P-05

Influence of Ionomycin and Cycloheximide Activation Treatments on *In Vitro* Maturation of Canine Oocytes

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Despite the rapid advancement of *in vitro* embryo production techniques in domestic animals, the efficiency in canine still remains very low. Moreover, the poor developmental competence of *in vitro* produced embryos might be the consequence of low maturation rates. The objective of the present study was to determine the effects of calcium ionophore, ionomycin with or without a protein synthesis inhibitor, cycloheximide (CHX) treatments on *in vitro* maturation (IVM) of canine oocytes. Ovaries were collected from twelve bitches of mixed breed (aged 2~6 years) at various stages of the estrous cycle by ovariohysterectomy. COCs were recovered by ovarian slicing and matured in TCM-199 containing 10% FBS, 10 μg/mL FSH and LH, 0.57 mM cystein and 10 ng/mL epidermal growth factor (EGF) for 72 h at 39℃ in a humidified atmosphere of 5% CO_2 in air. In experiment Ⅰ, the nuclear status of oocytes at different time points (0, 24, 48 and 72 h) of IVM was assessed using 1% aceto-orcein staining. Nuclear morphology was classified as follows: germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI) and metaphase Ⅱ (MⅡ) stages. Experiment Ⅱ evaluated the effects of ionomycin and CHX treatments on nuclear maturation rate of oocytes. Oocytes matured for 24 h and 48 h were exposed to 5μM ionomycin for 5 min. Subsequently, they were exposed to 10 μg/mL CHX for 0, 3 and 24 h each. With progression of time, there was a decrease in GV stage (52, 20, 11 and 6%) with a corresponding increase in MI and MII stages (8, 10, 29 and 31%; 0, 2, 6 and 12%). Oocytes

of 24 h matured group exposed to ionomycin for 5 min followed by CHX for 24 h reached significantly ($p<0.05$) higher MII stage (19.6%) than other groups. In conclusion, exposure of *in vitro* matured oocytes at 24 h to ionomycin (5 min) followed by CHX (24 h) was found suitable to enhance the nuclear maturation rate in canines.

Key words) *Oocyte, In vitro maturation, Ionomycin, Cycloheximide, Nuclear morphology, Canine*