Optimizing a protocol for isolating extracellular vesicles from medium conditioned by bovine embryos in vitro

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Study question

So far, different isolation procedures have been used for extracting extracellular vesicles (EVs) from body fluids or conditioned medium. But there is no standard isolation method for isolating EVs from limited volumes of medium conditioned by bovine embryos.

Summary answer

We used three different methods (Optiprep[™]density gradient (ODG), Differential ultracentrifugation(DU), Size exclusion chromatography (SEC)) for isolating EVs from bovine embryo conditioned medium. Based on our data, we propose to use the SEC method which is quick and efficient. Moreover, only a limited volume is sufficient for isolating EVs.

Experimental Design

OptiPrep™ density gradient (ODG) ultracentrifugation Routine bovine IVM and 1 mL IVF methods conditioned Fractions (1-4,5-7,8-9,10-12,13medium 16) diluted with 16 mL PBS Ultracentrifugation Ultracentrifugation **ODG 1-4** 100,000xg; 18 h at 100,000xg; 3 h at 4°C 4°C ODG 5-7 ODG Presumed zygotes were cultured in ultracentrifuged 8-9 ODG 10-12 SOF+ITS+BSA until Day 8 at ODG 13-16 38.5°C 5% CO₂, 5% O₂ EV pelleted with 100 µL PBS conditioned Differential ultracentrifugation (DU) medium Centrifugation Centrifugation Ultracentrifugation Size exclusion 1,000xg; 7 min 30,000xg; 30 min 100,000xg; 3 h at at 4°C at 4°C 4°C 1 mL chromatography(SEC) conditioned medium EV pelleted with EV pelleted with 100 µL PBS 1000 µL PBS

Electron microscopy

EVs derived by DU EVs derived by SEC EVs derived by ODG

Western Blot







