Assessing the effects of bovine embryo-derived extracellular vesicles on the development of individually cultured bovine embryos

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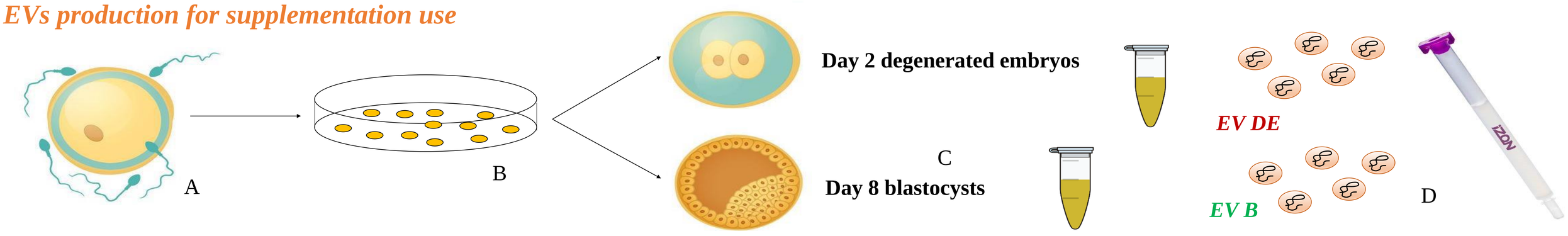
Background

In vitro embryo production requires an enriched microenvironment with various vital cell-secreted factors.

- *In vitro* cultured single bovine embryos have demonstrated lower blastocyst rate compared to grouped cultured embryos.
- We assumed that extracellular vesicles (EVs) within an embryo culture system may affect normal *in vitro* development.
- This study aimed to assess the supplementation effects of bovine embryo-derived EVs on the development of individually cultured bovine embryos.

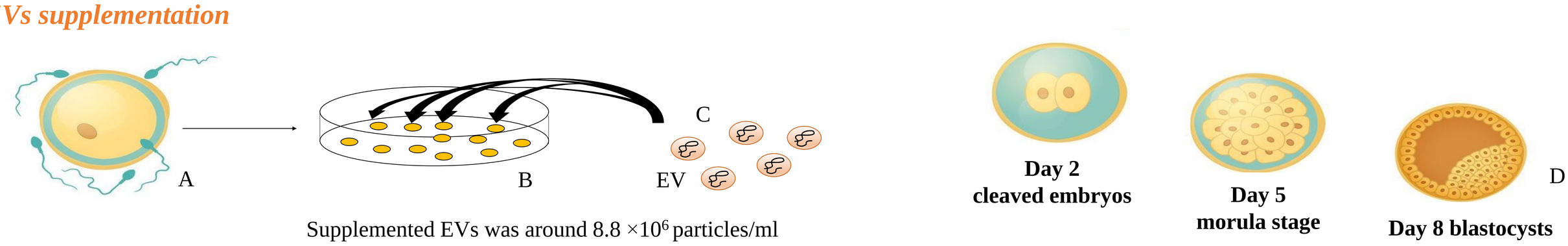
Methods

EVs production for supplementation use



Bovine oocytes were *in vitro* maturated (IVM) for 24 h, then *in vitro* fertilized (IVF) (A). Embryos were cultured individually in droplets of 60 µl of Bovine Serum Albumin (BSA) culture media under mineral oil for 8 days (B). Conditioned medium was collected from each droplet (50 µl) on day 5 from embryos which reached the blastocyst stage and embryos which cleaved on day 2 then degenerated and pooled together (C). EVs were purified, using Izon columns (D) and purified EVs were characterized by nanoparticle tracking analysis and transmission electron microscope (TEM).

EVs supplementation



Supplemented EVs was around 8.8 $\times 10^6$ particles/ml

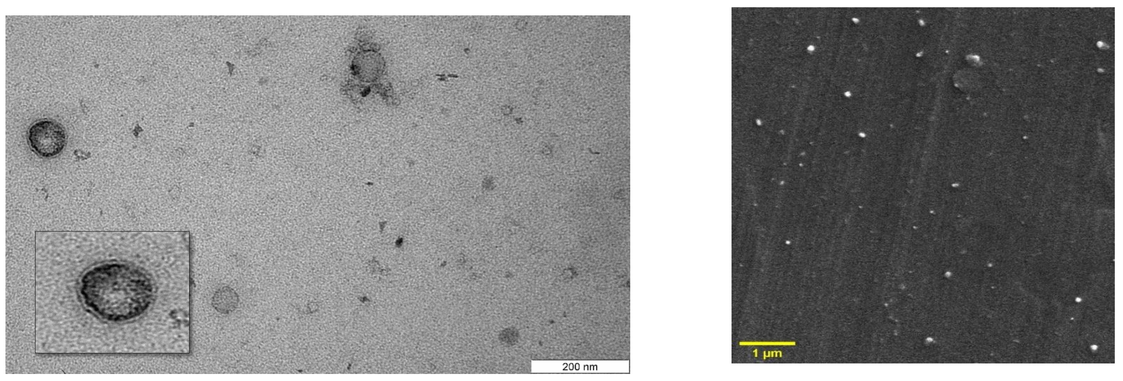
Bovine oocytes were *in vitro* maturated (IVM) for 24 h, then *in vitro* fertilized (IVF) (A). Embryos were cultured individually in droplets of 25 µl of EV depleted BSA culture media under mineral oil for 8 days (B). Purified EVs (5ul) were supplemented to individual embryos on day 4 post fertilization (C). Embryo quality was assessed at day 2, 5 and 8 (D). Non-EV supplemented single embryos cultured in BSA media were considered as control.

Results

Day 2
Cleaved embryos
EVs DE group = 70 %
EVs B group = 80%
Control = 86%

Day 5
Morula stage
EVs DE group = 40 %
EVs B group = 47%
Control = 47%

Day 8
Blastocysts
EVs DE group = 0 %
EVs B group = 0 %
Control = 33 %



Electron microscopy based characterization of EVs.
(A)Transmission Electron Microscopy (TEM) imaging of EVs isolated from media conditioned by individually cultured bovine embryos developed to morula by day 5.
(B) Scanning Electron Microscopy (SEM) imaging of EVs isolated from media conditioned by individually cultured bovine embryos developed to morula by day 5

Conclusion

Our study suggests that single embryo needs a significant amount of EVs to complete their development and reach the blastocyst stage. More research is needed to understand the role of EVs in culture media in supporting the development of single embryos.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 713714