SEASON TWO
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Proteomic analysis of pig seminal extracellular vesicles reveals differences between subsets

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Extracellular vesicles (EVs) have emerged as crucial messengers for intercellular communication, participating in both physiological and pathological processes. Seminal plasma (SP), the fluid that accompanies sperm during ejaculation, contains a heterogeneous population of EVs, which play a key role in sperm function, ultimately influencing the fertilization process. However, little is known about of the composition and function of SP-EV-subsets. This study aimed to evaluate the proteomic profile of EV-subsets isolated from pig SP. Three pools of SP (containing 3 ejaculates each one from boars used in artificial insemination) were used. The pools were centrifuged (3,200g/15 min and 20,000g/30 min at 4°C), and the pellets (containing large EVs [L-EVs]) were washed and solubilized in PBS, and supernatants (containing small EVs [S-EVs]) were 0.22µm-filtered and concentrated with Amicon®. Both, pellets and supernatants, were subjected to size-exclusion chromatography for EVs isolation. The fractions 7–9 were selected and mixed. The isolated EVs were analyzed by Dynamic light scattering (DLS), nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). Quantitative proteomics was performed using a SWATH-MS strategy. Proteins were considered quantitatively different with a $p < 0.05$ and a Log2 fold-change $> \pm 2$. Gene Ontology (GO) enrichment analysis of differentially abundant proteins was performed using database DAVID. The concentration (mean ± SD) of EVs was 13.6 x 1011 ± 3.65 x 1011 and 3.7 x 1011 ± 9.06 x 1010 particles/mL in S-EVs and L-EVs samples, respectively. DLS analysis revealed differences ($P<0.001$) in EVs size distribution (mean ± SD) between L-EVs (243.8 ± 25.7nm) and S-EVs (124 ± 7.65nm). NTA confirmed differences in size-distribution of EVs between S-EVs (peaks between 75 and 194 nm) and L-EVs samples (peaks between 167 and 354 nm). TEM also confirmed the above differences, showing L-EVs samples contained larger EVs than S-EVs samples. A total of 737 proteins were identified and quantified in both EV-subsets. Quantitative proteomic analysis revealed that 168 proteins were upregulated and 29 downregulated in L-EVs compared to S-EVs samples. GO enrichment revealed that while L-EVs samples were enriched in proteins related to metabolic processes, detoxification, and localization S-EVs samples were involved in cell killing, immune system process and multiorgan process. Molecular function analysis showed that the most abundant proteins in L-EVs samples had binding and catalytic activity, while the most abundant proteins in S-EVs samples had catalytic and regulatory activity. Regarding differentially abundant proteins involved in reproduction-related processes, SOD1, CLIC4, PAFAH1B2, ELSPBP1, GPX4, PGK2, GRB2, SLC26A3, SRC and PRKACA were more abundant in L-EVs and B4GALT1 in S-EVs samples. This study showed clear differences in the proteomic profile between EV subsets of pig SP, suggestive of different function.

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