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SOME FACTORS INFLUENCING THE DEVELOPMENT OF BOVINE OOCYTES MATURED AND FERTILIZED *IN VITRO*

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This study was carried out to examine a) the effect of the pre-incubation time of spermatozoa (3.0 h and 5.0 h) in relation to the maturation time of oocytes (20.0 h and 26.5 h) and, b) cleavage of in-vitro fertilized oocytes co-cultured with bovine oviductal epithelial cell monolayers up to the morula or blastocyst stage.

Brackett and Oliphant medium (1975) supplemented with 10mM caffeine was used for sperm pre-incubation while modified TCM–199 supplemented with 10% inactivated fetal bovine serum was used for oocyte maturation and co-culture. Oocytes were fertilized in-vitro by the method of Hanada et al. (1985). The rate of fertilization, polyspermy and cleavage rate were compared. The embryos were allocated to their respective monolayers of bovine oviductal epithelial cells at 44.0～62.5 h post culture. Oviductal epithelial cells were dissociated and cultured by the method of Hishinuma et al. (1989). The cells were composed of ciliated and non-ciliated cells. The epithelial cell monolayers were used for co-culture after reaching about 60～80% confluency.

There were no significant differences between the rates of normal fertilization, polyspermy and cleavage of oocytes matured for 26.5 h and fertilized in vitro by spermatozoa preincubated for 3.0 h or 5.0 h. However, the rate of fertilization and cleavage was higher in oocytes matured for 26.5 h rather than 20.0 h and fertilized in-vitro by spermatozoa pre-incubated for 3.0 h. A significant difference in the cleavage rate was observed between the two maturation times (20.0 h : 32.7%, 26.5h : 50.0% ; $P<0.05$).

The oocytes matured for 26.5 h cleaved to the morula or blastocyst stage when co-cultured with bovine oviductal epithelial cell monolayers. However, the cleavage rates to morulae (2.6～14.3%) and blastocysts (0～9.5%) were low. Nevertheless, the oocytes matured for 20.0 h and fertilized in-vitro by spermatozoa pre-incubated for 3.0 h cleaved to the morula (42.1%) or blastocyst (26.3%) stage even without co-culturing with oviductal epithelial cell monolayers. The numbers of blastomeres for expanded and hatched blastocysts were 74 and 200, respectively. The cell divisions were also observed.

This study indicated that bovine oocytes matured and fertilized in-vitro can undergo normal development up to the blastocyst stage. Comparatively, however, the rates of normal fertilization and cleavage were lower than in other reports. In addition, the effects of the pre-incubation time of spermatozoa and co-culturing with a bovine oviductal epithelial cell monolayer were difficult to determine due to the significant influence of the maturation time of oocytes.