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Seminal small extracellular vesicles modulate the expression of steroidogenesis-associated genes in pig cumulus cells

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:
Yentel Mateo-Otero, I.B. (2022). Seminal small extracellular vesicles modulate the expression of steroidogenesis-associated genes in pig cumulus cells. REPRODUCTION IN DOMESTIC ANIMALS, 57(S5), 144-144 [10.1111/rda.14227].

Availability:
This version is available at: https://hdl.handle.net/11585/909791 since: 2022-12-16

Published:
DOI: http://doi.org/10.1111/rda.14227

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(Article begins on next page)

12 October 2024

This is the accepted version of an abstract published:

Yentel Mateo-Otero, Isabel Barranco, Marc Llavanera, Diego Bucci, Marc Yeste, Jordi Roca, Giovanna Galeati, Marcella Spinaci

Seminal small extracellular vesicles modulate the expression of steroidogenesis-associated genes in pig cumulus cells

Reproduction in Domestic Animals. 2022; 57 (S5), 144.

The final published version is available online at:

https://doi.org/10.1111/rda.14227

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Seminal small extracellular vesicles modulate the expression of steroidogenesis-associated genes in pig cumulus cells

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Seminal plasma (SP) contains a large diversity of extracellular vesicles (EVs), which are involved in several reproductive processes. In pigs, SP has been found to modulate the ovary function, in terms of hormone secretion. This study aimed to evaluate whether SP-EV subsets induce changes in the expression of steroidogenesis-related genes in cumulus cells (CCs). Two SP-EV subsets (small-[S-EVs] and large-EVs [L-EVs]) were isolated from SP (pool from ejaculates from five boars), following the protocol described by Barranco et al. (2021) based on size-exclusion chromatography. EVs were characterized by total protein concentration, transmission electron microscopy (TEM), dynamic light scattering and flow cytometry (albumin assessment). Cumulus-oocyte complexes (COCs) were supplemented with each SP-EV subset (total protein concentration: 0.1 mg/ml) or without SP-EVs (control) during in vitro maturation (IVM). COCs were mechanically decumulated and the IVM medium containing CCs was centrifuged to obtain CCs, which were stored at −80°C for gene expression. Three replicates (400 COCs each one) were performed. mRNA levels of key steroidogenic enzymes (CYP11A1, HSD3B1 and CYP19A1) were assessed by qPCR. TEM confirmed the presence of both EV-subsets and flow cytometry revealed its high purity degree (albumin < 4.5%). Differences ($p < 0.01$) in size between S- vs L-EVs were found (119.71 ± 7.13 vs. 301.79 ± 2.63 nm, respectively). Interestingly, only S-EVs induced changes ($p < 0.05$) in transcript levels of HSD3B1 in CCs compared to the control (9.43 ± 5.88 *vs.* 1.00 ± 0.00, respectively). This study highlights the potential of seminal S-EVs to modulate hormone secretion in CCs.
Fundings: EC (H2020-MSCA-IF-2019-891382); MCIN/AEI/10.13039/501100011033 (PID2020-113493RB-I00, PID2020-113320RB-I00); AGAUR (2020-FI-B-00412).