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The Production of Cloned Embryos with Cooled and Frozen-Thawed Adult Ear Cells in Bovine

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This study was designed to investigate the *in vitro* developmental ability and apoptosis of embryos nuclear transferred (NT) with frozen-thawed (FT) or cooled donor cells in bovine. Cultured adult bovine ear cells were used as donor cells at confluent condition (CC), after cooling at 4°C for 48 hour, or after FT. Oocytes were enucleated 22 hour post *in vitro* maturation and electrofused with one of the donor cells from different culture condition by membrane to membrane fusion method using two pulses (DC) of 2.1 KV/cm for 30 msec. Reconstructed oocytes were activated by treatment 10 mM ionomycin for 5 minutes and 6- Dime-thylaminopurine for 3 hours and *in vitro* cultured in CR1aa medium supplemented with 0.3% fatty acid free bovine serum albumin for 3 days and subsequently in CR1aa medium supplemented with 10% fetal bovine serum for 5 days. The blastocysts were evaluated for apoptosis by terminal deoxynucleotidyl transferase mediated dUTP nick end labeling method. The fusion, cleavage and blastocyst rates were 69.0 (167/242), 68.8 (115/167) and 29.9% (50/167) with CC cells, 70.4 (88/125), 69.3 (61/88) and 29.6% (26/88) with cooled cells, and 66.1 (117/177), 70.1 (82/117) and 13.6% (16/117) with FT cells, respectively. The blastocyst rate of NT embryos with FT cells was significantly lower ($P<0.05$) than those with CC or cooled cells. Moreover, the rate of apoptosis in NT blastocysts with FT ($6.4 \pm 4.0\%$) cells was significantly higher ($P<0.05$) than those with CC ($2.8 \pm 1.7\%$) or cooled ($2.3 \pm 1.3\%$) cells. However, interestingly there were no significant differences in the development and apoptosis rates between NT embryos with cooled and CC cells as a donor karyoplast. From these data, we suggest that the use of FT cells as donor cells reduces significantly the development rate and increases the apoptosis rate of NT embryos. However, cooled cells may be good candidate donor cells for the production of cloned cow.

Key words: *Frozen-thawing, Cooled, confluent ear cell, Nuclear Transfer, bovine*