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

Isabel Barranco [a], Christian M Sánchez-López [b], Antonio Marcilla [b], Diego Bucci [a], Carlo Tamanini [a], Jordi Roca [c]

[a] Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy [b]Joint Research Unit on Endocrinology, Nutrition and Clinical Dietetics, Health Research Institute La Fe, Universitat de Valencia, Burjassot, Valencia, Spain [c] Department of Medicine and Animal Surgery, Faculty of Veterinary Science, University of Murcia, Spain.

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).