Title: Disrupting the CXCL12-CXCR4 chemokine axis at the fetal-maternal interface during implantation in sheep results in less placental VEGFA at midgestation

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Failure of proper placental vascular remodeling and development results in placental insufficiency which is a root cause of preeclampsia, fetal growth restriction, and fetal morbidity and mortality. The causes of placental insufficiency are poorly understood but origin of disease is thought to occur during placental development (placentation). Specifically, dysregulation of placental angiogenesis has emerged as one of the main pathophysiological features in the development of placental insufficiency and its clinical consequences. The chemokine, CXCL12 working through its receptor CXCR4 plays pivotal roles during implantation and placentation by stimulating synthesis of vascular endothelial growth factor (VEGFA), considered the most potent inducer of angiogenesis and chief player in placental growth and vascularization. Interestingly, VEGF in turn drives expression of both CXCL12 and CXCR4 creating a powerful feed forward angiogenesis loop. Dysregulation of the CXCL12-CXCR4 axis is implicated in pregnancy complications including miscarriage and preeclampsia but precise roles of this axis during placentation are unclear, especially at the fetal-maternal interface. Using a sheep model, we previously demonstrated suppressing CXCL12-CXCR4 signaling with a pharmalogical inhibitor (AMD3100, 1X dose) at the fetal-maternal interface during implantation diminishes placental vascularization with drastic reduction in VEGFA by d20 of gestation. Ewes also present with reduced trophoblast invasion and compromised uterine remodeling. If allowed to continue gestating, at midgestation, dams exhibit placental insufficiency. In the current study a more in depth investigation into the effects of CXCL12-CXCR4 on placental VEGFA production was conducted by delivering increasing concentrations of AMD3100 at the fetal-maternal interface during implantation and investigating placental VEGFA on d90. We hypothesized increasing the amounts of AMD3100 (1X, 1.5X, and 3X doses) during implantation would cause dose-response impacts to placental vascularization evidenced by altered VEGFA production. 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 for 24h and embedded in paraffin according to standard histological procedures. Immunofluorescence staining and analysis was conducted on placentomes to quantify VEGFA presence and subsequent vascularization. Images obtained were analyzed via Image J and integrated densities were analyzed in a mixed procedure via SAS. Differences in VEGFA expression between male and female fetuses was observed only in control placentomes with females displaying more (P<0.05) VEGFA than male fetuses. Ewes exposed to 3X AMD3100 exhibited less (P=0.008) VEGF compared to control. Immunoreactive levels of VEGFA tended to decrease in the 1X and 1.5X AMD3100 treated ewes compared to control. Our data underscore the importance of CXCL12-CXCR4 axis during placentation and provide evidence that altering CXCL12-mediated signaling during implantation induces enduring placental vascular effects manifesting later in gestation. As serious inadequacies in placental formation and vascularization 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.