

POSTERS

EXTRACELLULAR VESICLES RELEASED BY CUMULUS CELLS CONTRIBUTE TO THE ACQUISITION OF MOUSE OOCYTE'S DEVELOPMENTAL COMPETENCE

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Bidirectional communication between the oocyte and its surrounding cumulus cells (CCs) within the cumulus-oocyte complex is crucial for shaping oocyte developmental competence.

We previously demonstrated that immature GV-oocytes acquire developmental competence when matured on a feeder layer of CCs derived from competent oocytes (FL-SN-CCs), whereas maturation on CCs from incompetent oocytes (FL-NSN-CCs) fail to support preimplantation development [1]. We also showed that these feeder layers release extracellular vesicles (EVs) carrying distinct miRNA cargos, including 7 key regulators of genes involved in oocyte maturation pathways [2].

Here, with the purpose to verify whether EVs contribute to the acquisition of oocyte developmental competence, we established a microinjection protocol to deliver EVs released by FL-SN-CCs in the perivitelline space of GV oocytes. In a typical experimental setting, naked GV oocytes isolated from 4-6 week-old mouse females were divided into two groups, one was injected with FL-SN-CCs EVs, the other

was injected with medium (CTRL). Four hours after insemination, successful fertilization was confirmed by the presence of both female and male pronuclei assessed by orcein staining and by time-lapse monitoring.

Across six independent experiments, only $5.8 \pm 8.5\%$ of CTRL samples reached the blastocyst stage, with the remaining arresting development at the 2-cell stage. Instead, $49.3 \pm 7.3\%$ of oocytes injected with FL-SN-CCs EVs reached the blastocyst stage with the remaining arresting development between the 2-cell and the morula stage. In contrast, oocytes injected with EVs from FL-NSN-CCs retained poor developmental competence, with only $14 \pm 4\%$ developing to blastocysts.

These findings provide an evidence that EVs released by competent CCs are potential active mediators of developmental competence.

References

1. Cavalera F et al. *Reprod Fertil Dev.*2019;31:1068-77.
2. Fiorentino G et al. *Mol Hum Reprod.*2024;30(6):gaae019