Title: Effects of inhibiting CXCL12-CXCR4 during implantation on pregnancy-associated glycoproteins (PAGs) in the sheep placenta at midgestation.

Authors: Lauren H. Butler[1], Katherine E. Ropp[1], Lyndi R. Bryant[1], Ryan L. Ashley[1]

[1]Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM, United States

Failure of trophoblast cells to properly attach and invade uterine endometrium leads to placental insufficiency, a root cause of preeclampsia, fetal growth restriction, and fetal morbidity and mortality. Further, placental insufficiency also predisposes offspring to cardiovascular disease, type 2 diabetes, insulin resistance, obesity, hypertension, and stroke during adulthood. Currently, no therapy exists to treat placental insufficiency and due to ethical standards study of this disorder in humans is difficult, necessitating the need for animal models. Research using livestock animal are proving to be effective models to address placental insufficiency pathogenesis. Using a sheep model, we previously demonstrated that suppressing the signaling of a chemokine, CXCL12, and its receptor, CXCR4 at the fetal-maternal interface during implantation with a pharmalogical inhibitor (AMD3100, 1X dose) results in placental insufficiency by d90 of gestation. Interestingly, investigation of the placenta on d20 of gestation after suppressing CXCL12 revealed fewer trophoblast multinucleated giant cells expressing pregnancy-associated glycoproteins (PAGs) in the placenta compared to control. Trophoblast giant cells, similar to human syncytiotrophoblast cells, form a syncytium and invade the ovine endometrium, albeit to a lesser degree than in humans. PAGs are essential for successful pregnancy in ruminant animals, playing multiple roles in placental development, maternal-fetal communication, and pregnancy maintenance. Based on our results with fewer PAGs on d20 we hypothesized that ewes with placental insufficiency at d90 of gestation would present with fewer PAGs in the placenta compared to control. To test, on d12 post-breeding, osmotic pumps were surgically installed in ewes to deliver three different amounts (1X, 1.5X or 3X) of AMD3100 or saline (control) into uterine lumen ipsilateral to corpus luteum for 14 days. On d90 placentomes were collected and immediately immersed in 4% paraformaldehyde and later embedded in paraffin according to standard histological procedures. Immunofluorescence staining and analysis was conducted on placentomes to quantify PAG presence. Compared to control, ewes treated with 1X AMD3100 at the fetal-maternal interface during implantation had fewer ($P<0.05$) PAG expressing cells on d90. However, similar levels of immunoreactive PAGs in placentomes were observed between control ewes and those subjected to higher doses of AMD3100 (1.5X and 3X) during implantation. Our data underscore the importance of CXCL12-CXCR4 axis during placentation and provide evidence that altering CXCL12-mediated signaling during implantation induces enduring placental effects manifesting later in gestation as placental insufficiency. As serious inadequacies in placental formation, such as trophoblast invasion, underpin many cases of implantation failure and contribute to pregnancy complications, further characterization of our sheep model may prove useful for studying pregnancy complications stemming from compromised implantation. Research supported by National Institute of General Medical Sciences (NIGMS) of the National Institutes of Health (NIH). Grant number 1SC1GM139712-01.