Pig Conceptuses Utilize Extracellular Vesicles for Interferon Gamma-mediated Paracrine Communication with the Endometrium

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Interferon-gamma (IFNG) is a pro-inflammatory cytokine secreted by the trophectoderm cells of porcine conceptuses (embryo and associated extra-embryonic membranes) during the peri-implantation period of pregnancy. IFNG is a type 2 interferon that, unlike the type 1 IFN-tau of ruminants, does not act as a signal for pregnancy recognition. Instead, IFNG modifies the inflammatory immune response within the endometrium and, when deleted from conceptuses through gene editing, disrupts implantation and survival of conceptuses. The mechanism by which IFNG is secreted or released by conceptus trophectoderm and transported into the endometrium for communication with cells in the endometrial stroma has not been determined. In the present study, we performed immunofluorescence staining to localize IFNG to specific cells at implantation sites on Day 13 (n=4) and Day 15 (n=4) of pregnancy. Immunoreactive IFNG protein was detected in both trophectoderm and endometrial luminal epithelium (LE) on Day 15 of pregnancy, although we previously reported expression of IFNG mRNA only by conceptus trophectoderm suggesting IFNG protein in endometrial LE is of conceptus origin. We next cultured endometrial explants (n=3) from Day 11 of pregnancy with increasing doses of IFNG (0, 10, 50, 100 ng/mL). The explant culture procedure removed the endometrial LE barrier present *in vivo*, allowing IFNG to directly contact stromal cells. Real-time PCR analyses revealed that IFNG increased (P<0.001) expression of mRNAs CXCL9, CXCL10, CXCL11, B2M. These effects were not detected when IFNG was infused into the uterine lumen *in vivo* by McLendon et al. (*Biology of Reproduction.* 2020;103(5):1018-1029). These observations suggest a delivery mechanism that permits IFNG to be taken up by endometrial LE during pregnancy. Extracellular vesicles (EVs) are released from cells, contain molecular signals for intercellular communication, and can cross significant biological barriers including the plasma membranes of cells. We hypothesized that the trophectoderm of pigs releases EVs that contain IFNG for paracrine communication with the endometrium. We isolated EVs from the uterine fluid of gilts on Day 11 (n=4), 13 (n=4), and 15 (n=6) of pregnancy and Day 15 of the estrous cycle (n=5). Using Western blotting, IFNG was detected in EVs from Day 15 of pregnancy, but not in EVs from Day 15 of the estrous cycle. Mean size and total numbers of EVs increased from Day 11 to Day 15 of pregnancy. Real-time PCR demonstrated increased expression of IFNG-stimulated genes in endometrial explants treated with EVs from Day 15 of pregnancy (n=4) including CXCL9 (p<0.0001), CXCL10 (p<0.0001), CXCL11(p=0.001), IRF1 (p=0.0002), B2M(p=0.0150), CTLA4 (p=0.0071), PDL1 (p<0.0001), and IDO1 (p=0.003). EV-mediated transport of IFNG was confirmed by the presence of immunoreactive IFNG protein in the endometrial LE of explant cultures of whole uterine sections cultured with EVs from Day 15

of pregnancy. Results of this study suggest that EVs are involved in IFNG transport into the endometrial LE which enables paracrine communication between the conceptus and cells within the endometrial stroma, during the peri-implantation period of pregnancy. This project was supported by Agriculture and Food Research Initiative Competitive Grant no. 2016-67015-24955 from the USDA National Institute of Food and Agriculture.