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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™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 HSP90β 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±SEM, 98.82±0.11 *vs* 98.43±0.10 to S- and L-EVs, respectively); HSP90β (83.67±3.11 *vs* 84.0.3±2.60) and CD63 (83.11±1.31 *vs* 77.35±2.21); and low for CD9 (26.32±5.54 *vs* 28.56±5.12) and CD81 (29.66±3.45 *vs* 22.66±2.42). These results would indicate that both pig SP-EV-subsets show similar EV-specific protein profiles and CD44, CD63 and HSP90β labelling allows for accurate characterization. Fundings: MCIN/AEI/10.13039/501100011033 (PID2020-113493RB-I00); EC (H2020-MSCA-IF-2019-891382).