Placental GLUT3 (SLC2A3) RNA interference: Impact on fetal growth at mid-gestation

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ABSTRACT

Glucose is the predominant energy substrate for fetal oxidative processes and growth, and is taken up by the placenta and transported to the fetus by the facilitative transporters GLUT1 (SLC2A1) and GLUT3 (SLC2A3). SLC2A1 is the most abundant placental transporter, and as such is believed to be the primary glucose transporter in human and sheep placenta. However, SLC2A3 exhibits a six-fold greater glucose transport capacity, and in sheep, SLC2A3 is localized to the apical trophoblast membrane, whereas SLC2A1 is predominantly localized to the basolateral membrane, indicating that both may be required for optimal fetal development. It was our objective to use placenta-specific RNA interference (RNAi) to diminish SLC2A3, and determine the impact at mid-gestation (75 dGA) in sheep. Single hatched blastocysts were harvested and the trophectoderm was infected with lentiviral constructs expressing either a scramble control (SC) or SLC2A3-specific (GLUT3-RNAi) short-hairpin RNA, and then surgically transferred into a synchronized recipient. The resulting pregnancies underwent ultrasound Doppler velocimetry and fetal measurements at 70 dGA, and a terminal surgery at 75 dGA for collection of uterine and umbilical arterial and venous blood, fetal and placental measurements and tissue samples. Due to a lack of fetal sex x treatment interactions, statistical comparisons between SC (n=6) and GLUT3-RNAi (n=6) pregnancies were made by Student's T-test. At 70 dGA, while umbilical artery velocimetry was not impacted, biparietal diameter (P≤0.10), femur length and tibia length (P≤0.05) were reduced in GLUT3-RNAi pregnancies. These results were confirmed at 75 dGA surgery, as GLUT3-RNAi fetuses had reduced fetal weight (P≤0.10), head circumference (P≤0.05), femur length (P≤0.05), and tibia length (P≤0.05). While it has been suggested that GLUT3 is predominantly important in late gestation, these preliminary data indicate that GLUT3 is important for normal fetal development during the first-half of gestation as well. Supported by NIH grant HD094952.

INTRODUCTION

- Beginning in the maternal vasculature, oxygen, glucose, and amino acids must be transported across the placenta to the fetus in order to support fetal development and growth. In order for glucose to be used as the primary energy substrate for placental and fetal oxidative processes, it must be transported by the two major facilitative glucose transporters in the placenta: SLC2A1 (GLUT1) and SLC2A3 (GLUT3).
- Based on the refined localization study by Wooding et al.[1], these facilitative glucose transporters are localized similarly in cattle and sheep in which SLC2A3 is localized on the apical microvillous membrane of the trophoblast cells whereas SLC2A1 is localized at the maternal-fetal syncytial layer around the maternal blood capillaries as wells as on the basolateral membrane of the trophoblast cells (**Figure 1**). This arrangement of the facilitative glucose transporters in the ruminant placenta infers that in order for glucose to be transported from the maternal to the fetal circulation, SLC2A1 and SLC2A3 must be utilized sequentially. While SLC2A1 appears to be more abundant and prominent in the ruminant placenta, SLC2A3 has both a higher affinity and a five-fold greater transport capacity than SLC2A1[2].
- Thus, due to SLC2A3 being located on the apical microvilli membrane of the trophoblast cells and its greater transport capacity, we hypothesize that placental SLC2A3 deficiency will significantly impact the placental transfer of glucose as well as impairing placental development and function.

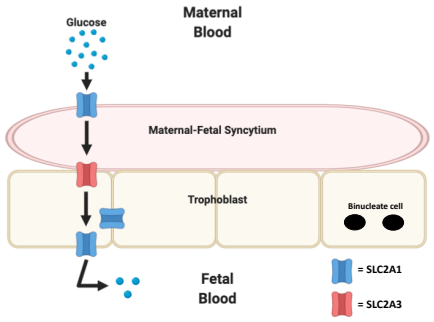


Figure 1. Illustration of the location of the facilitative glucose transporters in the ruminant placenta.

METHODS

Day 9 blastocysts were harvested from naturally mated donor ewes and then infected with either our control (LL3.7 SC shRNA) or targeting (LL3.7 472 shRNA) lentiviral vectors. After a 5-hour incubation period, single blastocysts were surgically transferred to a synchronized recipient ewe. At 70 dGA, the resulting pregnancies underwent fetal ultrasound measurements as well as ultrasound Doppler velocimetry. At 75 dGA, the ewes were anesthetized and fetal blood was collected from the umbilical artery and vein and maternal blood was collected from the uterine artery and vein ipsilateral to the fetus. A complete hysterectomy was then performed and all placentomes were excised from the ewe's uterus and dissected to separate the fetal cotyledon away from the maternal caruncle (**Figure 2**). These samples were stored at -80°C.

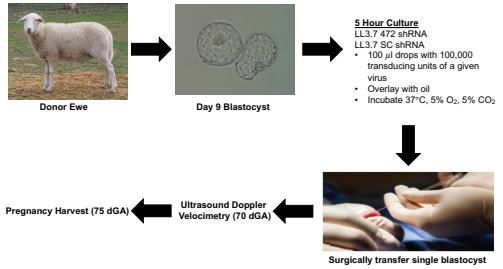
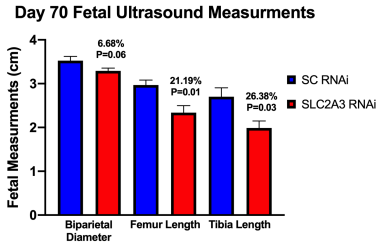
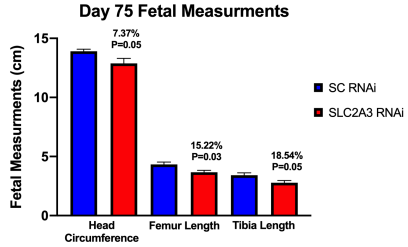
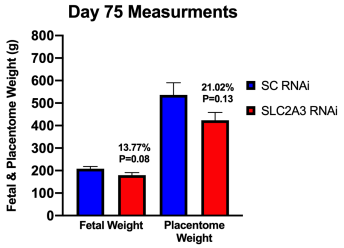


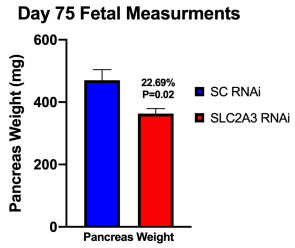
Figure 2. Outline of experimental design.

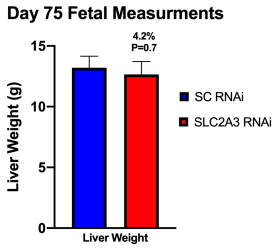
RESULTS



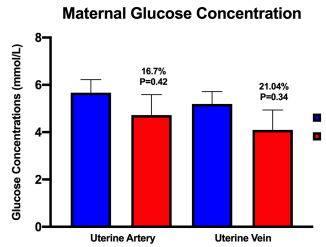


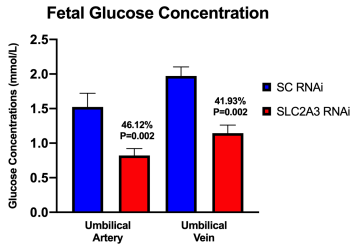


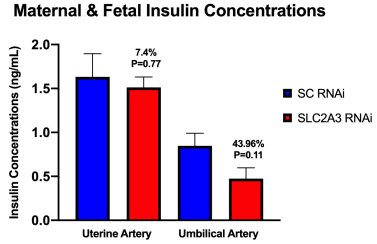




RESULTS (Continued)







DISCUSSION

- Our 70 dGA fetal ultrasound measurements indicated there were significant reductions in biparietal diameter, femur length, and tibia length. This was then confirmed at 75 dGA when the pregnancies were harvested.
- At 75 dGA, we also observed a notable decrease in fetal and placentome weight as well as a significant decrease in fetal pancreas weight in the SLC2A3 deficient pregnancies as compared to the controls. However, there were no effects seen on the fetal liver weight.
- While there were no significant reductions in maternal glucose or insulin concentrations, we observed a notable 44% reduction in fetal insulin concentrations as well as a significant 42% and 46% reduction in umbilical vein and umbilical artery glucose concentrations, respectively.
- Ehrhardt & Bell (1997)[3] reported that as pregnancy progresses in the sheep, from mid- to late gestation, SLC2A3 mRNA and protein levels increase, and thus suggested that SLC2A3 is more important as pregnancy advances[3]. However, the substantial differences we observed in fetal measurements, fetal glucose concentrations, and fetal insulin concentrations in this cohort of SLC2A3 deficient pregnancies suggests that SLC2A3 plays an important role for normal fetal development during the first half of gestation.

REFERENCES

1. Wooding FB, Fowden AL, Bell AW, Ehrhardt RA, Limesand SW, Hay WW. Localisation of glucose transport in the ruminant placenta: implications for sequential use of transporter isoforms. *Placenta*. 2005;26(8-9):626-640. doi:10.1016/j.placenta.2004.09.013
2. Simpson IA, Dwyer D, Malide D, Moley KH, Travis A, Vannucci SJ. The facilitative glucose transporter GLUT3: 20 years of distinction. *Am J Physiol Endocrinol Metab*. 2008;295(2):E242-E253. doi:10.1152/ajpendo.90388.2008
3. Ehrhardt RA, Bell AW. Developmental increases in glucose transporter concentration in the sheep placenta. *Am J Physiol*. 1997;273(3 Pt 2):R1132-R1141. doi:10.1152/ajpregu.1997.273.3.R1132