

3736140 SCAN/D : MAJOR : ANATOMY ; Ph.D. (ANATOMY)

KEYWORD : BOVINE, OOCYTE, MATURATION, MEIOTIC ARREST
CHROMOSOME CONDENSATION

BUSABA PANYARACHUN : STUDIES ON THE EFFECT OF INHIBITING FACTOR-HYPOXANTHINE ON THE *IN VITRO* MATURATION OF BOVINE OOCYTES USING LIGHT AND TRANSMISSION ELECTRON MICROSCOPY. THESIS ADVISOR : KANOK PAVASUTHIPASIT, M.D., Ph.D., REON SOMANA, Ph.D., M.D., AHNOND BOONYARATAVEJ, Ph.D., PRAPEE SRETARUGSA, Ph.D., SANGCHAI PRUTTIPUN, M.D., PRAKONG TANGPRAPRUTGUL, Ph.D.

The inhibitory effects of hypoxanthine (HX) on nuclear and cytoplasmic maturation of cumulus bovine oocyte complexes (COCs) were studied by light and transmission electron microscopy (LM and TEM). COCs were exposed to 4 mM HX in TCM 199 for 0, 8, 16 and 24 h. Nuclear stages during maturation of normal oocytes occur during various time were compared with those oocytes treated with HX in the same intervals. To study the reversibility of HX action, COCs were washed out of HX after 24 h treatment and re-cultured in the inhibitory-free culture medium (TCM 199) for 24 h. At 0, 8, 16 and 24 h of HX treatment, the percentages of oocytes in GV stage were 95, 93, 81 and 76, respectively whereas in the untreated group, all oocytes underwent GVBD by 8 h culture. GVBD occurred completely after 5 h incubation which was twice as fast as in the control medium (9 h). However, seventy-six percent of oocytes could reach metaphase II (M II) after 24 h incubation whereas 80% of control oocytes reached M II. However, the percentages of oocytes reaching M II in both groups at 24 h were not statistically significant. The morphological study at the beginning of treatment with HX (0 h) showed that GV was intact without chromosome condensation. But after 8, 16 and 24 h treatments, oocytes were maintained in the GV stage with condensed chromosome and normal intact nuclear membrane. During the maturation period, the oocytes underwent a series of clearly defined nuclear meiotic events. Oocyte maturation was also characterized by cytoplasmic changes. The cortical granules (CG) were composed of heterogenous and homogenous particles demonstrated by both the different electron densities and the different sizes similar to those immature oocytes. In contrast, the oocytes after washing out of HX and recultured in control medium. There were more dense electron particles and similar size granules as observed in the normal mature oocytes. These results indicated that 4 mM HX could inhibit the nuclear membrane breakdown but it could not prevent chromosome condensation at 8, 16 and 24 h after treatment. Furthermore, HX could also inhibit the cytoplasmic maturation of CG. The HX treated oocytes could be recultured to mature and normally reached M II after washing out of HX. The morphology of both nuclear and cytoplasmic maturation of HX treated oocytes and their reversibility was demonstrated by TEM. The distribution of CG and the stages of DNA during normal oocyte maturation were also revealed by the confocal laser scanning microscope.