A0207

In Vitro Development of Somatic Cell Nuclear Transferred Bovine Embryos Following Activation Timing in MII Enucleated Oocytes Cryopreservation

김선균, 김은영, 길광수, 박세영, 윤지연, 박세필, 정길생[1], 임진호[2]

마리아 기초의학연구소/마리아 생명공학연구소, [1]건국대학교, [2]마리아 병원

This study was to evaluate the *in vitro* survival of vitrified-thawed bovine MII enucleated (MIIe) oocytes according to activation timing and minimun volume cooling (MVC) method and their *in vitro* development after somatic cell nuclear transfer (SONT). Bovine oocytes were recovered from slaughtered bovine ovary and matured in TCM-199 supplemented with 10% FBS. After incubation for 20 h in IVM medium, recipient oocytes were stained using 5 ㎍/㎖ Hoechst and their 1st polar body and MII plate were removed by enucleation micropipette under UV filter. MIIe oocytes were subjected to activation before (pre-activation group) or after (post-activation group) vitrification in 5 uM ionomycin added CR1aa medium for 5 min. For vitrification, MIIe oocytes were pretreated in EG10 for 5 min, exposed in EG30 for 30 sec. Thawing was taken by 4-step procedures [1.0 M sucrose (MS), 0.5 MS, 0.25 MS, and 0.125 MS added PBS, for 1 min per each step] at 37℃. Survived MIIe oocytes were subjected to NT with cultured adult bovine ear cells. Reconstructed oocytes were cultured in 10 ug/ml of cycloheximide and 2.5 ug/ml of cytochalasin D added CR1aa medium for 1 h, and then in 10 ug/ml of cycloheximide added CR1aa medium for 4 h. Subsequently, the reconstructed oocytes were washed three times and incubated in CR1aa medium for 48 h. The cleaved embryos were then selected and further cultured on cumulus-cell monolayer drop in CR1aa supplemented with 10% FBS for 7 days. Survival rates of bovine vitrified-thawed MIIe oocytes in pre-activation and post-activation groups were 84.9% and 80.9%, respectively. However, nuclear transferred MIIe oocytes in post-ativation group indicated significantly higher rates of cytoplasmic fragment than those in pre-activation group with significant difference. Fusion rates of cytoplasts and oocytes in pre-activation group or post-activation group resulted in 70.0% and 69.1%, respectively. Also, their subsequent development into cleaved embryos (55.8% vs. 53.5%) and blastocysts (11.7% vs. 6.5%) with no significant difference. This result suggested that MVC method was appropriate freezing method for the bovine MIIe oocytes and that vitrified MIIe oocytes after pre-activation could support *in vitro* embryonic development after SONT as equally well as fresh oocytes.

Key words) ***Nuclear transfer, MVC method, Enucleated oocytes, Activation***