THESIS TITLE : BOVINE OOCYTE AND OVIDUCTAL EPITHELIAL CELL CO-CULTURE ON THE DEVELOPMENT OF *IN VITRO* MATURATION AND FERTILIZATION

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ABSTRACT

The objectives of this experiment were to modify methodology and study the effects of co-culture of bovine oocytes with bovine oviductal epithelial cells (BOEC) and condition medium (CM) on maturation and fertilization rate. Immatured bovine oocytes obtained from a local slaughter house, were aspirated and sliced into Hank's solution with 0.3 % albumin. Oocytes with compact cumulus cells were chosen to be cultured in TCM-199 with 10 % FCS under 39 ^{o}C, 5 % C_2O in air and 99 % humidity.

The methodology modification involved culturing of BOEC, evaluation technique for the oocyte maturation, and sperm preparation for the *in vitro* fertilization (IVF). These resulted in gaining larger number of live epithelial cells, more precise culturing time for the oocyte development to metaphase2 and higher fertilization rate than the original methods.

The effect of co-culture on the *in vitro* maturation (IVM) and IVF were carried out under 5 experiments.

Experiment 1 was to determine appropriate culturing time for the *in vitro* maturation of oocytes. The experiment consisted of three treatments including 24, 27 and 30 hours culturing time. Culturing time for 24 hours showed a lower maturation rate ($P<0.01$) than those cultured for 27 and 30 hours (4.08, 48.00 and 51.79 %, respectively). However, the maturation rate of the oocytes that cultured for 27 and 30 hours were not significantly different ($P>0.05$).

Experiment 2 was to compare the effect of co-culture oocytes with BOEC and CM with TCM-199 . The result showed that the maturation rate of the oocytes in all treatments were not significantly different ($P>0.05$).

Experiment 3 was to study interaction effect between culturing time and co-culture on maturation rate of oocytes. The result showed that the culturing time but not the co-culture had significant effect on the maturation rate of the oocytes ($P<0.01$) and there was no interaction between the two factors.

Experiment 4 was to study the effect of TCM-199 and Brackett and Oliphant media (BO) on IVF. After incubation of the matured oocytes with sperm for 18 hours, fertilization rate of the oocytes in TCM-199 (80.3 %) was higher ($P<0.01$) than BO (66.31 %).

Experiment 5 was to study the effect of oocyte co-culture on fertilization rate. The fertilization rate of oocytes cultured in TCM-199, CM and BOEC were 78.64, 81.12 and 85.75 % respectively and there was no significantly different ($P>0.05$).

In conclusion, the culturing time but not the co-culture of oocytes with BOEC and CM, had effect on the maturation rate.There was no interaction between the culturing time and the co-culture. Types of fertilization medium had effect on the fertilization rate but the co-culture of oocytes with BOEC and CM did not improve the fertilization rate.