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Flow cytometry characterization of porcine seminal extracellular vesicles (EVs) based on the content of EV-specific protein markers

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Isabel Barranco, Alberto Álvarez-Barrientos, Lorena Padilla, Ana Parra, Xiomara Lucas, Jordi Roca

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## **Flow cytometry characterization of porcine seminal extracellular vesicles (EVs) based on the content of EV-specific protein markers**

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Porcine seminal plasma (SP) contains a heterogeneous population of EVs that when isolated by a protocol based on size exclusion chromatography (SEC) can be grouped into two subsets showing size differences, namely large (L) and small (S) EVs. This study evaluated the expression of EV-specific proteins in S- and L- pig SP-EVs. 105 SP-samples (one per boar) were randomly arranged in 21 pools (5 SP-samples/pool) and EV-subsets were isolated by sequential centrifugations, SEC and ultrafiltration. The S- and L-EVs were characterized by total protein (BCA<sup>TM</sup>assay), morphology (TEM), size distribution (DLS) and purity (flow cytometry determination of albumin). The profile of the EV-specific proteins CD9, CD44, CD63, CD81, and HSP90 $\beta$  was analyzed using a flow cytometer (CytoFLEX S). S- and L- SP-EVs showed similar total protein concentration, morphology, and purity (albumin <7%). DLS revealed differences ( $P < 0.0001$ ) in size (median, 25–75<sup>th</sup> percentiles; 120.8, 115.3–126.2 nm and 260.8, 235.3–300.7 nm to S- and L-EVs, respectively). There was no difference between S- and L-EVs in the expression of EV-specific proteins. Both EV-subsets showed high positivity for CD44 (mean $\pm$ SEM, 98.82 $\pm$ 0.11 vs 98.43 $\pm$ 0.10 to S- and L-EVs, respectively); HSP90 $\beta$  (83.67 $\pm$ 3.11 vs 84.03 $\pm$ 2.60) and CD63 (83.11 $\pm$ 1.31 vs 77.35 $\pm$ 2.21); and low for CD9 (26.32 $\pm$ 5.54 vs 28.56 $\pm$ 5.12) and CD81 (29.66 $\pm$ 3.45 vs 22.66 $\pm$ 2.42). These results would indicate that both pig SP-EV-subsets show similar EV-specific protein profiles and CD44, CD63 and HSP90 $\beta$  labelling allows for accurate characterization. Fundings: MCIN/AEI/10.13039/501100011033 (PID2020-113493RB-I00); EC (H2020-MSCA-IF-2019-891382).