

# Bovine embryos release extracellular vesicles into the medium during group culture in vitro

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## Introduction

Communication between embryos is evidenced by

- Individual Culture has lower blastocysts than Group Culture
- Signaling pathways are involved in embryo communication
- Extracellular vesicles (EVs) may serve as vehicles for signals

## Media with BSA and with PVP

Table 1 Blastocyst development and hatching rate after culturing presumed zygotes in Normal Conditioned media

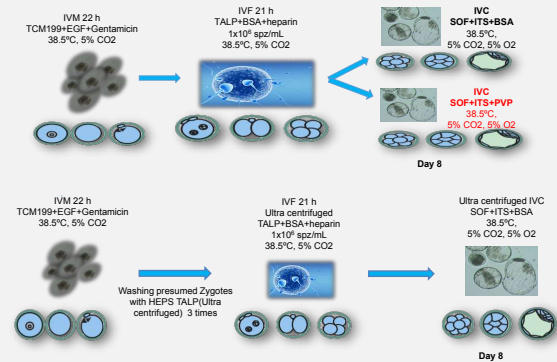
Culture system	IVC	No of presumed zygotes	Cleavage rate (%)		Blastocyst yield (%)		Hatching <sup>a</sup>
			Day2	Day7	Day 8	Day 8	
Group	SOF+BSA+ITS	625	87.07±7.25	31.68±3.05	40±3.43	25.20±1.16	
Group	SOF+PVP+ITS	515	87.96±5.90	29.32±2.72	38.64±2.88	15.07±0.80*	

Data are expressed as mean ± s.e.m. Differences at P<0.05 were considered to be significant and are marked with an asterisk. <sup>a</sup>Hatching rates are expressed as the percentage of hatching or hatched blastocysts at Day 8 compared with the total number of blastocysts.

## Objectives

- ❑ Can bovine embryos release EVs in *in vitro* conditions, and can they take up EVs?
  - Optimizing a standardized protocol for isolation and characterization of EVs from media conditioned by bovine embryos.

## Experimental Design



## Media with BSA depleted from EVs

Table 2 Blastocyst development and hatching rate after culturing presumed zygotes in EVs depleted SOF+BSA medium.

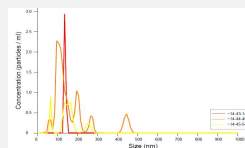
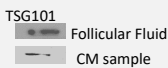
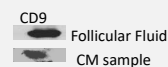
Culture system	IVC	No. of presumed zygotes	Cleavage rate (%)		Blastocyst yield (%)		Hatching <sup>c</sup>
			Day2	Day7	Day 8	Day 8	
Group	SOF+BSA+ITS <sup>a</sup>	450	88.00±5.71	29.11±1.64	38.88±1.48	24.0±0.83	

Data are expressed as mean ± s.e.m. Differences at P<0.05 were considered to be significant and are marked with an asterisk.

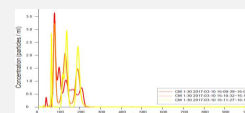
<sup>a,b</sup> The culture medium SOF+BSA+ITS was ultra-centrifuged at 100,000 g for 18 hr at 4°C and supernatant of the medium was used for IVC. <sup>c</sup>

Hatching rates are expressed as the percentage of hatching or hatched blastocysts at Day 8 compared with the total number of blastocysts.

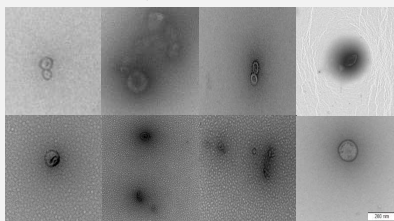
## Results



EVs extracted by OptiPrep™ density gradient protocol from the culture medium (SOF+BSA+ITS) conditioned by bovine embryos was analyzed by Nano particle tracking system. The concentration obtained from 10μl of the EVs extracted sample is 9.18x10<sup>10</sup> particles/ml.

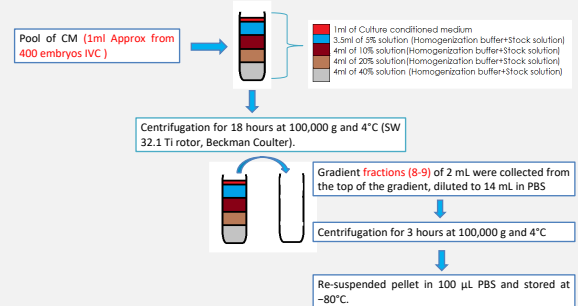


EVs extracted by OptiPrep™ density gradient and size exclusion chromatography protocol from the culture medium (SOF+BSA+ITS EVs depleted) conditioned by bovine embryos was analyzed by Nano particle tracking system. The concentration obtained from 10μl of the EVs extracted sample is 1.36x10<sup>10</sup> particles/ml.

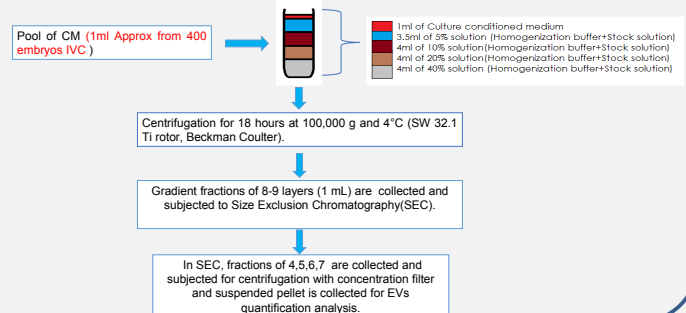


EVs extracted by OptiPrep™ density gradient and size exclusion chromatography protocol from the culture medium (SOF+BSA+ITS EVs depleted) conditioned by bovine embryos was analyzed by electron microscopy. EVs were varying from 40 to 200nm in size.

### EVs ISOLATION TECHNIQUE 1



### EVs ISOLATION TECHNIQUE 2



\*Please ask the presenting author for further experimental details

## Discussion and Conclusions

- After performing two different isolation techniques, we were able to optimize a standardized protocol for isolation of Extracellular Vesicles (Evs) from the media conditioned by bovine embryos.
- We could provide evidence that bovine embryos could release EVs into the medium, as part of embryo-embryo communication.