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Effect of different activation protocol on in vitro development of somatic cell nuclear transfer (SCNT) pig embryos[1]

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This study was conducted to examine the effect of activation methods and post-activation treatment on in vitro development of SCNT pig embryos. In vitro maturation of immature oocytes and nuclear transfer was done by a common protocol in our laboratory. Adult skin fibroblasts with hDAF gene were used as donor cell. Reconstructed oocytes were electrically (2V AC for 2 sec and 1.6 kv/cm, 50 usec, 2 pulses) fused in 0.28 M mannitol solution with (for simultaneous fusion and activation; SFA) or without calcium (for delayed activation; DA). In DA, fused oocytes were electrically (1.2 kv/cm, 60 usec, 2 pulses) activated in 0.28 M mannitol medium with 0.1 mM calcium chloride (Exp. 1). Fused and activated oocytes were treated with cytochalasin B (7.5 ug/ml), demecolcine (0.4 ug/ml) and both for 4 hrs (Exp. 2). SCNT embryos were cultured in a NCSU-23 medium for 6 days.

DA method showed higher developmental rate to cleavage and blastocyst stage than SFA method (Table 1). When fused and activated embryos were treated with cytochalasin B and/or demecolcine, cleavage rate decreased but blastocyst formation tended to increase compared to control (Table 2). This result suggested that activation protocol and post-activation treatment affected developmental ability of SCNT pig embryos.

Table 1. In vitro development of SCNT pig embryos after simultaneous fusion and activation (SFA) and delayed activation (DA) protocol

Activation protocol	Manipulated	Fused (%)	No. cultured	≥2-cell (%)	Blastocyst (%)	Cell No.
SFA	250	77.2	109	57.1	11.0	36.7
DA	262	82.4	167	77.1	17.7	36.5

Table 2. Effect of post-activation treatment on the development of cloned pig embryos

Post-activation	No. cultured	≥2-cell (%)	Blastocyst (%)	Cell No.
None	38	84.2	23.7	41.3
Cytochalasin B (CB)	40	77.5	32.5	36.8
Demecolcine (D)	40	77.5	37.5	43.4
CB+D	36	75.0	38.9	38.4

Keywords: *nuclear transfer, activation, embryo development, cytochalasin, pig*

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[1]This work was supported by the Research Project on the Production of Bio-organs, Ministry of Agriculture and Forestry, Republic of Korea.