How Small Extravesicles (sEVs) from PCOS follicular fluid modified granulosa cells activity. Use of proteomic and miRNA approach

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The development of the ovarian follicle and the maturation of the oocyte both require the presence of several factors which come from the blood and follicular cells. Among these factors, extracellular vesicles (EVs) represent an original communication pathway inside the ovarian follicle and the follicular fluid (FF). Recently, EVs have been show to present potential roles in follicular development and reproduction-related disorders including the Polycystic ovary syndrome (PCOS), the most common hormonal disease among women of childbearing age. To our knowledge, only one study has focused on protein content of FF exosomes and has detected one protein associated to PCOS (Li et al. 2020). The objective of our project was to better decipher the role of EVs derivated from FF. We have performed a functional study with an incubation of small EV from PCOS FF and control FF on the human granulosa cell line (KGN). In order to obtain a "global" view of the proteins content, 6 sEV from PCOS (BMI< 25) and 6 sEV from control (BMI< 25) were analysed by proteomic and miRNA approaches.

First results, sEVs purified from FF expressed exosomal markers such as CD9, CD63 and hsp70. The proteomic analysis of sEVs derived from FF in comparison to sEVs derived from blood has shown that FF sEVs possess proteins expressed by steroidogenic cells (StAR, Cyp11a1, 3βHSD) such as granulosa cells. The characterization by using nanoparticle tracking has shown that sEVs from control and PCOS patients presented same morphological parameters. However, we have identified difference in the functionality of EVs in cell culture when they were purified from control some PCOS patients. Both sEVs (PCOS FF and control FF, $p< 0.05$) stimulated granulosa cell proliferation. But, proliferation was more increased (+192%) with control sEVs in contrast to PCOS sEVs (+131%, $p< 0.05$). In addition, sEVs from FF (PCOS FF and control FF) increased cell migration of granulosa cells which was enhanced when sEVs were derived from PCOS patients. The elevated migration rate was associated with a lower β-catenin protein cell content. Moreover, the progesterone and estradiol secretion were more produced by granulosa cells after a 48h of exposition with control sEVs. The increase in steroids secretion was associated with an elevate expression of proteins involved in steroidogenesis (StAR, 3βHSD). However, the stimulating activity of the steroid production was lost when the sEVs derived from PCOS patients were used.

In order to identify difference in sEVs content (PCOS FF and control FF), the proteomic approach was performed and has identified 706 proteins in sEVs including 68 proteins that were significantly enriched in PCOS sEVs. These 68 proteins are involved in immunity functions, cell-cell and matrix adhesion and regulation of actin cytoskeleton. Analysis of the miRNA content has revealed 239 miRNA specific of the FF sEVs targeting genes involved in steroid

biosynthesis, cell cycle, Oocyte meiosis, tight junctions and Insulin signaling pathway. Specific miRNAs were expressed specifically in PCOS sEVs, showing a specific signature.

In conclusion, this study demonstrated that EVs are able to modify the granulosa activity suggesting a role in the follicle development. The results show that proteins and miRNA content in sEVs are significantly different in PCOS patients leading to perturbation of the granulosa cells functions. The miRNA and proteins profile in sEVs could be used as biomarkers of the follicle health.

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