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A protocol for isolation of extracellular vesicle-subtypes from pig seminal plasma

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Seminal plasma (SP) contains subsets of extracellular vesicles (EVs) and reliable procedures to isolate them separately have not been reported. This study describes a protocol based on size-exclusion chromatography (SEC) to isolate EV-subsets from pig SP. Fifteen SP pools from 60 ejaculates (4 per pool) from AI-boars were used. SP (6 mL) was sequentially centrifuged (3,200g 15min and 20,000g 30min at 4°C). The resultant pellets were resuspended in PBS and supernatants were diluted in PBS (1:2), filtrated (0.22 µm) and concentrated (Amicon[®]-10K). EVs were isolated from both samples by SEC, using filtration tubes stacked with 10 mL of Sepharose[®]-CL2B. Twenty sequential 500 µL-fractions were collected and the fractions 7 to 10 (EVs-enriched) were selected and pooled. The EVs were assessed by dynamic light scattering (DLS), nanoparticle tracking analysis (NTA), transmission electron microscopy (TEM) and total protein concentration (MicroBCA[™]). DLS showed differences (P<0.001) in EVs size distribution (mean±SD) between pellets (243.8±25.7nm) and supernatant (124±7.65nm), which were confirmed by TEM and NTA. The protein concentration also differed (P<0.001) between pellet (105.5±88.07µg/mL) and supernatant (332.2±191.5µg/mL). The results demonstrated that the pellets contained larger EVs (mainly microvesicles) and the supernatant smaller vesicles (mainly exosomes). In sum, this study describes a protocol to separately isolate microvesicles and exosomes from pig SP. Funded by EU (H2020-MSCA-IF-2019-891382); MICINN/FEDER (PID2020-113493RB-100, PID2019-105713GB-I00 and RED2018-102411-T), Spain; Seneca Foundation, Murcia (19892/GERM-15); Generalitat Valenciana (PROMETEO/2020/071).