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# *IN VITRO* MATURATION, FERTILIZATION AND SUBSEQUENT DEVELOPMENTAL POTENTIAL OF BOVINE OOCYTES WITH DIFFERENT MORPHOLOGICAL CHARACTERISTICS

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Bovine cumulus-oocyte complexes (COCs) derived from follicles from slaughtered cattle show various morphological characteristics under a stereomicroscope. In this study, the COCs were classified into two groups on the basis of the quality of their ooplasm and cumulus investments : 1) homogeneous ooplasm and compact multilayered cumulus investment and 2) ooplasm granulated with dark clusters and less compact multilayered cumulus investment.

The COCs in groups 1 and 2 were cultured in a maturation medium, 25 mM Hepes-buffered TCM199 supplemented with 10% fetal calf serum, 0.02 U/ml follicle-stimulating hormone, 1 $\mu$g/ml estradiol-17$\beta$ and 0.2 mM sodium pyruvate at 39°C and in a humidified atmosphere of 5% $CO_2$ in air. In experiment 1, all oocytes were fixed after 12 and 22 hrs of culture for maturation, respectively. The nuclear status of the two groups were not significantly different. In experiment 2, the COCs were cultured for 22 hrs in the maturation medium. Then they were co-incubated with $5\times10^6$ sperm/ml in the medium defined by Brackett and Oliphant (1975), supplemented with 2.5 mM theophylline and 3 mg/ml bovine serum albumin for 20 hrs at 39°C in a humidified atmosphere of 5% $CO_2$ in air. Although there were no differences in the total sperm penetration rates between the two groups, the normal fertilization rate of the oocytes in group 2 (93.8%) was significantly higher than that of the oocytes in group 1 (86.2%, $P<0.05$). The rate of polyspermy of the oocytes in group 1 (8.3%) was significantly higher than that of the oocytes in group 2 (2.2%, $P<0.01$). In experiment 3, *in vitro* fertilized presumptive zygotes were cultured in modified synthetic oviduct fluid at 39°C in a humidified atmosphere of 5% $O_2$, 5% $CO_2$ and 90% $N_2$. Cleavage rates at 72 hrs post-insemination were significantly higher in the embryos in group 2 (89.8–91.6%) than in group 1 (81.4–81.9%, $P<0.05$). The embryos in group 2 were more advanced in developmental stage than those in group 1 at 30 and 72 hrs post-insemination ($P<0.05$). However, cleaved embryos in groups 1 and 2 showed the same developmental rate to the blastocyst stage and the average cell numbers of blastocysts were not different between the two groups at 168 to 170 hrs post-insemination.

The results of the experiments showed that the COCs in groups 1 and 2 had the same nuclear maturation rate but that the ability to block polyspermy was higher in

group 2. This may due to the more advanced cytoplasmic maturation of the oocytes in group 2 than in group 1. Cleaved embryos in groups 1 and 2 had the same development potential to the blastocyst stage. In conclusion, the oocytes in group 2 had more potential for *in vitro* fertilization than those in group 1.