

Morphological Interaction of Porcine Oocyte and Cumulus Cells Study on in vitro Oocyte Maturation Using Electron Microscopy

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Abstract : Morphological interaction of porcine cumulus-oocyte complexes (pCOCs) was investigated on in vitro condition using electron microscope (SEM and TEM). The totals of 1,923 oocytes were round in shape, surrounded by zona pellucida with layer of cumulus cells ranging between 59.29-202.14 μm in size. They were classified into intact-, multi-, partial cumulus cell layer oocyte, and completely denuded oocyte, at the percentage composition of 22.80% 32.70%, 18.60%, and 25.90 % respectively. The pCOCs classified as intact- and multi cumulus cell layer oocytes were further culturing at 37°C with 5% CO₂, 95% air atmosphere and high humidity for 44 h in M199 with Earle's salts supplemented with 10% HTFCS, 2.2 mg/mL NaHCO₃, 1 M Hepes, 0.25 mM pyruvate, 15 $\mu\text{g}/\text{mL}$ porcine follicle-stimulating hormone, 1 $\mu\text{g}/\text{mL}$ LH, 1 $\mu\text{g}/\text{mL}$ estradiol with ethanol, and 50 $\mu\text{g}/\text{mL}$ gentamycin sulfate. On electron microscope study, cumulus cells were found to stick their processes to secrete substance from the sac-shape end into zona pellucida of the oocyte and also communicated with the neighboring cells through their microvilli on the beginning of incubation period. It is believed that the cumulus cells communicate with the oocyte by inserting the microvilli through this gap and embedded in the oocyte cytoplasm before secreting substance, through the sac-shape end of the microvilli, to inhibit primary oocyte development at the prophase I. Morphological changes of the complexes were observed after culturing for 24-44 h. One hundred percentages of the cumulus layers were expanded and cumulus cells were peeling off from the oocyte surface. In addition, the round-shape cumulus cells transformed themselves into either an elongate shape or a columnar shape, and no communication between cumulus neighboring cells. After 44 h of incubation time, diameter of oocytes surrounded by cumulus cells was larger than 0 h incubation. The effect of hormones in culture medium is exerted by their receptors present in porcine oocyte. It is likely that all morphological changes of the complexes after hormone treatment were to allow maturation of the oocyte. This study demonstrated that the association of hormones in M199 could promote porcine follicle activation in 44 h in vitro condition. This culture system should be useful for studying the regulation of early follicular growth and development, especially because these follicles represent a large source of oocytes that could be used in vitro for cell technology.

Keywords : cumulus cells, electron microscopy, in vitro, porcine oocyte

Conference Title : ICBESE 2015 : International Conference on Biological, Ecological and Environmental Sciences, and Engineering

Conference Location : Boston, United States

Conference Dates : April 20-21, 2015