

Abstract # 2239

Gene Regulation by LIN28-let-7 miRNA Axis in Sheep Trophoblast Cells. Asghar Ali, Colorado State University, USA

Normal placental development is critical for fetal and maternal health in both humans and animals. Reduced conceptus elongation is a major cause of embryonic mortality and reduced fertility in domestic ruminants. Trophoblast proliferation is critical for successful placentation and establishment of pregnancy, therefore, there is a need to better understand the molecular mechanisms that regulate trophoblast proliferation. LIN28 is an RNA binding protein and has two paralogs, LIN28A and LIN28B. Its major function is to repress let-7 miRNAs biogenesis. Let-7 miRNAs are markers of cell differentiation and high let-7 levels reduce cell proliferation. We previously reported that LIN28A and LIN28B were significantly lower and let-7 miRNAs were significantly higher in term human IUGR vs normal placenta. LIN28A and LIN28B double knockout in human first trimester trophoblast (ACH-3P) cells led to a significant increase in let-7 miRNAs, significantly decreased expression of proliferation-associated genes including ARID3A, ARID3B, HMGA1, c-MYC, VEGF-A and WNT1 and significantly reduced cell proliferation. ARID3A, ARID3B and KDM4C make a tri-protein complex (the ARID3B-complex) which binds to promoter regions of HMGA1, c-MYC, VEGF-A and WNT1. ARID3B knockout in ACH-3P cells disrupted the ARID3B-complex leading to a significant decrease in these proteins and cell proliferation. In this study we hypothesized that LIN28-let-7 axis regulates proliferation of ovine trophoblast cells in vivo by targeting proliferation-associated genes. To test this hypothesis, day 9 hatched sheep blastocysts were incubated with lentiviral particles to deliver shRNA targeting LIN28A or LIN28B specifically to trophoblast (TE). At day 16, conceptus elongation was significantly reduced in LIN28A and LIN28B knockdown conceptuses compared to control, suggesting reduced proliferation of trophoblast cells. Let-7 miRNAs were significantly increased and proliferation-associated proteins IGF2BP1-3, HMGA1, ARID3B and c-MYC were significantly decreased in trophoblast from knockdown conceptuses compared to control. This suggests that the LIN28-let-7 axis regulates proliferation of sheep trophoblast cells by targeting proliferation-associated genes. To further test this hypothesis, ovine trophoblast (OTR) cells were derived from day 16 trophoblast. Surprisingly, after only a few passages LIN28 was significantly reduced and let-7 miRNAs significantly increased compared to day 16 TE suggesting that passaged OTR cells represent a more differentiated phenotype of trophoblast cells. To create an OTR cell line more similar to day 16 trophoblast we overexpressed LIN28A and LIN28B, which significantly decreased let-7 miRNAs, significantly increased IGF2BP1-3, HMGA1, ARID3B and c-MYC and significantly increased cell proliferation. These results suggest that reduced LIN28 during early placental development may decrease trophoblast proliferation at a critical period for successful establishment of pregnancy. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2017-67015-26460 from the USDA National Institute of Food and Agriculture.