P29 A comparative study of exosome isolation methods for miRNA profiling in semen

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Introduction & Objectives: Both healthy and pathological cells release into the biological fluids, including semen, various types of membranous structures that are called extracellular vesicles (EVs). The most studied of them are exosomes, small cell-derived vesicles (40-150nm) of endocytic origin. These vesicles contain proteins, lipids and nucleic acids (DNA, mRNA, miRNA). Specifically, miRNAs act as negative post-transcriptional regulators of gene expression. The tissue-specific expression profile and the high stability of these molecules within exosomes lead miRNAs to be considered as potential biomarkers with diagnosis or prognosis purposes.
Currently, the most extensively used method to isolate exosomes from biofluids is differential ultracentrifugation which can be technically challenging. We aim to evaluate the effectiveness and effect on downstream miRNA analysis for prostate cancer of several exosome isolation reagents compared with the use of ultracentrifugation in semen.

Materials & Methods: The semen was centrifuged to remove the cells and cell debris. Exosome isolation was performed by using differential ultracentrifugation and three commercial reagents. Additionally, a non-commercial kit was used. Exosomes were characterized in terms of physical properties (size, concentration) by NanoSight NS300 (Malvern Instruments) and in terms of the RNA quality (OD260/280) and quantity (QUBIT fluorometer). The RNA was extracted from exosomal fractions using miRNeasy micro kit (Qiagen). The expression of 16 miRNAs was analyzed (3 of them were used as normalizers) by RT-qPCR using the miRCURY LNA™ Universal RT miRNA kit (Exiqon) and the Lightcycler 96 (Roche). The relative expression values were determined by the 2ddCq strategy. For the statistical analysis of evaluation of differences between groups, the nonparametric Mann-Whitney test was used.

Results: Our results evidenced differences in exosome quality and quantity among the exosome isolation techniques used. Additional differences in exosomal RNA quality and miRNA content are also observed.

Conclusions: Several exosome isolation kits are viable alternatives to ultracentrifugation with semen samples. However, exosome miRNA profile seems to be affected by the EV isolation method used.