PF08.11 | Pig seminal extracellular vesicles (sEVs) load cytokines showing quantitative differences between sEV subsets

Lorena Padilla[1]; Isabel Barranco[2]; Ana Parra[1]; Xiomara Lucas[1]; Jordi Roca[1]
[1]*Department of Medicine and Animal Surgery, Faculty of Veterinary Science, University of Murcia, Murcia, Spain, Murcia, Spain;* [2]*Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy, Bologna, Italy*

Introduction: Seminal plasma (SP) contains cytokines, which are involved in the immunoregulation of female genital tract environment, a key event for successful embryo development. This study aimed to (1) evaluate whether pig sEVs load cytokines and (2) assess putative quantitative differences between sEV subsets.
Methods: sEVs were isolated from five SP pools (three ejaculates per pool) from artificial insemination boars following the procedure described by Barranco et al. (2021), which includes SP centrifugation (20,000xg/30 min at 4°C), and SEC of resultant pellets (large sEVs) and supernatants after ultrafiltration (small sEVs). Fractions (7–10) were selected, mixed, concentrated and used for sEVs characterization (DLS, TEM and total protein concentration) and cytokine measurement (Luminex® Technology). Thirteen cytokines (GMCSF, IFNγ, IL-1α, IL-1β, IL-1ra, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-α) were measured on intact and lysed sEVs (0.1% Triton and 0.1% Sodium Dodecyl Sulfate) and the difference between lysed and intact sEVs was considered the cytokine load in sEVs. Data are showed as mean±SD.
Results: Isolated structures showed the typical structure of EVs. The diameter and total protein concentration differed ($P<0.001$) between large (237±12.01 nm and 83.96±53.92 μg/mL) and small sEVs (121.6±7.89 nm and 306.9±169 μg/mL). sEVs loaded all 13 cytokines, with amounts ranging from 2558±505.4 pg/mL for IFNγ to 0.25±0.08 pg/mL for IL-12. Small sEVs loaded higher ($P< 0.05$) than large sEVs for all cytokines except for IL-1ra.
Summary/Conclusion: Pig sEVs load cytokines, the amount being higher in small sEVs than in large ones. Then, pig sEVs would be involved in modulating the immune environment of the sow genital tract after mating or insemination, a role that would be played mainly by small sEVs, as they load higher amounts of most cytokines.
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PF08.12 | Effects of extracellular vesicles derived from steroids-primed oviduct epithelial cells on porcine in vitro embryonic development

Seonggyu Bang; Xun Fang; Bereket Molla Tanga; Gyeonghwan Seong; Islam M. saadeldin; Sanghoon Lee; Jongki Cho
Chungnam national univ., Daejeon, Republic of Korea

Introduction: Extracellular vesicles (EVs) including exosomes and microvesicles provide a means of communication for delivering bioactive cargoes between cells. In the past decade, EVs have been shown to contribute to the transport of proteins, lipids, mRNA, and microRNA. In vivo, embryos receive nutrients and specific signals through the oviduct to enhance embryonic development.
Methods: Therefore, we aimed to isolate EVs from porcine oviduct epithelial cell (pOECs) that were primed with steroid hormones to mimic the in vivo conditions of reproductive cycle and studied their effects on the in vitro produced embryos development. pOECs were treated with formulated estardiol (E2) and progesterone (P4) combinations in two treatment groups: 50 pg/ml E2 + 0.5 ng/ml P4 (group H1), and 10 pg/ml E2 + 35 ng/ml P4 (group H2). The control group was not supplemented with hormones. Embryos were prepared after in vitro maturation and parthenogenetic activation. Embryos were randomly distributed into 4 groups: control, non-primed pOECs-derived EVs treated group (EV group), and two primed pOECs-derived EVs groups (H1 and H2 groups). EVs were isolated through targeted filtration commercial kits. Data were analyzed by ANOVA test and $P < 0.05$ was considered statistically different.
Results: pOECs-derived EVs were supplemented with embryo culture medium after measuring the protein concentration (260.6 μ/ml, 284.3 μ/ml, 283 μ/ml, 279.6 μ/ml, for EV, H1 and H2 groups, respectively). The number and concentration of the EVs was measured through nanoparticles tracking analysis. EVs uptake by the embryos was investigated after staining of the EVs with lipophilic fluorescent dye, PKH26. Results showed that EVs from hormone primed pOECs improved the blastocyst formation rate compared to the control group (20.3 ± 0.5 %, 22.8 ± 0.7, 25.0 ± 0.6, and 25.1 ± 1.1 %, for control, EV, H1 and H2 groups, respectively, $P < 0.05$). TUNEL assay showed that EVs-supplemented embryos contained less apoptotic cells when compared with the control group (11.8 ± 2.7 %, 6.9 ± 2.2 %, 6.09 ± 2.04 %, and 2.4 ± 0.7 %, for control EV, H1 and H2 groups, respectively, $P < 0.05$).
Summary/Conclusion: In conclusion, EVs derived from pOECs cultured in hormonal conditions that simulate the in vivo environment have a positive effect on porcine blastocysts formation and reduced the embryonic cell apoptosis, which would improve the porcine in vitro embryo production such as cloned animals.