**Embryo-induced alterations in the protein profile of bovine oviductal extracellular vesicles *in vitro***

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The oviduct provides the ideal environment for early embryo development. Maternal-embryonic communication within the oviduct, important for ensuring embryo quality, is partially facilitated through extracellular vesicles (EVs). Bovine oviductal explants offer an *ex vivo* alternative for studying this communication between the embryo and the female reproductive tracts via EVs. This study aimed to identify the protein profile of EVs resulting from the interaction between embryos and maternal tissue using this model. For this, five synchronized cyclic heifers were slaughtered 3.5 days after heat. Six 0.25 $mm^2$ oviductal explants were obtained from each heifer and cultured individually in 750 µL of protein-free synthetic oviduct fluid (SOF): three were cultured in medium alone (Expl), and three were co-cultured with 10 in vitro-produced 8-16-cell stage bovine embryos each (Expl+Emb). Also, a group of 100 in vitro-produced 8-16-cell stage embryos was cultured alone (Emb) in 500 µL of SOF. All groups were cultured for six hours at 5% $CO_2$, 38.5°C, and conditioned medium (CM) was collected for EV isolation. EVs were isolated using size exclusion chromatography and their presence was confirmed by the detection of CD63, CD81, and CD44 EV markers with flow cytometry. Proteomic analysis was carried out using nanoLC-MS/MS with spectral counting for protein identification and quantification. The EVs from 5 replicates (representing each cyclic heifer) of CM from the “Expl” and “Expl+Emb” groups, as well as 5 replicates from the “Emb” group, were analyzed. Data were compared between pairs of groups using the Student’s t-test, with a *p*-value threshold of 0.05. Bioinformatic analysis was performed with PANTHER. We identified 859 proteins in the CM-EVs from Expl, 627 in the CM-EVs from Expl+Emb, and 111 proteins in the CM-EVs from Emb. In the qualitative analysis, 81 proteins were detected in the three experimental groups, 485 were common to Expl and Expl+Emb, 17 were common to Expl and Emb, 5 were common to Expl+Emb and Emb (MUC16, LMNB1, TSN, MELTF, TTC26), 5 were unique to Expl (CAP2, LGALS3BP, PLIN4, MOSPD2 and CRP), and none were unique to Emb. Additionally, six unique proteins (UPK3BL2, PTPRD, TOM1L1, S100A11, PTPA, and PRKAB1) were identified as being exclusively present when there is an interaction between the oviduct and the embryo *in vitro* (Expl+Emb). Among these, S100A11, a member of the calcium-binding protein S100 family, might act as a modulator of the embryonic transcriptome controlling processes such as cell cycle progression and differentiation. Two protein-modifying enzymes (A0A4W2DFR9 and PSMB8) related to metabolic process proteins were overabundant in the Expl group compared to the Expl+Emb group. Twenty-one proteins, mainly associated with cellular processes such as cell motility, adhesion, and communication, were overabundant in Expl compared to Emb. Additionally, when comparing Emb with Expl+Emb, seven proteins were overabundant in Expl+Emb, some of which are related to the Wnt signaling pathway, a regulator of embryonic cell proliferation and differentiation. Furthermore, the transcriptional factor YBX1, related to mRNA decay during the pre-implantation period, was found to be present only in the Expl+Emb group. Our data indicate that the oviduct can respond to embryos as early as the 8-16-cell stage, exhibiting distinct protein profiles within its EVs when cultured alone or in the presence of embryos. Funded by the Spanish Ministry of Science and Innovation PID2019-111641RB-I00.