Isolation and Characterisation of Extracellular Vesicle Subpopulations in Human Follicular Fluid

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Extracellular vesicles (EVs) are lipid-bilayered particles present in various body fluids, including follicular fluid (FF). The cells within the ovarian follicle release EVs of endosomal and plasma membrane origin into the extracellular environment. EVs participate in intercellular communication, influence folliculogenesis and oocyte development. Based on their biogenesis, EVs are classified into three subpopulations: exosomes, microvesicles, and apoptotic bodies.

The aim of the current study was to optimize EV subpopulation isolation techniques from human FF and to identify protein markers associated with EVs. The primary focus was to optimize the EV yield while ensuring the preservation of their qualitative characteristics.

Ethical approval was granted by the Research Ethics Committee of the University of Tartu, Estonia and follicular fluid samples were collected from preovulatory follicles of 10 patients at Nova Vita Clinic in Tallinn, Estonia. Size-exclusion chromatography was implemented to isolate EVs from FF, followed by tangential flow filtration to separate EV subpopulations based on their size (cut-off value 200 nm). EV subpopulations were concentrated with ultracentrifugal filters and characterized by Western blotting. Additionally, the size and concentration of the isolated particles were determined with nanoparticle tracking analysis to assess the EV yield at each purification step. The presence of EVs was confirmed by transmission electron microscopy. Subsequently, human ovarian granulosa-like tumour cell line KGN was treated with isolated EV subpopulations for 24 hours. Following this, cell viability and proliferation were assessed using a bioluminescent assay.

The techniques for isolating EV subpopulations, such as size-exclusion chromatography and concentration, were effectively optimized to achieve the highest possible yield. The average diameter of small nanoparticles was approximately 100 nm, whereas the diameter of large nanoparticles ranged from 250 to 350 nm. Notably, the concentration of small nanoparticles from the same amount of FF was observed to be 15 times higher than the large ones. Immunoblotting confirmed the presence of EV-specific surface markers, such as CD9 and CD81, in both subtypes. The purity of the isolated EVs was validated by the reduced levels of albumin and lipoprotein ApoA1. Isolated EVs, even at high concentrations, demonstrated no toxicity to KGN cells. This makes them suitable for analysing their effect on steroidogenesis and gene expression changes. Furthermore, EVs enter KGN cells without significantly affecting the viability or proliferation in a statistically significant manner.

The results set the stage for future functional experiments with EV subpopulations to investigate the effects of follicular fluid EVs on the proliferation, viability, steroidogenesis, and metabolism of various follicular cell types. The optimized isolation procedure does not only establish the groundwork for

identifying biomarkers to assess and predict the quality of oocytes but also provides opportunities for applying EVs in the diagnosis and treatment of reproductive diseases.