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Extracellular vesicles from early pregnant uterine fluids promote expression of implantation-related markers in ovine endometrium

Laurent Galio, Kais Hussain Al-Gubory, Juliette Cognie, Pierrette Reinaud, Corinne Giraud-Delville, Soahary Jean-Rene, Mariam Raliou, Audrey Lesage, Gilles Charpigny, Christine Longin, et al.

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uterine stroma of serum protein-infused control gilts. Similar accumulation of immune cells was observed in the uterine stroma of Day 15 pregnant gilts, representing the period of IFNG secretion by porcine conceptuses, but not Day 15 cyclic gilts. Double immunofluorescence staining for proliferating cell nuclear antigen (PCNA) and CD45 demonstrated co-localization within the same cells, suggesting that IFNG induces proliferation of some immune cell subsets within the endometrium. Real-time PCR analyses indicated that a co-signaling molecule on T cells was altered in the absence of changes in the corresponding ligands expressed by antigen presenting cells. IFNG infusion increased endometrial expression of inducible T cell co-stimulator precursor (ICOS), a T cell co-signaling molecule that activates and increases proliferation of T cells. There were no significant changes in other T cell co-signaling molecules including programmed cell death protein 1 (PDCD-1), CD28, and cytotoxic T-lymphocyte associated protein 4 (CTLA4) and their ligands including CD274, CD80, CD86, and ICOS ligand (ICOSLG). Because ICOS is not expressed on resting T cells and is rapidly upregulated upon T cell activation, our results suggest that IFNG activates T cells through ICOS co-signaling receptors. Therefore, we hypothesize that pig conceptus IFNG activates T cells to induce an inflammatory environment within the uterine stroma during the peri-implantation period, and this environment may be favorable for stromal remodelling, including tissue repair and extensive vascular development required for successful development of early placentation in the pig. Research supported by Agriculture and Food Research Initiative Competitive Grant no. 2015-06857 from the USDA National Institute of Food and Agriculture.

P84. Extracellular Vesicles from Early Pregnant Uterine Fluids Promote Expression of Implantation-Related Markers in Ovine Endometrium.

Laurent Galio, Kais Hussain Al-Gubory, Juliette Coggié, Pierrette Reinaud, Corinne Giraud-Delville, Soahary Jean-René, Mariam Raliou, Audrey Lesage-Padilla, Gilles Charpigny, Christine Péchoux, Pierre Adenot, Corinne Cotinot, Olivier Sandra. UMR BDR, INRA, ENVA, Université Paris Saclay, Jouy en Josas, France; PRC, CNRS, IFCE, INRA, Université de Tours, Nouzilly, France; GABI, INRA, Agroparistech, Université Paris-Saclay, Jouy-en-Josas, France; MIMA2 Platform, INRA, Jouy en Josas, France.

Extracellular vesicles (EVs) are crucial for intercellular communications and they may play important role in the delivery of molecular messages during the pre-implantation period of pregnancy. EVs have been isolated from uterine luminal fluid (ULF) in human and sheep. Recent data have provided evidence that EVs contained in ovine ULF can penetrate endometrial epithelial cells after a 6-days infusion *in vivo*. Nevertheless, embryo implantation involves rapid and dynamic changes in molecular interactions with the endometrium. Our present work aims to determine whether EVs collected from ULF interact with endometrial epithelial cells and modify cell physiology after a short time of *in vitro* and *in vivo* incubation conditions. Primary cultures of endometrial epithelial cells were derived from ovine uteri on day 12 post-oestrus. EVs were purified from ovine ULFs on Day 14 of pregnancy (2 days before conceptus implantation). The presence of EVs in ULF was confirmed by transmission electron microscopic observation. ULF EVs were labeled with lipophilic PKH26 fluorescent dye and then incubated with primary cultures of epithelial cells during 30 min to 24h. Confocal microscopy analyses revealed an uptake of EVs as early as 30 minutes after incubation, followed by a progressive increase of intracellular fluorescence up to 6 hours. For the *in vivo* study, ovine ULF EVs isolated on Day 14 of pregnancy were labeled with PKH26 fluorescent dye and infused into the uterine lumen of cyclic ewes on Day 12 post-oestrus. After 24h, numerous epithelial cells from the luminal layer and superficial glands exhibited an intensive fluorescence signal, whereas deep endometrial glands displayed few fluorescent cells. No signal was detectable in the stroma. The impact of EVs on endometrial function was investigated by quantifying transcript expression of a selection of endometrial genes. First data pointed out that expression of two genes, including the Myxovirus-Resistance Protein (MX1) was up-regulated following EVs uptake by the endometrial epithelium. This work provides first evidence that ovine EVs from pregnant ULF can (i) enter in endometrial epithelial cells within 30 min *in vitro* or 24h *in vivo* (ii) modulate expression of endometrial gene expression known to be critical for embryo implantation. These results suggest a critical role for EVs in the preparation of endometrium when implantation initiates. Acknowledgments: Dr. Isabelle Hue for helpful discussion; Martine Letheule (MIMA2, INRA) for confocal observations; Noémie Perrot (UCEA, INRA) and Edouard Guitton (PFIE, INRA) experimental units for the animal; Gilles Gomot (CIRE, INRA) surgery Platform; Aude Dos Santos for preliminary results and Philippe Bolifraud for logistics. This research was supported by PHASE Division of INRA.

P87. Interferon Tau (IFNT)-Dependent and -Independent Effects of the Bovine Conceptus on the Endometrium During Early Pregnancy.

Daniel J. Mathew, Jose M. Sánchez, Claudia Passaro, Gilles Charpigny, Susanta Behura, Thomas E. Spencer, Patrick Lonergan. School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland; INRA, Biologie du Développement et Reproduction, Jouy en Josas, France; Division of Animal Sciences, University of Missouri, Columbia, Missouri, USA.

Establishment of pregnancy in mammals requires reciprocal molecular communication between the conceptus and endometrium that modifies the endometrial transcriptome and uterine luminal milieu to support pregnancy. After hatching from the zona pellucida, the bovine conceptus grows and elongates within the uterine lumen and produces the maternal recognition of pregnancy signal, interferon tau (IFNT). In-turn, IFNT induces endometrial expression of IFN-stimulated genes (ISGs) and disrupts endometrial production of luteolytic prostaglandin F2alpha (PGF2A) pulses. In addition, the conceptus releases other factors such as PGs and various proteins that are hypothesized to modify the endometrial microenvironment. Here, we tested the hypothesis that conceptus regulation of gene expression in the endometrium involves factors other than IFNT. Due to the small size of the early elongating conceptus relative to the volume of the uterine lumen, collection of endometrium adjacent to the developing conceptus is difficult following conventional uterine flushing methods. Thus, a conceptus-endometrial explant co-