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Size distribution of porcine seminal extracellular vesicles (EVs) is related to fertility

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## Size distribution of porcine seminal extracellular vesicles (EVs) is related to fertility

L. Padilla<sup>1,2</sup>; I. Barranco<sup>3</sup>; A. Parra<sup>2</sup>; D. Bucci<sup>3</sup>; X. Lucas<sup>2</sup>; J. Roca<sup>2,\*</sup>

<sup>1</sup> Biotechnology of Animal and Human Reproduction (TechnoSperm), Department of Biology, Faculty of Sciences, Institute of Food and Agricultural Technology, University of Girona, Girona, Spain; <sup>2</sup> Department of Medicine and Animal Surgery, Faculty of Veterinary Science, University of Murcia, Spain; <sup>3</sup> Department of Veterinary Medical Sciences, University of Bologna, Ozzano dell'Emilia, Bologna, Italy

Porcine seminal plasma (SP) contains large amounts of EVs that are not yet known to be involved in fertility. This study evaluated the size distribution (SD) of the SP-EVs of 20 artificial insemination boars that showed differences in fertility, both in farrowing rate (FR) and litter size (LS). Boars were classified as having high (n = 5) or low (n = 5) FR or small (n = 5) or large (n = 5) LS. SP samples from each group of boars (15 samples, 3 per boar) were divided into 3 mixtures and SP-EVs were isolated by a protocol combining sequential centrifugations, size exclusion chromatography and ultrafiltration that allows to obtain 2 EV-subsets, small (S) and large (L) EVs. The S- and L-EVs were characterized by total protein (BCA™ Assay), morphology (TEM) and purity (flow cytometric determination of albumin). The SD of EVs was analyzed by dynamic light scattering (Zetasizer Nano ZS system operating at 633 nm and recording backscattered light at 173°). TEM revealed the presence of both EV-subsets and flow cytometry revealed their high purity (albumin <5%). No differences in total protein were found between both EV-subsets. The SD differed ( $p < 0.0001$ ) between S-EVs and L-EVs, irrespective of fertility group (median, 25–75th percentiles; 120.9, 119.0–129.2 nm vs. 243.2, 228.8–267.1 nm for S-EVs and L-EVs, respectively). Regarding fertility, SD differed ( $p < 0.01$ ) between S-EVs of boars showing high and low FR (116.0, 113.8–121.0 nm vs. 127.1, 126.1 vs. 130.2 nm, respectively). This study demonstrates that the size of seminal EVs differs according to boar fertility, when measured in terms of farrowing rate. Fundings: MCIN/AEI/10.13039/501100011033 (PID2020- 113493RB-I00); EC (H2020-MSCA-IF-2019-891382)