Placenta-Specific RNA Interference of Transthyretin in Sheep

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During the first half of gestation, both the fetus and placenta are reliant on maternally derived thyroid hormone (TH). Maternal hypothyroidism is associated with miscarriage, fetal growth restriction, and preeclampsia; even mild hypothyroidism may negatively impact development. The predominant TH in both fetal and maternal circulation is thyroxine (T4) which can be converted to triiodothyronine (T3) or reverse triiodothyronine (rT3) by the iodothyronine deiodinase enzymes 2 (DIO2) and 3 (DIO3), respectively. DIO2 and DIO3 are abundantly present in the placenta. Transplacental T4 delivery to the fetus is critical, but how T4 evades placental metabolism is not well understood. Interestingly, the placenta also produces transthyretin (TTR) which binds T4. Evidence exists for TTR-T4 trophoblast uptake, and TTR has been hypothesized to protect and shuttle maternal T4 to fetal circulation; however, this shuttling mechanism has not been directly tested *in vivo*. We have detected TTR, DIO2, and DIO3 mRNA within cotyledons in sheep and have localized their proteins in placentomes. The purpose of this study was to use lentiviral-mediated RNA interference (RNAi) to directly investigate the role of TTR in TH transport across the placenta at mid-gestation (day 75 of gestation) in sheep. At this timepoint, the fetal thyroid has not yet begun producing substantial amounts of T4. Blastocysts were collected from Dorper ewes on day 9 and infected with lentivirus targeting either TTR (TTR RNAi) or a non-targeting sequence (NTS) before being surgically transferred into recipient ewes. At gestational day (GD) 50 and 70, ultrasound doppler velocimetry was utilized to assess pregnancy status and umbilical artery blood flow. At GD 75, ewes carrying TTR RNAi (n=4) or NTS (n=4) singleton pregnancies were weighed and anesthetized for collections. Uterine and umbilical blood was collected for T3 and T4 quantification by ELISA. TTR, DIO2, and DIO3, mRNA quantification by qPCR was conducted in cotyledon samples. Fetal measurements, qPCR, and ELISA results were compared by two-tailed t tests in GraphPad Prism 10 with a p-value of ≤0.05 denoting statistical significance. Lentiviral-mediated RNAi of TTR in ovine placental tissues resulted in a significant decrease in TTR mRNA in cotyledons (p=0.01) and resulted in an approximate 30% reduction in umbilical vein total T4 at GD 75 (0.658 µg/dL vs. 0.915 µg/dL; p=0.021). No significant differences were detected in umbilical artery, uterine vein, or uterine artery T4, and no significant differences in T3 were observed. T3 and T4 were significantly lower in fetal plasma compared to maternal plasma, regardless of treatment. TTR RNAi fetuses (TTR RNAi vs. NTS) did not have significantly different fetal weights (209g vs. 199g; p=0.57) or liver weights (14.2g vs.12.3g; p=0.15), but they tended towards shorter femur lengths (4.25cm vs. 4.6cm; p=0.086) at GD75. Major gaps exist in our understanding of how transplacental TH transport occurs, but these results suggest that our *in vivo* approach provides a model to study the role of TTR in placental TH transport. Overall, these results indicate that placental inhibition of TTR leads to significantly less T4 availability for fetal use, supporting the hypothesized shuttling mechanism of TTR, and while the TTR RNAi fetuses may experience hypothyroidism, significant fetal phenotypes may not be detected until later in gestation. This work was supported by NIH Grant HD093701 and Agriculture and Food Research Initiative Competitive Grant no. 2019-67015-29000 from the USDA National Institute of Food and Agriculture.