Extracellular vesicles isolation method can influence its contents and the bovine in vitro oocyte maturation upon supplementation.

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The quality of oocytes derived from in vitro maturation (IVM) culture systems is inferior to those matured in vivo, which can result in a low conversion to transferable embryos. This can be attributed to the fact that, during IVM, the oocyte obtained from small antral follicles loses crucial contact with theca and granulosa cells and is stimulated to undergo the early resumption of meiosis. Extracellular vesicles present in follicular fluid (ffEVS) function as carriers of molecular messages and play a crucial role in cell-to-cell communication, participating in both follicular development and oocyte maturation. However, there is limited understanding regarding the impact of the isolation methods on ffEVs contents and its use as supplement during IVM. Therefore, we initially collected follicular fluid from 3-6 mm follicles harvested from bovine ovaries from a slaughterhouse and used the same sample and volume to isolate ffEVs using ultracentrifugation (UC) or size exclusion chromatography (SEC). Next, the ffEVs isolated by UC or SEC were characterized by their miRNA profile (n= 5 pools of follicular fluid from 6 ovaries). MiRNAs were considered present when they presented an appropriate melting curve, cycle threshold (CT) lower than 35, and were expressed in all replicates. The CT data generated by amplification were normalized using an endogenous gene (bta-miR-99b) in technical duplicate. After the characterization, cumulus-oocytes complex (COCs) retrieved from 3-6 mm follicles from abattoir ovaries were submitted to IVM under different medium conditions: (i) C (Control), consisting of base medium TCM 199; (ii) UC (base medium supplemented with ffEVs separated by UC) and (iii) SEC (base medium supplemented with ffEVs separated by SEC). The COCs were matured for 9 hours to evaluate the number of transzonal projections (TZPs) and 22 hours to evaluate the maturation rate. The ffEVs miRNA profile data was analyzed using t-test analysis, while the supplementation data was analyzed using ANOVA followed by the Tukey test. Statistical significance was determined at a p-value < 0.05. We identified a total of 44 miRNAs differently expressed, being 16 up-regulated in SEC and 28 up-regulated in UC. The bioinformatic analysis indicates that these miRNAs play a role in modulating pathways associated with to the regulation of TZPs, such as the regulation of actin cytoskeleton, as well as pathways related to the oocyte maturation, such as PI3K-Akt signaling pathway, respectively. Next we supplemented COCs during IVM with ffEVs separated by the different methods, and observed lower number of TZPs ($p= 0.04$) and an increased maturation rate (78,9% ± 7,19) in the SEC group compared to the C group (59,34% ± 7,53; $p= 0.003$). In conclusion,

our results demonstrate that the isolation method influence the contents of ffEVs, and that SEC isolated ffEVs affect the cellular responses. Funding: FAPESP 2021/06645-0 and 2021/12560-7