Loss of Function Mutations in *ALPL* Alter Nutrient Abundance in Fetal Plasma, and Allantoic and Amniotic Fluids in Sheep

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Tissue-nonspecific alkaline phosphatase (TNSALP; encoded by the *ALPL* gene) has an important role in the regulation of phosphate homeostasis postnatally. We recently reported spatio-temporal alterations in expression of TNSALP and its enzymatic activity in ovine utero-placental tissues during gestation, yet the role of TNSALP in the regulation of placental development and function is unclear. To determine the role of TNSALP during pregnancy in ruminants, wildtype (WT) ewes and ewes with heterozygous (Het) or homozygous (Hom) loss-of-function mutations in *ALPL* (c.1077 C>G; generated by CRISPR/Cas9) were bred with fertile rams. Ewes were euthanized and hysterectomized on Day 97 of gestation. Placentae from Het ewes were longer, and had abnormally large and irregularly shaped placentomes, while placentomes from Hom ewes were smaller and more numerous. These results suggest that loss of function mutations in *ALPL* affect placental morphology and surface area, both of which are critical for placental transport of nutrients. We hypothesized that these alterations in placental morphology and surface area in response to loss of function mutations in *ALPL* altered placental nutrient transport and thus the abundance of nutrients in fetal plasma and fetal fluids that are important nutrient reservoirs for the fetus. To test this hypothesis, the abundance of key nutrients required for fetal development were quantified in fetal plasma, and amniotic and allantoic fluids from WT, Het, and Hom fetuses on Day 97 of gestation. Het fetuses had more urea nitrogen (P=0.07) and less 25(OH)D, aspartate aminotransferase (AAT) and gamma-

glutamyl transferase (GGT) activity ($P<0.05$) in plasma compared to WT fetuses. Het fetuses had less creatine phosphokinase (CPK) activity compared to Hom fetuses ($P<0.05$) in plasma. Hom fetuses had more glucose ($P<0.001$) and potassium ($P<0.01$), but a lower sodium: potassium ratio ($P<0.01$) and AAT activity in plasma compared to WT and Het fetuses. TNSALP activity in fetal plasma was positively correlated with amounts of bilirubin ($P<0.05$), magnesium ($P<0.05$), AAT ($P<0.01$), and CPK ($P=0.058$) in fetal plasma. Fetal fluid analyses demonstrated that Het fetuses had greater concentrations of glucose in amniotic fluid compared to WT ($P<0.01$) and Hom ($P<0.05$) fetuses. Similarly, Het fetuses had greater concentrations of glucose in allantoic fluid compared to WT fetuses ($P<0.05$). Allantoic fluid from Hom fetuses contained more calcium than for WT ($P<0.05$) and Het ($P=0.10$) fetuses, which is consistent with decreased fetal skeletal mineralization. Allantoic and amniotic fluids from Het fetuses had greater concentrations of urea nitrogen than WT fetuses ($P<0.05$). Amniotic fluid from Het fetuses contained more bilirubin ($P=0.05$) and creatinine ($P<0.01$) compared to WT fetuses. In contrast, amniotic fluid of Het ($P<0.05$) and Hom ($P=0.10$) fetuses contained less chloride than WT fetuses. The significant correlations between fetal *ALPL* genotype and fetal TNSALP activity with specific nutrient abundance in plasma and fetal fluids suggest significant alterations in fetal development in response to loss of function mutations in TNSALP by mid-gestation. Collectively, these findings suggest loss of function mutations in TNSALP alters the nutritional composition of fetal plasma, and allantoic and amniotic fluids, and positions TNSALP as an important regulator of placental development and function, far beyond what is widely accepted as simply regulating phosphate homeostasis.