16 - Miscellaneous

OC-028 - (23966) - EFFECTS OF CULTURE SYSTEMS ON IN VITRO DEVELOPMENT OF SHEEP OOCYTES FROM EARLY ANTRAL FOLLICLES

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In female mammals, only a small proportion of follicles reach ovulation, while most undergo atresia. Given the abundance of immature follicles, their utilization could enhance fertility preservation for valuable genetic animals and cancer patients. This study evaluates whether a group culture system, compared to a single culture system, better supports the development of cumulus-oocyte complexes (COCs) from early antral follicles (EAFs), improving oocyte maturation yield.

COCs (GV stage) were isolated from sheep EAFs and subjected to a long in vitro culture (LIVC) for five days, either individually or in groups. Ovaries were collected from Sarda sheep during the non-breeding season. COCs were cultured in TCM199 (38.5°C, 5% CO_2) in a 96-well plate, either singly or in groups of three per well. Freshly isolated COCs, along with post-LIVC COCs from single and group cultures, were analyzed by transmission electron microscopy (TEM).

Before LIVC, oocytes had a thick, continuous zona pellucida (ZP) with transzonal projections, no perivitelline space, and microvilli extending into the ZP. Post-LIVC, single-cultured oocytes retained a thick, continuous ZP, while group-cultured oocytes displayed a thinner ZP with distinct layers. Both groups exhibited reduced transzonal projections. Group-cultured oocytes had a cortical ooplasm rich in vacuoles and mitochondria, whereas single-cultured oocytes showed irregular organelle distribution and hooded mitochondria. Microvilli structures also differed between the groups.

Group-cultured oocytes exhibited thinner ZPs, peripherally located mitochondria, and fewer vesicles, suggesting enhanced metabolic activity. These findings indicate that group culture might optimizes granulosa cell interactions and mitochondrial distribution, contributing to improved oocyte developmental competence.

Palavras-chave : Cumulus-oocyte complexes (COCs), In vitro culture, Transmission Electron Microscopy (TEM)