P-10

Production of Cloning Embryos with Activated Oocytes in Rabbits

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Large scale production of cloned embryos requires the technology of nuclear transfer (NT). In this work we investigated comparatively the effects of enucleated oocytes treated with ionomycin and 6-DMAP on the electrofusion rate and *in vitro* developmental potential of NT embryos.

The embryos of 16-cell stage were collected from the mated does by flushing oviducts with Dulbecco's phosphate buffered saline (D-PBS) containing 10% fetal calf serum (FCS) at 47 hours after hCG injection. The recipient cytoplasms were obtained by removing the nucleus and the first polar body from the oocytes collected at 15 hours after hCG injection. The enucleated oocytes were activated by 5 min incubation in 5 μM ionomycin and 2 hours incubation in 2 mM 6-DMAP at 19-20 hours post-hCG before microinjection. the unsynchronized 16-cell stage embryos were used as nuclear donor. The separated donor blastomeres were injected into the enucleated activated recipient oocytes by micromanipulation and were electrofused by electrical stimulation of single pulse for 60 μsec at 1.25 kV/cm in Ca^{++}, Mg^{++} - free 0.28 M mannitol solution. In the non-activation group, the electrofusion and electrical stimulation was given 3 pulses for 60 μsec at 1.25 kV/cm in 100 μM Ca^{++}, Mg^{++} 0.28 M mannitol solution. The fused oocytes were co-cultured with a monolayer of rabbit oviductal epithelial cells in TCM-199 solution containing 10% FCS for 120 hours at 39℃ in a 5% CO_2 incubator. The results obtained were summarized as follows:

1) In the NT embryos, the electrofusion rate of preactivated and non-activated oocytes (80.4 and 87.8%) was not significantly different.
2) In the NT embryos, the developmental potential to blastocyst stage was significantly ($P<0.05$) higher in the activated oocytes (40.3%) than in the non-activated oocytes (16.0%).

In conclusion, it may be efficient to use the oocytes preactivated with ionomycin and 6-DMAP for the multiple production of cloned embryos by nuclear transfer.