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

I. Barranco^{1,*}; A. Álvarez-Barrientos²; L Padilla^{3,4}; A. Parra⁴; X. Lucas⁴; J. Roca⁴

¹Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy; ²Applied Bioscience Facility, University of Extremadura, Badajoz, Spain; ³Biotechnology of Animal and Human Reproduction (TechnoSperm), Department of Biology, Faculty of Sciences, Institute of Food and Agricultural Technology, University of Girona, Girona, Spain; ⁴ Department of Medicine and Animal Surgery, Faculty of Veterinary Science, University of Murcia, Murcia, Spain

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 (BCATMassay), 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 β 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, 25–75th 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 β (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 β labelling allows for accurate characterization. Fundings: MCIN/AEI/10.13039/501100011033 (PID2020-113493RB-I00); EC (H2020-MSCA-IF-2019-891382).