Nutrient transporters in bovine utero-placental tissues on days 16 to 50 of gestation

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The objectives of this study were to determine the effect of the day of early gestation on messenger ribonucleic acid (mRNA) expression of nutrient transporters known for their roles in transporting arginine (SLC7A1, SLC7A2 and SLC7A3) and glucose (GLUT-1 and GLUT-3) across the uterine endometrium and fetal membranes to the fetus. The results indicate that the expression of these transporters changes dramatically during the first 50 days of gestation and that the days of key importance to further investigation are days 16, 34 and 50 of gestation.

Summary

Our hypothesis was that transporters for glucose and amino acids in utero-placental tissues would be expressed differentially across days of early pregnancy. To test this hypothesis, crossbred Angus heifers (n = 46), were synchronized, bred via artificial insemination (AI) and then ovariohysterectomized on days 16, 22, 28, 34, 40 or 50 of gestation (n = 5 to 9/day), or were not bred and ovariohysterectomized on day 16 of the synchronized estrous cycle (n = 7) to serve as nonpregnant (NP) controls. Utero-placental tissues (caruncular, CAR; intercaruncular, ICAR; and fetal membranes, FM [chorioallantois, day 22 and later]) were collected from the uterine horn of pregnancy immediately following ovariohysterectomy. For NP controls, only CAR and ICAR were obtained. The relative mRNA expression of the glucose transporters GLUT1 and GLUT3, as well as cationic amino acid transporters SLC7A1, SLC7A2 and SLC7A3, was determined for each tissue from days 16 to 50 of gestation and also for NP controls. In CAR, the expression of GLUT1 was greatest ($P < 0.001$) on day 16, and the expression of GLUT3 was greatest ($P = 0.01$) on day 50 of gestation. The expression of cationic amino acid transporter SLC7A1 was greater ($P \leq 0.05$) in CAR on days 28, 34 and 40, compared with NP and days 16, 22 and 50. We found no effect of day on SLC7A2 expression in CAR. The expression of SLC7A3 was greatest ($P = 0.01$) in CAR on day 16. In ICAR, the expression of GLUT1 was greatest ($P < 0.001$) on day 16 of gestation. Relative expression of GLUT3

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tended to be greater ($P = 0.06$) in ICAR at days 34 and 40, compared with NP. Intercaruncular expression of SLC7A1 and SLC7A2 was greatest on day 34 ($P < 0.001$ and $P = 0.02$, respectively). The relative expression of SLC7A3 was greater ($P \leq 0.05$) in ICAR on days 28, 34 and 40 compared, with days 16 and 22. In FM, GLUT1 was greater ($P \leq 0.05$) on day 22, compared with days 34, 40 and 50. We found no effect of day on expression of GLUT3 in FM. The expression of SLC7A1 was greatest ($P < 0.001$) in FM at day 22. We founds no day effect for SLC7A2 or SLC7A3 in FM. These results support our hypothesis that an effect of day on the expression of glucose and amino acid transporter mRNAs in utero-placental tissues of heifers does exist during early pregnancy.

Introduction

To meet the projected food requirements of the growing population, the world needs to increase its output of meats significantly by 2050 (Elliot, 2013). Currently, fertilization rates for first-service AI are approximately 90 percent in beef heifers (Bridges et al., 2013); however, by day 30 of gestation, only 50 to 60 percent are viable embryos. Moreover, Thatcher et al., (1994) indicated that up to 40 percent of all embryonic loss occurs before day 40 of gestation.

We recently developed a standing, flank ovariohysterectomy procedure that allows for a detailed and accurate assessment of expression of utero-placental nutrient transporters during the early stages of gestation (NP to day 50 of gestation).

The presence of nutrient transporters and nutrient flow to the growing embryo is crucial for proper development and growth. During this time, the placenta is developing, and the fetus begins to utilize increasing quantities of glucose and amino acids, which are supplied via uterine secretions (Gardner, 1998; Groebner et al., 2011; Bazer et al., 2014). Thus, the expression of glucose and amino acid transporters in the utero-placenta becomes essential to the viability of the conceptus.

The main utero-placental glucose transporters are *GLUT1* and *GLUT3*. The *GLUT1* isoform is the main glucose transporter; it is present in most tissues throughout the body and is ubiquitous across species. The *GLUT3* is a specific neural and placental glucose transporter.

The main cationic utero-placental amino acid transporters are *SLC7A1, SLC7A2* and *SLC7A3*. The luminal and glandular epithelium of the endometrium have a greater prevalence of *SLC7A1* and *SLC7A2*, with *SLC7A3* also being located in stromal cells (Bazer et al., 2011). The substrates for these transporters are amino acids such as arginine and lysine, which are linked directly to angiogenesis and cell proliferation.

In this study, we tested the hypothesis that mRNA for glucose and amino acid transporters in utero-placental tissues is expressed differentially across days of early pregnancy.

Experimental Procedures

Animals

Protocols described herein were approved by the North Dakota State University Institutional Animal Care and Use Committee. Crossbred Angus heifers (n = 46, ~15 months of age; body weight [BW] = 798.7 ± 76.5 pounds) were exposed to the five-day CO-Synch + CIDR estrus synchronization protocol. Seven heifers were not inseminated to serve as nonpregnant (NP) controls, but they received ovariohysterectomy on day 16 of the synchronized estrous cycle. The remaining heifers (n = 5 to 9/day) were AI bred at 12 hours after observed estrus and ovariohysterectomized at days 16, 22, 28, 34, 40 or 50 of gestation.

Sample Collection

Immediately following ovariohysterectomy, utero-placental tissues (caruncle, CAR; intercaruncular endometrium, ICAR; and fetal membranes, FM [chorioallantois]) were obtained from the uterine horn containing the conceptus, as previously described (Grazul-Bilska et al., 2010, 2011). Fetal membranes also were collected only from day 22 and later days due to inadequate quantities of FM on day 16 and the absence of FM in NP controls. Once collected, all tissues were snap frozen in liquid nitrogen-cooled isopentane and stored at minus 112 F.

Real-time Reverse Transcription Quantitative PCR

The RNA was extracted and purified from each tissue. The level of mRNA expression of each transporter within the tissue was established using polymerase chain reaction (PCR) to determine differences in mRNA expression of the transporters across days of early gestation.

Results and Discussion

Maternal Caruncules (CAR)

The expression of *GLUT1* mRNA was greatest at day 16, with a 7.8-fold increase ($P < 0.001$), compared with NP, and also exhibited a 4.3-fold increase ($P < 0.05$) at day 22, with the remaining days of early pregnancy being similar to NP (Table 1). The expression of *GLUT3* mRNA was 13.68-fold greater ($P < 0.01$) at day 50, compared with NP, intermediate at day 34 and similar ($P > 0.05$) among NP on days 16, 22, 28 and 40 of gestation.

The expression of *SLC7A1* mRNA was greater ($P < 0.05$) on days 28, 34 and 40, compared with NP on days 16, 22 and 50 of gestation (Table 1). The stage of gestation did not influence *SLC7A2* mRNA expression ($P = 0.20$). The expression of *SLC7A3* mRNA was greatest at day 16 (6.89-fold greater), de-

creasing to 0.69-fold by day 22 and maintaining a relative expression level less than NP through day 50 of gestation ($P = 0.01$; Table 1).

By day 50, the mRNA expression of *GLUT1, SLC7A1, SLC7A2* and *SLC7A3* all had returned to similar levels as observed in NP heifers. With *GLUT3* being a low Km (high affinity) glucose transporter (Illsley, 1999), its linear and dramatic increase (13.7-fold by day 50; $P = 0.01$) in mRNA expression indicates it may play a pivotal role in supporting the increased nutrient demands of the conceptus as early pregnancy progresses.

Maternal Intercaruncular Endometrium (ICAR)

The expression of *GLUT1* mRNA followed a similar expression trend in ICAR as in CAR, with day 16 being greater ($P < 0.001$) than all other days measured (Table 1). At day 22, *GLUT1* mRNA expression was greater than NP on days 34, 40 and 50 of gestation. The expression of *GLUT3* mRNA tended ($P = 0.06$) to be greater at days 34 and 40, compared with NP. On day 34 of gestation, the mRNA expression of *SLC7A1* peaked at 16-fold greater ($P < 0.001$) than NP, and on day 40 it still was greater ($P \leq 0.05$) than NP or days 16, 22, 28 and 50 of gestation.

The expression of *SLC7A2* mRNA was greatest on day 34, intermediate on day 28, and least in NP on days 16, 22 and 50 ($P \leq 0.05$; Table 1). In contrast to CAR, *SLC7A2* in ICAR reached its greatest mRNA expression on day 34 ($P = 0.02$; Table 1). The expression of *SLC7A3* mRNA was greater in NP on days 28, 34 and 40 ($P \leq 0.05$), compared with days 16 and 22 (Table 1).

Fetal Membranes (FM)

The expression of *GLUT1* mRNA was greatest on day 22 and decreased to day 50, indicating a linear decline as pregnancy progressed, but it still remained at relatively high levels, compared with B-actin ($P = 0.009$; Table 2). The expression of *GLUT3* mRNA remained consistent from days 22 to 50 ($P = 0.76$; Table 2). The expression of *SLC7A1* mRNA was greatest on day 22 (9.57) and showed a cubic pattern, decreasing by day 50 ($P < 0.001$; Table 2). The expression of *SLC7A2* and *SLC7A3* mRNA was consistent throughout early gestation (~5.5 and ~11.5, and $P = 0.60$ and $P = 0.52$, respectively, compared with B-actin; Table 2).

Due to *GLUT3*'s known function as a placental and neural glucose transporter, we expected that the mRNA expression would be greater

Table 1. Expression of nutrient transporters *GLUT 1, GLUT3, SLC7A1, SLC7A2* and *SLC7A3* mRNA in CAR and ICAR tissue in nonpregnant controls and from days 16 to 50 of gestation.

			Day of Gestation[1]							
Tissue[2]	Gene of Interest[3]	NP	16	22	28	34	40	50	SEM[4]	*P* - value[5]
CAR	*GLUT 1*	1.00^{a}	7.77^{c}	4.34^{b}	2.06^{a}	1.38^{a}	1.20^{a}	1.70^{a}	0.99	<0.001
	GLUT 3	1.00^{a}	3.89^{a}	5.62^{ab}	4.80^{ab}	9.13^{bc}	4.93^{ab}	13.68^{c}	2.28	0.01
	SLC7A1	1.00^{a}	1.54^{a}	1.99^{a}	5.12^{b}	6.82^{b}	5.10^{b}	0.98^{a}	0.92	<0.001
	SLC7A2	1.00	2.38	1.37	0.68	3.24	2.95	0.35	1.01	0.20
	SLC7A3	1.00^{a}	6.89^{b}	0.69^{a}	0.42^{a}	0.52^{a}	0.54^{a}	0.14^{a}	1.74	0.01
ICAR	*GLUT 1*	1.00^{a}	13.24^{c}	6.07^{b}	2.48^{ab}	1.65^{a}	1.04^{a}	0.66^{a}	2.22	<0.001
	GLUT 3	1.00^{abc}	0.93^{b}	1.67^{ac}	4.32^{ce}	4.90^{de}	3.51^{acd}	0.73^{ab}	1.65	0.06
	SLC7A1	1.00^{a}	2.03^{a}	1.63^{a}	6.30^{b}	16.03^{d}	12.02^{c}	5.73^{b}	2.03	<0.001
	SLC7A2	1.00^{a}	1.25^{a}	1.23^{a}	7.84^{bc}	10.25^{c}	4.08^{ab}	2.15^{a}	3.11	0.02
	SLC7A3	1.00^{abc}	0.50^{a}	0.20^{a}	1.87^{c}	1.49^{bc}	1.46^{bc}	0.61^{ab}	0.54	0.03

[1]Day of Gestation = number of days after insemination. Day 0 is a nonbred, nonpregnant control and serves as the baseline of expression for that gene. Each gene expression is given as a fold change in relation to NP level of expression.

[2]CAR = caruncular tissue (caruncles taken from the uterine horn containing the conceptus in pregnant heifers), ICAR = intercaruncular tissue (endometrial tissue not including caruncles; taken from the horn containing the conceptus in pregnant heifers).

[3]Gene of Interest = *GLUT1* and *GLUT3* - Glucose transporter solute carrier family 2 member 1 and 3. *SLC7A1, SLC7A2* and *SLC7A3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2 and 3.

[4]The most conservative SEM was used within gene.

[5]Probability values for effect of day on level of expression of individual genes. For those with a value 0.0001, values are <0.0001.

[a-e]Means within a row without a common superscript differ ($P < 0.05$).

Table 2. Expression of nutrient transporters *GLUT 1*, *GLUT 3*, *SLC7A1*, *SLC7A2* and *SLC7A3* mRNA in fetal membranes from days 22 to 50 of gestation.

Gene of Interest[2]	Day of Gestation[1]					SEM[3]	*P* - value[4]
	22	28	34	40	50		
GLUT 1	6.72^{c}	6.35^{bc}	5.58^{a}	5.78^{ab}	5.32^{a}	0.30	0.009
GLUT 3	6.38	6.06	5.80	6.35	5.66	0.51	0.76
SLC7A1	9.57^{c}	8.16^{b}	7.05^{a}	8.86^{bc}	6.99a	0.43	<0.001
SLC7A2	6.27	5.3	5.71	4.77	5.57	0.72	0.60
SLC7A3	12.25	10.49	10.99	11.61	13.08	1.19	0.52

[1]Day of Gestation = number of days after insemination. Values for expression of genes are provided as ΔCt values for that gene after being normalized to B-Actin.

[2]Gene of Interest = *GLUT1* and *GLUT3* - Glucose transporter solute carrier family 2 member 1 and 3. *SLC7A1, SLC7A2* and *SLC7A3* - Cationic amino acid transporters of arginine and lysine, solute carrier family 7 member 1, 2 and 3.

[3]The most conservative SEM was used within gene.

[4]Probability values for effect of day on level of expression of individual genes.

[a-e]Means within a row without a common superscript differ ($P < 0.05$).

than those of *GLUT1* and might even increase throughout gestation. Although neither *GLUT1* nor *GLUT3* mRNA expression increased, both were expressed at relatively high levels, compared with B-actin, which suggests their key role in glucose transport to the developing fetus.

Previous work by Bazer et al. (2011) examined the location of the various nutrient transporters within the endometrium in ewes. The *GLUT1, GLUT3, SLC7A1* and *SLC7A2* transporters were in the luminal and glandular epithelium of the uterus, and *SLC7A3* transporter was found in the luminal and glandular epithelium as well as the stromal cells.

Examining the cells or tissue compartments expressing these transporters in beef cattle could provide insight into the difference in mRNA expression among tissue types.

Although not all transporters showed differences across all tissues, for the most part, these data supported our hypothesis that day of early pregnancy has an effect on the mRNA expression of *GLUT1, GLUT3, SLC7A1, SLC7A2* and *SLC7A3* in CAR, ICAR and FM.

We interpret these data to imply that glucose and cationic amino acid transport capacity in utero-placental tissues is changing dramatically during the first 50 days of pregnancy in beef heifers. Moreover, implications are that our model provides an effective platform for additional studies investigating a plethora of mechanisms at play during early bovine embryo development. Ultimately, new knowledge in this area will facilitate increased efficiencies associated with beef cattle production and contribute to meeting projected world food demands.

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