

Update on Extracellular Vesicles in Porcine Reproductive Fluids: Isolation, Proteomics and Their Interaction with Spermatozoa

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Extracellular vesicles (EVs) are membrane particles produced by various cells, including those of the male and female reproductive tract. EVs ensure intercellular communication and cargo transfer between cells and gametes. Our study is focused on the isolation and characterization of EVs in porcine seminal plasma and oviductal fluid. EVs were isolated from the biofluids by ultracentrifugation and the presence of EVs in a size range between 20 and 500 nm was determined by transmission electron microscopy. The interaction between isolated seminal plasma EVs and spermatozoa (acrosome and flagellum) was uniquely confirmed by structure illumination microscopy (SIM) super-resolution technique (SR-SIM). Since one of the important roles of EVs is the transport of the specific proteins we investigated differences between protein profiles of EVs isolated from seminal plasma and oviductal fluid. These EVs' protein profiles were determined using mass spectrometry (MS), and the Western blot (WB) analysis showed the presence of spermadhesins originating from both aforementioned biofluids. The CD9 and CD81 as major EVs markers were proved by WB analysis, however, MS identified solely CD9 protein marker in the EVs originating from oviductal fluid. The potential applications of EVs in reproductive biology are significant, as they have been shown to play an essential role in cargo transport to the gametes influencing their maturation and fertilizing ability. In our study for the first time, we confirmed by SR-SIM the interaction of boar sperm with EVs delivered by seminal plasma. Moreover, presence of spermadhesins in the EVs cargo provide further insight to the EVs biology. Finally, different expression of CD9 and CD81 markers determined by various methodological approaches introduce important insight into the methods used to study of EVs.

Acknowledgement: This work was supported by GACR (GA22-31156S), by institutional support RVO (86652036), and by the BIOCEV project (CZ.1.05/1.1.00/02.0109) from the ERDF. We acknowledge the Imaging Methods Core Facility at BIOCEV, an institution supported by the MEYS CR (LM2018129 Czech-Biolmaging) for their support & assistance in this work, and Dr. Petr Pompach, Ph.D. from the Proteomic Core Facility, IBT CAS, BIOCEV for MS analysis.