Effects of Maternal Nutrition on Fructose and Expression of the Fructose Transporter *GLUT5* in Bovine Tissues and Fluids from Days 16 to 50 of Gestation

Nathaniel Greseth[1], Matthew Crouse[1], Kyle McLean[1], Mellissa Crosswhite[1], Nicolas Negrin Pereira[1], Alison Ward[1], Larry Reynolds[1], Carl Dahlen[1], Bryan Neville[2], Pawel Borowicz[1] and Joel Caton[1]

[1]Department of Animal Sciences, NDSU
[2]Central Grasslands Research Extension Center, NDSU

The objectives of this study were to determine the effects of maternal nutritional status on fructose concentration in maternal and fetal fluids and the mRNA expression of the fructose transporter GLUT5 in maternal and fetal tissues on days 16, 34, and 50 of gestation. The expression of GLUT5 was not influenced by maternal nutritional status; however, the concentration of fructose in amniotic fluids was influenced by day of gestation and maternal nutritional status.

Summary

We tested the hypothesis that the concentration of fructose in maternal and fetal fluids, and expression of *GLUT5* in utero-placental tissues, would be influenced by day of gestation and maternal nutritional status. Angus-cross heifers (n = 46, about 15 months of age; average initial body weight [BW] = 716 pounds) were estrus synchronized, bred via artificial insemination (AI) and ovariohysterectomized on day 16, 34, or 50 of their respective gestations (n = 6 to 9/day). Some heifers (n = 6) were not bred to serve as nonpregnant (NP) controls and were ovariohysterectomized on day 16 of the synchronized estrous cycle. Immediately after AI, heifers were assigned randomly to one of two treatment groups: Control (CON) received 100 percent of the National Research Council (NRC, 2000) requirements to gain 1 pound per heifer daily, and restricted (RES) received only 60 percent of the CON diet. Tissues collected included: caruncular tissue from the uterine horn ipsilateral to the corpus luteum (PC), from the uterine horn contralateral to the corpus luteum (NPC), inter-caruncular tissue from the uterine horn ipsilateral to the corpus luteum (PIC) and from the uterine horn contralateral to the corpus luteum (NPIC), as well as chorioallantoic tissue (FM). Fluids collected included: maternal serum, histotroph collected from horn ipsilateral to the corpus luteum (P histotroph) and from the horn contralateral to the corpus luteum (NP histotroph), allantoic fluid and amniotic fluid. Fetal membranes, allantoic fluid and amniotic fluid were not collected in NP heifers due to the lack of the presence of fetal tissues and fluids in NP animals. Serum fructose concentrations were greater ($P < 0.01$) in nonpregnant heifers, compared with pregnant heifers. Concentrations of fructose in P histotroph and NP histotroph were greater ($P < 0.01$) on day 50, compared with days 16 and 34. Amniotic fluid was influenced by a day × treatment interaction, with day 34 RES being greater ($P = 0.04$) than day 50 CON and RES heifers. Expression of *GLUT5* was greater on day 34 in PC ($P = 0.02$) and NPC ($P < 0.01$). In FM, day 16 was greater ($P = 0.04$), compared with days 34 and 50 of gestation. These results indicate that the expression of GLUT5 is not influenced by maternal nutritional status; however, concentrations of fructose in amniotic fluids are influenced by maternal nutritional status and day of gestation.

Introduction

First-service AI rates in beef cows are approximately 90 percent (Bridges et al., 2013); however, by day 30, only 50 to 60 percent are viable embryos in beef cows. Furthermore, fetal growth is vulnerable to maternal dietary nutrient deficiencies during the first trimester of gestation (Wu et al., 2004).

Currently, fetal undernutrition occurs in grazing livestock worldwide (Wu et al., 2004). Early in gestation, trans-placental exchange has yet to be established; therefore, nutrients must be transported to the conceptus via nutrient transporters in the uterus and developing placenta, such as the fructose transporter *GLUT5*.

Fructose is the most abundant hexose sugar in fetal blood and fetal fluids of ungulates (Kim et al., 2012), and maternal undernutrition has been implicated in altered fructose transport (Zhang et al., 2015). Having an understanding of how maternal nutrition affects the mRNA expression and supply of fructose to the conceptus could lead to future research that may directly influence the flux of fructose from the maternal to fetal systems in early gestation.

This research utilized a newly developed technique to ovariohysterectomize cattle without slaughter to allow for an accurate analysis of fetal growth and development, as well as utero-placental tissues and fluids on days 16, 34 and 50 of early gestation in beef heifers.

In this study, we tested the hypothesis that the concentration of fructose in maternal and fetal fluids, along with the relative mRNA expression of *GLUT5* in maternal and fetal tissues, would be influenced by day of gestation and maternal nutritional status.

Procedures

All animal procedures were approved by the North Dakota State University Institutional Animal Care and Use Committee (IACUC numbers A14053 and A16049). Crossbred Angus heifers (n = 49, about 15 months of age; average initial BW = 716 pounds) were exposed to the 5-day CO-Synch + CIDR estrus synchronization protocol. Six heifers were not inseminated to serve as nonpregnant controls, but received an ovariohysterectomy on day 16 of the subsequent synchronized estrous cycle. The remaining heifers (n = six to nine/day of gestation/treatment) were bred by AI to a common sire at 12 hours after observed estrus and ovariohysterectomized at days 16, 34, and 50 of gestation.

Immediately following the ovariohysterectomy, maternal caruncular (PC) and inter-caruncular tissue (PIC) were collected from the uterine horn ipsilateral to the corpus luteum, along with caruncular (NPC) and inter-caruncular tissue (NPIC) collected from the uterine horn contralateral to the corpus luteum. Also, fetal membranes (chorioallantois) were obtained.

Fetal membranes were collected only on days 16, 34, and 50 of gestation due to nonpregnant controls not having fetal membranes. All tissues were frozen immediately in liquid nitrogen-cooled isopentane and stored at minus 112 F.

Serum samples were obtained via jugular venipuncture on the day of ovariohysterectomy, and blood constituents were separated by centrifugation and stored at minus 20 F. Histotroph was obtained from the uterine horn ipsilateral to the corpus luteum (P histotroph) and from the uterine horn contralateral to the corpus luteum (NP histotroph) for pregnant and NP heifers.

Allantoic and amniotic fluids were collected on days 34 and 50 of gestation due to the limited presence of fetal fluids on day 16. Histotroph, allantoic and amniotic were snap-frozen in liquid nitrogen-cooled isopentane and stored at minus 20 F immediately after being obtained.

Fructose concentrations among fluid samples were determined by utilizing a colorimetric assay kit. Expression of *GLUT5* was determined by first isolating and purifying RNA from collected tissue samples, followed by real-time quantitative PCR (qPCR) to determine differences in mRNA expression of the fructose transporter in each tissue relative to a NP endometrium sample.

Results and Discussion

Concentrations of fructose in maternal serum were greater ($P < 0.01$) in NP heifers, compared with pregnant heifers (Table 1). In P histotroph, fructose concentrations were greater ($P < 0.01$) on day 50, compared with days 16 and 34 (1.34, 0.18, and 0.63 millimolar [mM], respectively; SEM = 0.22).

Fructose concentrations also were greater ($P < 0.01$) on day 34 + 50, when compared with day 16 (Table 1). In NP histotroph, day 50 (0.97 mM) was greater ($P = 0.01$) than days 16 and 34 (0.10, and 0.36 mM, respectively; SEM = 0.20).

Additionally, fructose concentrations were greater ($P = 0.02$) in NP histotroph on days 34 + 50, compared with day 16 (Table 1). Fructose concentrations in amniotic fluid were influenced by a day × treatment interaction, where day 34 RES (3.60 mM) was greater ($P = 0.04$) than the day 50 CON and RES treatments (2.57, and 1.55 mM, respectively; SEM = 0.29). Furthermore, day 34 and 50 CON (3.30 and 2.57 mM, respectively) were greater than day 50 RES (1.55 mM; SEM = 0.29).

In PC, a main effect of day was observed; day 34 was greater (44.40-fold greater than NP; $P = 0.02$; Table 2) than day 16 and day 34 (4.79 and 6.49-fold greater than NP, respectively; SEM = 10.88).

In NPC, the expression of *GLUT5* was greater ($P < 0.01$) on days 34 and 50, compared with day 16 (44.19, 36.60 and 3.22 -fold greater than NP, respectively; Table 2). Furthermore, the relative expression of *GLUT5* in pregnant heifers was greater ($P = 0.02$), compared with NP heifers.

A main effect of day was observed in FM where day 16 was greater ($P = 0.04$) than day 50 (80.17 and 27.39, respectively; SEM = 13.82; Table 2). Also, day 16 was greater, compared with day 34 + 50 ($P = 0.03$). The results show that as pregnancy advances, fructose and mRNA expression of *GLUT5* changes significantly among various tissues and fluids measured in this study.

The low fructose concentration found in maternal serum is expected because fructose is not a main physiological fuel for the dam. The cause of the NP heifers having a greater fructose concentration in serum samples could be due to fructose being utilized by the conceptus. The greater mRNA expression of *GLUT5* in pregnant heifers, compared with NP

Table 1: Fructose concentrations mM in Serum (maternal serum), P histotroph (histotroph from the horn ipsilateral to the corpus luteum), NP histotroph (histotroph from the horn contralateral to the corpus luteum), Allantoic (allantoic fluid) and Amniotic (amniotic fluid) on days 16, 34 and 50 of gestation.

			Day of Gestation[1]						*P* - values[2]					
Fluid[3]	Trt[4]		NP	16	34	50	Trt[5]	SEM[6]	NP vs. P	16 vs. 34 + 50	34 vs. 50	Day	Trt	Day × Trt
Serum	CON		0.13	0.08	0.075	0.071	0.07							
	RES		-	0.08	0.085	0.094	0.09	0.016	< 0.01	0.79	0.92	0.97	0.35	0.82
		Day[7]		0.08	0.080	0.083								
P Histotroph	CON		0.14	0.12	0.58	1.03	0.58							
	RES		-	0.23	0.69	1.64	0.86	0.310	0.09	< 0.01	0.03	< 0.01	0.27	0.65
		Day		0.18[h]	0.63[h]	1.34[g]								
NP Histotroph	CON		0.01	0.11	0.45	0.67	0.41							
	RES		-	0.08	0.26	1.27	0.54	0.277	0.13	0.02	0.02	0.01	0.58	0.33
		Day		0.10[h]	0.36[h]	0.97[g]								
Allantoic	CON		-	-	5.53	5.07	5.30							
	RES		-	-	4.56	4.83	4.69	0.76	-	-	0.95	0.90	0.44	0.64
		Day		-	5.04	4.95								
Amniotic	CON		-	-	3.30[ab]	2.57[b]	2.94							
	RES		-	-	3.56[a]	1.55[c]	2.55	0.29	-	-	-	< 0.01	0.21	0.04
		Day		-	3.43	2.06								

[1]Number of days after AI.

[2]Probability values for the effect of day, treatment and day × treatment on the concentration of fructose. Contrast statements comparing pregnant vs. nonpregnant concentration (NP vs. P), day pre-implantation vs. day post-implantation (day 16 vs. 34 + 50) and day post-attachment comparison (34 vs. 50).

[3]Fluids evaluated for fructose concentrations mM include maternal serum (Serum), histotroph flushed from the horn ipsilateral to the corpus luteum (P histotroph), histotroph flushed from the horn contralateral to the corpus luteum (NP histotroph), allantoic fluid (Allantoic) and amniotic fluid (Amniotic).

[4]CON = Heifers fed a TMR that meets 100 percent of NRC requirements to gain 0.45 kg daily. RES = Heifer restricted to 60 percent of CON diet.

[5]Mean fructose concentration of treatment groups across day of gestation within fluid.

[6]Average SEM for day × treatment interaction. Day 16 CON n = 7, day 16 RES n = 7, day 34 CON n = 6, day 34 RES n = 9, day 50 CON n = 7, day 50 RES n = 7.

[7]Mean fructose concentration across treatment within day of gestation.

[a-c]Means within fluid without common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without common superscript differ in main effect of day ($P \leq 0.05$).

heifers in NPC, may be explained by the conceptus's increasing need of fructose, which is partially supplied by maternal blood concentrations.

The consistent concentration of fructose in P histotroph and NP histotroph may be explained by the availability of fructose for transport into the uterine lumen. Fructose concentrations were found to be less than 1 mM in maternal circulation; therefore the total available fructose to be transported to the conceptus from the maternal system is low.

The placenta is a site of the conversion of glucose to fructose (Kim et al., 2012), which plays a role in the consistently high fructose concentration and the increase in fructose concentration found in fetal fluids compared with maternal fluids. This conversion of glucose to fructose indicates the

Table 2: Relative mRNA expression of *GLUT5* in PC (pregnant caruncle), PIC (pregnant inter-caruncle), NPC (non-pregnant caruncle), NPIC (non-pregnant inter-caruncle) and FM (fetal membranes from days 16, 34, and 50 of gestation as a fold change in relation to nonpregnant heifer samples set to 1.

			Day of Gestation[1]					*P* - values[2]					
Tissue[3]	Trt[4]		16	34	50	Trt[5]	SEM[6]	NP vs. P	16 vs. 34 + 50	34 vs. 50	Day	Trt	Day × Trt
PC	CON		6.61	22.06	9.07	12.58							
	RES		2.98	66.74	3.91	24.54	15.33	0.24	0.06	< 0.01	0.02	0.35	0.20
		Day[7]	4.79[h]	44.40[g]	6.49[h]								
PIC	CON		1.46	3.60	6.11	3.72							
	RES		3.59	3.82	1.20	2.87	2.36	0.36	0.58	0.97	0.85	0.66	0.31
		Day	2.52	3.71	3.66								
NPC	CON		4.24	36.44	53.54	31.41							
	RES		2.21	51.93	19.66	24.60	10.39	0.02	< 0.01	0.36	< 0.01	0.43	0.07
		Day	3.22[h]	44.19[g]	36.60[g]								
NPIC	CON		25.23	38.42	49.57	37.74							
	RES		14.29	27.02	6.99	16.10	16.90	0.17	0.46	0.82	0.74	0.13	0.57
		Day	19.76	32.72	28.28								
FM	CON		59.78	43.59	33.40	45.59							
	RES		100.57	46.63	21.38	56.19	19.54	-	0.03	0.30	0.04	0.52	0.44
		Day	80.17[g]	45.11[gh]	27.39[h]								

[1]Number of days after AI.

[2]Probability values for the effect of day, treatment and day × treatment on the mRNA expression of *GLUT5*. Contrast statements comparing pregnant vs. nonpregnant expression (NP vs. P), day pre-implantation vs. day post-implantation (day 16 vs. 34 + 50), and day post-attachment comparison (34 vs. 50).

[3]Tissues evaluated for mRNA expression of *GLUT5* include caruncular tissue collected from the uterine horn ipsilateral to the corpus luteum (PC), caruncular tissue collected from the uterine horn contralateral to the corpus luteum (NPC), inter-caruncular tissues collected from the uterine horn ipsilateral to the corpus luteum (PIC), inter-caruncular tissue collected from the uterine horn contralateral to the corpus luteum (NPIC), and chorioallantois (FM).

[4]CON = Heifers fed a TMR that meets 100 percent of NRC requirements to gain 0.45 kg daily. RES = Heifer estricted to 60 percent of CON diet.

[5]Mean *GLUT5* mRNA expression of treatment groups across day of gestation within tissue.

[6]Average SEM for day × treatment interaction. Day 16 CON n = 7, day 16 RES n = 7, day 34 CON n = 6, day 34 RES n = 9, day 50 CON n = 7, day 50 RES n = 7.

[7]Mean *GLUT5* mRNA expression across treatment within day of gestation.

[a-c]Means within tissue without a common superscript differ in day × treatment ($P \leq 0.05$).

[g-h]Means within row without a common superscript differ in main effect of day ($P \leq 0.05$).

essentiality of fructose for the growth and development of the conceptus. Furthermore, this consistently high concentration could be explained by the conceptus's hypoxic environment.

Vascularization of the fetal membranes is limited up to day 35 of gestation, which results in an oxygen-poor environment for the conceptus due to a lack of a transport of oxygen via a shared blood supply. Glucose thrives in a hyperoxic environment, while fructose thrives in a hypoxic environment.

This information is emphasized by our observed results in fetal fluids and FM. Fructose concentration decreased from day 34 to 50 (numerically in allantoic), while relative

expression of *GLUT5* decreased from day 16 to 50, indicating a decreased need of fructose transport.

These decreases could be due to vascularization intensifying after day 35, resulting in an increase in oxygen for the conceptus's environment, thereby decreasing the concentration of fructose (3.43 to 2.06 mM in amniotic fluid from day 34 to 50, respectively) and increasing the need of glucose (results from our lab not shown; 1.46 to 1.68 mM glucose in amniotic fluid from day 34 to 50, respectively).

In amniotic fluid, fructose concentrations differed between day 34 RES and day 50 CON and RES, as well as between day 50 CON and RES. We interpret these data to imply that a compensatory mechanism may be in action when examining the greater fructose concentration found in day 34 RES, compared with day 50 CON and RES.

Organogenesis takes place throughout the first 50 days of gestation, with most of the fetal organs having significantly developed by day 50. At this time, the conceptus could have a greater need of fructose. Therefore, a compensatory mechanism may have been in action, resulting in a greater amount of fructose being made available to the conceptus for day 34 RES to maintain a viable pregnancy.

When examining the greater concentrations found in day 50 CON, compared with day 50 RES, we interpret these data to imply that the conceptus could have a lower need of fructose at this time, which is supported by the decrease in fructose concentration observed from day 34 to 50 in amniotic fluid and the increased placental development and vascularization on day 34, compared with day 50.

This potential decreased need of fructose may have resulted in the lack of the aforementioned compensatory mechanism. Therefore, the greater concentration of fructose found in day 50 CON could be explained by the day 50 CON receiving 100 percent of NRC requirements, while the day 50 RES received only 60 percent of requirements.

In PC and NPC, relative *GLUT5* mRNA expression was greater on days 34 and 50, compared with day 16. We interpret these data to imply that the increase could be due to the critical period for maternal recognition (days 15 to 16) already passing (Senger, 2012), resulting in an increase in expression to compensate for the nutritional needs of the developing conceptus.

The FM had high mRNA expression of *GLUT5* relative to NP. We interpret this data to imply that the conversion of glucose to fructose may have an impact on the mRNA expression of *GLUT5* in FM.

When examining the main effect of day seen in FM, the fold change relative to NP decreases from 80.17 at day 16 to 27.39 -fold greater than NP by day 50. We interpret these data to imply that sugars such as fructose are highly important in supplying energy for the elongation of the conceptus on days 12 to15 to ensure maternal recognition of pregnancy to occur by days 15 to 16 (Senger, 2012).

In conclusion, these data partially support our hypothesis that day of gestation and maternal nutritional status would impact mRNA expression of *GLUT5* in utero-placental tissues and fructose concentration among maternal and fetal fluids. In partially keeping with our hypothesis, we found that day of gestation, but not a 40 percent global nutrient restriction, affects the relative expression of *GLUT5* in PC, NPC and FM.

In addition, day of gestation, and not a 40 percent global nutrient restriction, affects fructose concentration in histotroph. Furthermore, maternal nutritional status and day of gestation affect fructose concentration in amniotic fluid.

With the establishment of these data, future research can be aimed at increasing efficiency of maternal and fetal nutrition. Specifically, providing improvements to the dam's nutrition at certain points of gestation allows the conceptus to receive an appropriate amount of fructose throughout early gestation, which it needs for proper growth and development. Applications such as this may result in increased reproductive efficiency and, ultimately, aid in supporting the increasing need of food by the growing world population.

Literature Cited

Bridges, G.A., M.L. Day, T.W. Geary and L.H. Cruppe. 2013. Deficiencies in the uterine environment and failure to support embryonic development. J. Anim. Sci. 91:3002-3013

Elliot, I. Meat output must double by 2050. Feedstuffs. Accessed 8/5/2016: http://feedstuffsfoodlink.com/story-meat-ouput-must-double-by-2050-71-66920

Kim, J., G. Song, G. Wu and F.W. Bazer. 2012. Functional Roles of Fructose. PNAS. 109:E1619 – E1628.

Senger P.L. 2012. Early Embryogensis and Maternal Recognition of Pregnancy. In: Pathways to Pregnanncy and Parturition. Current Conceptions Inc. Redmond, Ore. P. 282.

Wu, G., F.W. Bazer, T.A. Cudd, C.J. Meininger and T.E. Spencer. 2004. Maternal nutrition and fetal development. J. of Nutrition. 9:2169-2172.

Zhang, S., T.R.H. Regnault, P.L. Barker, K.J. Botting, I.C. McMillen and C.M. McMillan, C.T. Roberts and J.L. Morrison. 2015. Placental adaptation in growth restriction. Nutrients. 7:360-389.