Long In Vitro Culture of Oocytes from Early Antral Follicles in Prepubertal Lambs

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Long in vitro culture (LIVC) of oocytes derived from early antral follicles (EAFs) has emerged as a potential reproductive technology for producing large numbers of competent oocytes. In a previous study, we demonstrated that LIVC of cumulus-oocyte complexes (COCs) from EAFs supports oocyte survival, growth, and acquisition of meiotic competence in adult sheep. This promising approach offers opportunities for preserving valuable and endangered animals, shortening generation intervals, and increasing genetic gain, especially when ovaries are collected from prepubertal animals as a source of EAFs. With this in mind, our objective was to investigate the efficacy of LIVC of COCs collected from EAFs in lambs. To achieve this, lamb ovaries were collected, and COCs were retrieved from EAFs (350-450 μm) by rupturing the follicle wall using a 21-gauge needle. Subsequently, they were cultured in a 96-well plate (one COC per well) containing TCM199 supplemented with 0.15 μg/mL zinc sulfate, 10^{-4} IU/mL FSH, 10 ng/mL estradiol, 50 ng/mL testosterone, 50 ng/mL progesterone, and 5 μM Cilostamide. After 5 days of culture, the following parameters were evaluated: COC morphology, oocyte diameter, chromatin configuration, gap junction communications, meiotic competence, levels of reactive oxygen species (ROS), mitochondrial activity, and distribution. Following the LIVC process, the results

indicate a significant increase in oocyte diameter (108.76 vs. 113.44 µm, $p<0.000$) and in mitochondrial activity ($p<0.005$), with a change in mitochondrial distribution pattern from 'fine' to 'granular' following the LIVC and IVM process ($p<0.01$). Furthermore, a notable transition in chromatin configuration was observed, characterized by a shift from a non-surrounded nucleolus (NSN) to a surrounding nuclear envelope (SNE) stage ($p<0.000$), coupled with spontaneous meiosis resumption after LIVC (MI/MII, 26.7%), and an increase in meiosis resumption after IVM ($p<0.000$). However, these results were accompanied by a significant increase in low quality COCs (based on morphological evaluation) ($p<0.05$) and an increase in ROS levels ($p<0.000$). Most COCs also exhibited closed gap junction communications before and after LIVC ($p<0.01$), indicating a defective cell coupling between oocytes and cumulus cells.

In conclusion, our study indicates that LIVC of COCs collected from prepuberal EAFs can only partially sustain oocyte growth. While significant improvements were observed in oocyte diameter, chromatin configuration, and meiotic resumption following the LIVC process, there were notable challenges, such as limited gap junction communication and a loss of oocyte-cumulus investment architecture. These findings highlight the need to refine the COCs culture system considering the intrinsic limited developmental potential of the oocyte in prepubertal animals.