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Effect of Conditioned Medium of Mouse Embryonic Fibroblasts Produced from EC-SOD Transgenic Mice in Nuclear Maturation of Canine Oocytes *In Vitro*

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Abstract

The rate of *in vitro* maturation (IVM) of canine oocytes has not improved in comparison to that of other mammalian species. This study aims to improve the efficiency of canine oocytes IVM using the antioxidant, extracellular superoxide dismutase (EC-SOD). Thus, the effect of conditioned medium of EC-SOD transgenicmouse embryonic fibroblasts cultured with MEF culture medium (DMEM+5% FBS) for *in vitro* nuclear maturation in canine oocyteswas investigated. In experiment I, oocytes were collected from the ovaries of domestic bitches, which were allotted to one of two groups: (1) TCM199+1% FBS (n=108) or (2) DMEM+5% FBS (n=112), cultured for 48 h and investigated for *in vitro* nuclear maturation of canine oocytes using Hoechst staining. Meiotic progression to metaphase II in group 1 was 1.8% compared to 1.8% in group 2. In experiment II, EC-SOD levels were examined in NTg-CMEF and Tg-CMEF at 0, 2, and 4 days obtained from EC-SOD transgenic mice generated in our laboratory. The concentration of EC-SOD in Tg-CMEF at day 2 (371.7±3.1ng/mL) was the highest for all groups ($p<0.05$). EC-SOD levels in Tg-CMEF were higher than in NTg-CMEF therefore, the efficiency of Tg-CMEF for IVM was investigated. In experiment III, oocytes were allotted to one of three groups: (1) Tg-CMEF at day 0 (n=84), (2) Tg-CMEF at day 2 (n=92) or (3) Tg-CMEF at day 4 (n=98), cultured for 48 h and the IVM of canine oocytes investigated. The

mean percentage of MII oocytes in IVM was 2.4, 4.4 and 2.0% for groups 1, 2 and 3, respectively. In experiment IV, the effects of conditioned medium of EC-SOD transgenicmouse embryonic fibroblasts (Tg-CMEF) cultured in MEF culture medium were compared with conditioned medium acquired from non-transgenic mouse embryonic fibroblasts (NTg-CMEF) on IVM of canine oocytes. In this experiment, meiotic progression to metaphase II was 7.1% in Tg-CMEF versus 0% in NTg-CMEF ($p<0.05$). Tg-CMEF was more effective than NTg-CMEF. In conclusion, it wasverified that canine oocytes were able to effectively progress to metaphase II in IVM when cultured in Tg-CMEF.

Key words) *EC-SOD, Transgenic mouse, Mouse embryonic fibroblast, In vitro maturation, Canine oocyte, Conditioned medium*