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In Vitro Maturation of Oocytes Derived from the Brown Bear (*Ursus arctos*)

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The brown bear (*Ursus arctos)*, a member of Ursidea family, is currently as threatened by the IUCN Red list. The oocytes collection and maturation are an important means to preserve genetic resources. This study was conducted to determine if meiotic maturation could be induced in ovarian oocytes of the American brown bear (*Ursus arctor*), a model for gamete "rescue" techniques for endangered ursids. Bear ovaries were obtained from two female brown bear that aged 15 years at anestrous. The bear was euthanasia and ovaries were transported to the laboratory within 2 hours. The mean ovarian size was 2.4/1.8 cm (range 2.0~3.3/1.5~2.2 cm). Ovaries obtained from 2 brown bears yielded 97 oocytes (48.5 per female), of which 88 (90.7%) were morphologically classified as excellent quality. Oocytes were *in vitro* matured at 39℃, 5% CO_2 in air atmosphere for 48 h in a IVM medium (TCM-199 supplement with 10% FBS, 0.6 mM cysteine, 0.2 mM pyruvic acid and 10 IU/mL HMG). Experiment I: the effect of maturation time on the evaluation of meiotic development was conducted. And the matured oocyte morphologic evaluation was conducted by measuring the diameter of oocytes with or without ZP and the diameter of cytoplasm. Experiment II: the matured oocytes were equilibrated in 0.28 M mannitol solution containing 0.1 mM Ca^{++}, then transferred to an electrofusion chamber containing the same medium. Activation was induced by applying two times 2.0 kv/cm 20 μsec DC pulses delivered by Electro Cell Fusion Generator (Nepagene, Japan). After the eletrical stimulation, the oocytes were cultured in TCM199 containing 2 mM of 6-dimethylaminopurine for

4 hours. The activated oocytes were cultured in CR1 for 3 days and changed to CR2 for remain 4 days. The diameter of matured bear oocytes (161.8 ± 6.0 μm) with ZP and cytoplasm(135.3 ± 7.5 μm) without ZP was significantly ($p<0.05$) bigger than that of bovine oocytes (150.7 ± 4.9 μm and 118.7 ± 7.5 μm). The maturation rate of bear oocytes were 59.4% and 17.6% at 48 and 24 hours *in vitro* maturation. The activated oocyte developed to 2, 4-cell stage was 31.6%, but no blastocyst observed. These results indicated that bear oocytes can be developed to MII in *in vitro* culture system, and activated oocyte can be develop to 2, 4-cell stage.

Key words) *Bear, Oocyte, In Vitro maturation, Oocyte size, Activation*

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