implications of a negative energy balance or undernutrition in early lactation of dairy cows have not been investigated. This insult in early gestation may or may not alter offspring characteristics at birth and later in life. Further, it is unknown as to when measurements should be collected in neonatal calves to best assess these impacts. Collecting measurements at birth are difficult due to timing and scheduling and variation may be increased due to difficulty of parturition as well as other factors. But it is not known as to whether measurements at one month of age, which are easier to schedule, are adequate representations of the neonatal calf.

Therefore, the first objective of this characterization study was to determine whether the nutritional status of the dam during early gestation was correlated with growth, development and hemodynamics of her offspring. The second objective was to determine if measurements collected from calves at birth and at 1 month of age were correlated. These results will facilitate the planning of future experiments designed to elucidate the mechanisms and impacts of undernutrition during early gestation on offspring.

2. MATERIALS AND METHODS

2.1. Cows

All procedures in this study were approved by the Institutional Animal Care and Use Committee of Mississippi State University.

Initially, 135 Holstein and Jersey cows from the Mississippi State University Bearden Dairy Research Center were evaluated from an estimated 3 weeks prior to calving until 90 days post-conception. Cows were housed in 2 free-stall barns with access to water ad libitum and were fed twice daily a total mixed ration formulated to meet or exceed dietary requirements of dry or lactating cows (NRC, 2001). All cows were subjected to a 60 day voluntary waiting period and then artificially inseminated after detected in standing estrus or as a timed artificial insemination after synchronization of ovulation, per normal herd procedures. Cows were inseminated with frozen thawed semen by trained technicians. Due to negative effects of heat stress in Mississippi during summer months, this herd was managed with a seasonal breeding season with no females being inseminated from the end of May to mid-November. Therefore, cows were evaluated between September, 2011 and August, 2012. Pregnancy diagnosis was performed by palpation per rectum on approximately 32 days post-AI by a herd veterinarian and confirmed between 60 and 90 days post-AI.

Beginning an estimated 3 weeks prior to calving and every 14 days after until 90 days in gestation, cows were assessed for body condition scores (Edmonson *et al*., 1989) by one of two trained observers. Subsequent to calving, blood samples were collected into evacuated tubes (Becton Dickinson, Franklin Lakes, NJ and USA) without anticoagulants every 14 days. After collection, samples were placed on ice until returned to the laboratory and then centrifuged at 3,000×g at 4°C for 20 min. Plasma was collected and frozen at-20°C until analysis. Due to a variation in days open for individual cows, the number of blood samples collected ranged from 9 to 19.

Milk production was recorded daily in the parlor via Westfalia Surge Milk Meters (GEA Farm Technologies, Bönen, Germany). For analysis, milk production was averaged between two consecutive milkings at four time points (date of artificial insemination and 30, 60 and 90 d post-artificial insemination) during the first trimester of pregnancy. The range in this calculated mean was 21 to 54 kg among cows with an overall mean of 40.4 kg per day.

2.2. Calves

Thirty nine calves (25 female, 14 male; 30 Holstein, 9 Jersey; 34 singletons, 5 twin calves) born from these dams were evaluated at birth and at 1 month of age (August, 2012 to February, 2013). Calves were weighed at birth. Within 2 days of birth, calves were measured for size characteristics (crown to rump length, heart girth and hip and wither height) and a blood sample was collected from the jugular vein. Samples were collected, processed and stored the same as samples from cows.

Blood pressure (systolic and diastolic pressures) and heart rate were evaluated using a blood pressure sphygmomanometer (Omron Healthcare, Inc., Forrest Lake, IL, USA) while the calf was in a lateral position. From these measurements, Mean Arterial Pressure (MAP; diastolic + [0.33333*(systolic-diastolic)]) and pulse pressure (systolic-diastolic) were calculated. Hemodynamic characteristics of the carotid artery were evaluated using Doppler ultrasonography (Micromaxx, SonoSite, Inc., Bothell, WA, USA). Carotid artery hemodynamic values were recorded by placing the probe (L52x, Micromaxx, Sonosite, Inc) of the ultrasound on the skin near the jugular groove. Three cardiac cycle waveforms were used to calculate systolic velocity (s; cm/second), diastolic velocity (d; cm/second) and s: D ratio, Pulsatility Index (PI) and Resistance Index (RI) using preset functions on the Doppler ultrasound. Following these calculations, vessel area of the carotid artery was determined. From these measurements, mean blood velocity ((s-d)/PI) and blood flow (mean blood velocity *cross sectional area of vessel *60 sec) of the carotid artery were calculated. These measurements (size, blood pressure and hemodynamics) were recorded at birth and when the calf was 1 month (±4 days) of age.

2.3. Analysis of Serum

Serum samples from cows and calves were analyzed for concentrations of glucose (Stanbio Glucose Liqui-UV, Boerne, TX and USA) and concentrations of β-hydroxybutyrate (BHBA; Randox Ranbut, Ireland, UK) to assess nutritional status. For both assays, absorbance was measured via a spectrophotometer (SpectraMax Plus384, Sunnyvale, California and USA) following respective manufacturers' instructions.

2.4. Statistical Analysis

The GLM Procedure of SAS (SAS Institute, Cary, NC) was used for all ANOVA analyses and LS Mean's ± SEM are presented. Pearson correlation coefficients were derived using the CORR procedure of SAS (SAS Institute) and means ± SD are presented. For all models tested within GLM, independent variables were categorized. For BHBA data, cows were categorized into 8 groups: 420 to 599, 600 to 699, 700 to 799, 800 to 899, 900 to 999, 1,000 to 1,099, 1,100 to 1,199 and 1,200 to 2,293 µmol L^{-1} (there were no values between 1,174 and 2,065 µmol L^{-1}). For maternal glucose data, cows were categorized into 3 groups: < 2.9, 3.0 to 3.9 and ≥4.0 mmol L^{-1} and for milk production data, cows were categorized into 4 groups: < 31, 31 to 40, 41 to 45, ≥46 kg/day. Significance was set at p≤0.05 and tendencies were discussed when p>0.05 but ≤0.10.

3. RESULTS

3.1. Cows

In cows that had calves included in the study, the average milk yield across the four time points (time of artificial insemination, 30, 60 and 90 days after conception) was 40.4±8.3 kg of milk (range 21 to 54 kg). The range of days in milk at the time of conception was 43 to 167 days. The concentration of glucose among all samples from included cows was 3.5±0.7 mmol L^{-1}. The concentration of BHBA among all samples from included cows was 905.3±505.2 µmol L^{-1}. Mean length of gestation was 279.7±3.8 days.

Mean concentrations of BHBA were not different (P = 0.503) between Holstein and Jersey cows, with Holsteins having a mean of 898.6±107.4 µmol L^{-1} and Jerseys having a mean of 1,007.8±161.9 µmol L^{-1}. Mean concentrations of BHBA were also not different (P = 0.985) in cows which had twin calves (951.2±195.7 µmol L^{-1}) compared to those which had singletons (955.2±84.3 µmol L^{-1}). Mean concentrations of glucose were not different (P = 0.122) between the two breeds, with Holstein cows having a mean of 3.4±0.2 mmol L^{-1} and Jersey cows having a mean of 3.8±0.3 mmol L^{-1}. Mean concentrations of glucose were similar (P = 0.766) between cows which had twin calves (3.5±0.4 mmol L^{-1}) and those which had singletons (3.6±0.2 mmol L^{-1}). Average milk production during the evaluation period did differ (P = 0.003) between Holsteins (41.6±1.8 kg/day) and Jerseys (31.7±3.0 kg/day). However, milk production did not differ (P = 0.430) in cows which had twins (38.0±3.3 kg/day) compared to those which had singletons (35.3±1.6 kg/day).

3.2. Parameters in Calves at Birth

Concentration of glucose in dams tended to be related to heart girth in calves but was not related to birth weight, concentration of glucose, crown-rump length, hip height, wither height, mean heart rate, mean arterial pressure, mean RI, mean PI, mean blood velocity, carotid artery blood flow, or blood flow relative to body weight at birth (**Table 1**). Concentration of BHBA in dams was not related to calf birth weight, concentration of glucose, crown-rump length, heart girth, hip height, wither height, mean heart rate, mean arterial pressure, mean RI, mean PI, mean blood velocity, carotid artery blood flow, or blood flow relative to body weight (data not shown). Maternal milk production did significantly affect crown-rump length, heart girth, hip height and blood flow relative to body weight and tended to affect wither height, pulse pressure and carotid artery blood flow (**Table 1**). It did not affect calf birth weight, concentration of glucose, mean heart rate, mean arterial pressure, mean RI, mean PI, or mean blood velocity (**Table 1**).

At birth, calves born as twins weighed less (P = 0.017; 25.4±3.2 kg) than calves born as singletons (34.2±1.4 kg). In addition, female calves tended to have an increased (P = 0.063) heart rate (132±6 beats per min [bpm]) compared to male calves (119±6 bpm). All other parameters at birth (concentration of glucose, crown rump length, heart girth, hip height, wither height, mean arterial pressure, mean pulse pressure, mean RI, mean PI, mean blood velocity, mean blood flow and blood flow per kilogram of body weight were similar (p≥0.10) between singletons and twins and between male and female calves.

3.3. Parameters in Calves at One Month of Age

Concentration of glucose in dams was significantly related to crown rump length, heart girth, hip height, wither height and mean blood velocity and it tended to be related to blood flow in calves at one month of age (**Table 2**). Maternal glucose did not affect concentration of glucose in calves, mean RI, mean PI and blood flow (**Table 2**). Concentration of BHBA in dams was related to (P = 0.047) concentration of glucose in calves with dams in the mid-range of BHBA (699, 799, 899, 999 and 1099) having calves with decreased concentrations of glucose (4.1±0.3, 3.8±0.3, 4.1±0.2, 3.7±0.2 and 3.8 ± 0.2 mmol/L, respectively) than calves born from cows in the 599 category (4.8±0.4 mmol L^{-1}) and the 1199 and 1999 categories (4.1±0.3 and 5.0±0.3 mmol L^{-1}, respectively) of BHBA. It was not significantly related to crown rump length, heart girth and hip height, wither height, mean RI, mean PI, mean blood velocity and blood flow (data not shown). Maternal milk production was significantly related to crown rump length, heart girth, hip height and wither height but did not affect concentration of glucose, mean RI, mean PI, blood flow and mean blood velocity (**Table 2**).

Compared to singletons, calves born as twins had decreased (P = 0.008) crown rump length (84.0±3.0 Vs. 94.2±2.1 cm), decreased (P = 0.022) heart girth (77.4±3.0 Vs. 85.9±2.1 cm), decreased (P = 0.005) hip height (76.4±2.2 Vs. 84.4±1.6 cm) and decreased (P = 0.001) wither height (74.0±2.0 Vs. 82.7±1.4 cm). Mean blood velocity tended (P = 0.069) to be greater in twins (54.3±6.3 cm sec^{-1}) than in singletons (40.5±4.0 cm sec^{-1}) and greater (P = 0.098) in females (53.6±4.0 cm sec^{-1}) than in males (41.2±6.3 cm sec^{-1}). Mean blood flow was greater (P = 0.025) in twin calves (653.8±76.4 mL min^{-1}) than in singletons (445.0±48.1 mL min^{-1}) and greater (P = 0.048) in males (640.4±76.4 mL min^{-1}) than in female calves (458.3±48.1 mL min^{-1}). Males also had a greater (p<0.001) area of the carotid artery compared to females. All other parameters at one month of age (concentration of glucose, mean arterial pressure, mean pulse pressure, mean RI and mean PI were similar (p≥0.10) between singletons and twins and between male and female calves.

3.4. Correlations

Growth parameters including heart girth, hip height and wither height were positively correlated in calves at birth and at one month of age (p<0.001; **Table 3**). No other parameters measured were correlated between birth and one month of age (**Table 3**).

Heart girth in calves at birth was positively correlated to the mean milk yield of the dam (P = 0.05) and length of gestation (P = 0.02; **Table 4**). All other growth characteristics at birth were not significantly correlated to variables measured in the dam (**Table 4**). Mean arterial pressure in calves at birth tended to be positively correlated to mean concentration of glucose in the dam (P = 0.07; **Table 4**). Pulse pressure in calves at birth tended to be negatively correlated to the mean concentration of BHBA (P = 0.06; **Table 4**) in the dam. All other cardiovascular characteristics of the calves at birth were not correlated to the measurements in dams.

At one month of age, heart girth in calves was no longer correlated with mean milk yield of dams (P = 0.14; **Table 5**) but still tended to be (P = 0.09) positively correlated to gestation length. Hip height in calves at one month of age was (P = 0.02) positively correlated to milk yield in dams. Wither height in calves at one month of age was positively correlated to mean concentration of glucose (P = 0.02) and gestation length (P = 0.01) in dams. Wither height at one month of age also tended to be (P = 0.07) positively correlated to milk yield in dams.

Concentrations of glucose in calves at one month of age were positively correlated to concentrations of BHBA of their dam (P = 0.03; **Table 5**). Gestation length was negatively correlated to mean resistance index of calves at one month (P = 0.01; **Table 5**). Mean milk yield of dams tended (P = 0.08) to be negatively correlated to mean arterial pressure but tended to be positively correlated to mean pulsatility index of calves at one month (**Table 5**). Mean blood velocity at one month was negatively correlated to milk yield as well (P = 0.01; **Table 5**).

Table 1. LSMeans ± standard errors for parameters measured in calves at birth by categories of concentrations of glucose and daily milk production in dams

	Glucose				Milk Production		
Parameter at birth	< 2.9 mmol L^{-1}	3.0 to 3.9mmol L^{-1}	≥4.0 $mmol^{-1}L$	< 31 kg	31 to 40 kg00	41 to 45 kg	≥46 kg
Weight (kg)	31.3±2.500	34.3±2.200	27.8±4.300	27.9±3.3000	31.8±3.100	28.4±4.000	36.4±4.800
Glucose (mmol/L)	4.1±0.3000	4.1±0.300	4.0±0.400	4.0±0.3000	4.3±0.300	4.2±0.300	3.8±0.400
Crown rump length (cm)	85.2±2.200	84.0±1.500	84.7±3.800	79.8^{a}±1.700	86.7^{b}±2.00	82.8^{ab}±2.20	86.5^{b}±2.00
Heart girth (cm)	75.5^{y}±1.20	76.2^{y}±1.00	70.6^{x}±2.00	70.1^{ax}±1.400	75.4^{by}±1.4	71.8^{ax}±1.50	79.1^{bz}±1.6
Hip height (cm)	76.9±1.400	78.5±1.100	73.3±2.400	72.3^{a}±1.600	77.5^{b}±1.61	75.5^{ab}±1.80	79.6^{b}±1.90
Wither height (cm)	73.8±1.900	74.9±1.300	71.6±3.200	70.5^{x}±1.800	76.2^{y}±1.60	73.8^{xy}±1.70	76.4^{y}±2.00
Mean heart rate (beats per minute)	127.1±8.00	129.7±6.100	148.1±16.10	141.0±11.400	132.7±10.20	128.3±11.00	137.8±15.10
Mean arterial pressure (mmHg)	76.4±3.300	79.8±2.500	90.4±6.600	77.9±4.7000	87.4±4.200	80.0±4.500	83.5±6.200
Pulse pressure (mmHg)	43.5±2.300	44.2±1.700	49.5±4.600	43.7^{xy}±2.600	38.9^{x}±1.90	45.4^{y}±2.10	46.3^{y}±2.40
Mean RI	0.82±0.050	0.86±0.03	0.80±0.08	0.86±0.050	0.89±0.05	0.92±0.05	0.70±0.08
Mean PI	2.1±0.4000	2.6±0.300	1.7±0.800	2.3±0.5000	1.8±0.600	2.9±0.600	1.7±0.900
Mean blood velocity (cm/second)	46.5±6.000	42.4±4.200	48.4±10.20	45.4±5.2000	42.0±4.700	38.1±4.900	44.9±6.000
Carotid blood flow (mL/minute)	386.8±58.8	377.4±48.00	426.3±98.70	483.8^{y}±65.40	380.0^{xy}±58.5	239.0^{x}±61.6	445.4^{xy}±75.5
Carotid blood flow/BW(mL/minute/kg)	16.4±2.100	14.6±1.900	16.1±5.100	22.9^{b}±2.500	9.1^{a}±2.700	10.6^{a}±2.70	19.0^{b}±3.600

[a,b] Within parameters, means without a common superscript letter differ (p≤0.05)
[x,y,z] Within parameters, means without a common superscript letter differ (p≤0.10)

Table 2. LSMeans ± standard errors for parameters measured in calves at one month of age by categories of concentrations of glucose and daily milk production in dams

	Glucose				Milk Production		
Parameter at one monthof age	<2.9mmol/L	3.0 to 3.9mmol/L	≥4.0mmol/L	<31kg	31 to 40kg	41 to 45kg	≥46kg
Glucose (mmol/L)	4.2±0.2	4.3±0.1	3.8±0.3	4.1±0.3	3.9±0.3	4.0±0.3	4.2±0.7
Crownrump length (cm)	89.3^{a}±1.9	93.6^{a}±1.5	78.3^{b}±3.7	83.2^{a}±2.2	87.3^{a}±2.1	84.9^{a}±2.6	93.0^{b}±2.6
Heart girth (cm)	81.9^{b}±1.7	85.2^{b}±1.3	69.6^{a}±3.3	76.2^{a}±2.0	79.4^{ab}±1.9	76.5^{a}±2.4	83.6^{b}±2.3
Hip height (cm)	81.5^{b}±1.4	84.0^{b}±1.1	73.9^{a}±2.8	72.3^{a}±1.6	77.5^{b}±1.6	75.5^{ab}±1.8	79.6^{b}±1.9
With rheight (cm)	78.6^{b}±1.0	81.3^{c}±0.8	71.0^{a}±1.9	75.5^{a}±1.1	77.4^{a}±1.1	73.9^{a}±1.4	81.0^{b}±1.3
Mean RI	0.80±0.06	0.90±0.04	0.88±0.09	0.87±0.05	0.81±0.06	0.97±0.07	0.83±0.09
Mean PI	3.4±0.6	2.5±0.5	2.4±0.9	1.9±1.0	2.2±1.0	2.3±1.2	2.9±1.5
Mean blood velocity (cm/second)	51.3±5.1	44.7±4.1	45.3±7.6	52.2±6.4	55.3±5.6	42.4±7.6	42.8±7.4
Carotid blood flow (mL/minute)	508.6^{z}±63.8	342.3^{y}±51.1	535.5^{z}±95.7	337.2±84.8	542.0±73.4	493.9±100.8	421.3±97.1

[a,b,c] Within parameters, means without a common superscript letter differ (p≤ 0.05)
[y,z] Within parameters, means without a common superscript letter differ (p≤ 0.10)

Table 3. Measurements of calves at birth and one month of age (mean ± SD)

	Measurements of calves	
Parameter	At birth	At 1 mo of age
Glucose	4.2±1.1 mmol L^{-1}	4.2±0.5 mmol L^{-1}
Heart girth***	77.0±4.4 cm	86.6±5.2 cm
Hip height***	79.5±4.1 cm	85.3±3.9 cm
Wither height***	76.2±4.3 cm	82.8±4.1 cm
Mean heart rate	129.5±21.3 beats per min	110.2±26.5 beats per min
Mean arterial pressure	80.1±8.8 mmHg	95.3±16.6 mmHg
Pulse pressure	42.8±7.0 mmHg	49.2±9.5 mmHg
Mean resistance index	0.8±0.2	0.8±0.1
Mean Pulsatility index	2.4±1.3	2.4±1.0
Mean blood velocity	41.8±15.2 cm/second	46.3±15.9 cm/sec
Carotid blood flow	395.5±202.2 mL^{-1} min	431.5±172.9 mL^{-1} min

***Parameters positively correlated (p<0.001) between birth and one month of age

Table 4. Pearson correlations (r) between growth and cardiovascular measurements in calves at birth and in dams during early gestation

	Measurements in dams during early gestation			
Measurements at birth in calves	Mean milk yield	Mean glucose	Mean BHBA	Gestation length
Heart girth	0.41*	0.11	0.01	0.45*
Hip height	0.310	0.30	-0.09	0.22
Wither height	0.230	0.19	0.15	0.29
Glucose	-0.31	0.07	0.30	-0.18
Mean heart rate	-0.29	0.06	-0.06	-0.14
Mean arterial pressure	0.010	0.38†	0.07	0.07
Pulse pressure	0.260	-0.26	-0.38†	0.13
Mean resistance index	-0.32	-0.21	0.01	-0.14
Mean pulsatility index	-0.06	-0.26	-0.13	0.06
Mean blood velocity	-0.19	0.17	0.08	0.13
Carotid blood flow	-0.21	0.26	0.19	0.09

*p≤0.05
†p>0.05 but ≤ 0.10

Table 5. Pearson correlations (r) between cardiovascular measurements in calves at one month of age and in dams during early gestation

	Measurements in dams during early gestation			
Measurement at 1 month in calves	Mean milk yield	Mean glucose	Mean BHBA	Gestation length
Heart girth	0.390	0.39	-0.10	0.43†
Hip height	0.60*	0.06	-0.14	0.39
Wither height	0.47†	0.57*	-0.17	0.62*
Glucose	0.180	-0.09	0.58*	-0.38
Mean heart rate	0.170	-0.39	0.02	-0.23
Mean arterial pressure	-0.46†	-0.25	-0.05	0.01
Pulse pressure	0.080	0.31	0.19	-0.11
Mean resistance index	0.110	-0.19	0.27	-0.59*
Mean pulsatility index	0.44†	-0.14	0.03	-0.39
Mean blood velocity	-0.61*	-0.13	0.13	0.02
Carotid blood flow	-0.30	0.31	0.30	0.10

*p≤0.05
†p>0.05 but ≤ 0.10

4. DISCUSSION

A period of negative energy balance at some point in early lactation is common among high-producing dairy cows. The effects on offspring which were conceived during a nutritional insult (less than adequate nutrient intake) have yet to be investigated in the dairy industry. This characterization study was designed to initiate that avenue of research and to potentially provide evidence of changes in the offspring to be further evaluated. In this current study, Holstein and Jersey breeds of cows were included and concentrations of BHBA and glucose were not different between the two breeds. Five twin calves were also included and concentrations of BHBA and glucose did not differ between cows that had singletons versus those with twins. Milk production, as expected, did differ between Holstein and Jersey cows but did not differ between cows with singletons and those with twin calves.

Ewes gestating singleton versus multiple fetuses have been identified as a compromised pregnancy model due to the intrauterine growth restriction that occurs (Grazul-Bilska *et al*., 2006). For example, gestating multiple fetuses causes a 30% decrease in fetal weight and a 23% decrease in uterine blood flow (Reynolds *et al*., 2006). In addition, this decrease in fetal weight specific to twins has been associated with an attenuated postnatal hypothalamic-pituitary-adrenal response (Bloomfield *et al*., 2007). In beef cattle, post-natal average daily gain is increased in singleton calves versus twins (Gregory *et al*., 1996); however, these differences in growth rates are more than likely a combination of physiological limitations of the uterus and mammary gland. A paucity of information exists on cardiovascular function in twins versus singletons during the neonatal period. Although the current experiment contained a limited number of twins it is still interesting to note the differences in blood flow at one month of age. This developmental plasticity of the cardiovascular system may allow for specific adaptations to promote blood flow and growth during early life.

Maternal glucose was measured as an indicator of nutritional availability to the developing embryo/fetus and placenta. Dams with the greatest concentration of glucose tended to have calves with the smallest hearth girth at birth and mean arterial pressure in calves at birth tended to be positively correlated to mean concentration of glucose in the dam. Wither height in calves at one month of age was positively correlated to mean concentration of glucose. Maternal undernutrition in early pregnancy may cause hypertension in offspring as human adults (Hult *et al*., 2010; Ravelli *et al*., 1976) as well as metabolic syndrome (impaired glucose tolerance, hypertension and increased triglycerides) in humans (Barker *et al*., 1993; Boney *et al*., 2005; De Rooij *et al*., 2007; Yarbrough *et al*., 1998). It is yet to be determined if concentration of glucose in the dam during early gestation might impact calves as they grow and join the lactating herd or whether a method of compensation takes place instead.

Undernutrition during early to mid-pregnancy followed by dietary realimentation alters place tome vascularity, antigenic factor expression and uterine blood flow in beef cattle (Vonnahme *et al*., 2007; 2013). Specifically, undernutrition during early pregnancy followed by realimentation increased uterine artery blood flow and placental artery sensitivity to the vasodilator bradykinin during late gestation when the majority of fetal growth occurs (Vonnahme and Lemley, 2012). Therefore, nutrient restriction during early pregnancy may improve late gestation placental functional capacity in cattle; however, the mechanisms behind this compensatory response remain elusive. Birth weight is often correlated with nutritional availability throughout pregnancy in ovine models of intrauterine growth restriction (Reynolds *et al*., 2005; 2006); however, similar nutrient restriction studies in cattle have shown limited alterations to birth weight and early post-natal growth (Mossa *et al*., 2013). Expectedly, concentration of glucose in dams did not cause a difference in calf birth weight in the current study. Moreover, undernutrition was not induced in this study; it could be that milk production alone did not create a stressed gestational environment. The mechanisms of the relationships identified in this current study are unclear; however it is likely that maternal nutrition does impact parameters in offspring to some extent.

β-hydroxybutyrate was measured as an indicator of the process of fat mobilization that dairy cows undertake during a time of negative energy balance, often in early lactation, to make up for their diet or intake not meeting their nutritional requirements. Cows included in this study had to conceive to artificial insemination and the first artificial insemination after calving occurred no sooner than 60 days post-calving. Because of typical conception rates to artificial insemination, there was a significant range in days in milk at the time of conception and this led, in part, to an extensive range in milk production during the collection period (time of artificial insemination, 30, 60 and 90 days after conception). The variation in milk production capacity and the average production for individual cows also increased the variation in milk production. Concentrations of BHBA were quite variable, no doubt due to the range in days in milk which is often tied to energy balance. Because of this range, only three cows were considered to have concentrations of BHBA high enough to constitute a subclinical case of ketosis. Regardless, concentrations of BHBA in dams did not alter any parameters in calves at birth but did affect concentration of glucose in calves at one month of age which was also positively correlated to concentrations of BHBA of their dam. Pulse pressure in calves at birth tended to be negatively correlated to the mean concentration of BHBA in the dam.

Although general undernutrition during pregnancy has been studied extensively, particularly in humans, particular micronutrient availability has been investigated less. Lacking iron during early gestation was associated with greater mean arterial pressure and systolic blood pressure in humans (Bourque *et al*., 2008; 2012; Gambling *et al*., 2003; Lewis *et al*., 2001; Lisle *et al*., 2003). Similarly, in cattle increased arterial pressure and enlargement of the aorta have been observed in offspring born to dams subjected to early gestational undernutrition (Mossa *et al*., 2013). As stated above, substantial evidence exists that supports that nutrient availability in early gestation can impact metabolic characteristics in offspring, particularly if nutrient availability dramatically changes between the environment in the uterus and out of the uterus (Lakshmy, 2013). However, it would be speculative to suggest that BHBA in the dams directly changed concentration of glucose in the calves at one month of age based on these data. It does warrant further investigation.

Maternal milk production is an indicator of the nutritional demands placed on the dam during early gestation. Production is related to the genetic capacity of the cow to produce milk and also stage of lactation and nutrition, among other things. Milk production tended to affect wither height, pulse pressure and carotid blood flow. It was also positively correlated to heart girth in calves at birth and this may be because larger cows tend to produce more milk and they also produce larger calves, although cow size was not measured in this experiment. It may also be related to feed intake; however, individual feed intake was not recorded. At one month of age, a correlation between milk production and heart girth no longer existed; however, a positive correlation between hip height developed and there tended to be a positive correlation between wither height. It was interesting that fewer correlations existed between size characteristics of calves and parameters of dams when the calves were born compared to when they were at one month of age. Mean milk yield of dams tended to be negatively correlated to mean arterial pressure but tended to be positively correlated to mean pulsatility index of calves at one month. Mean blood velocity at one month was negatively correlated to milk yield as well. Although explanations for these correlations or lack of correlations would be speculation, it is clear that further research is necessary to elucidate these explanations.

Although growth parameters were positively correlated in calves between birth and one month of age as expected, it was hypothesized that other parameters might also be correlated and this was not the case. For example, it was expected that heart rate would decrease from birth to one month of age as the neonatal calf matures but the lack of a correlation indicates this did not occur in a similar way among calves. A lack of research in this area is evident from the literature but determining when these potential changes in offspring are present and their impact are important components of developmental programming research.

5. CONCLUSION

These data lead to speculation that early gestational environment may impact growth and some hemodynamic parameters in young calves. Parameters measured at birth and at one month of age are not necessarily correlated and thus further experiments are necessary to understand how the early neonate changes and perhaps compensates for insults during early gestation. Further research can lead to understanding the impact of maternal nutritional status during early gestation and short and long-term implications to health and growth of offspring.

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