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The Effect of Oocyte Activation on Development of Porcine Cloned Embryos

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The successful development of embryos cloned by nuclear transfer (NT)have been dependent on a wide range of known factors including cell cycle of donor and recipient ooplast, oocyte quality, NT procedure and oocyte activation. The present study compared the development of cloned porcine embryos following different activation treatments. Cumulus-oocyte complexes (COCs) were aspirated from 2 6 mm follicles of slaughterhouse ovaries and cultured for 22 h in NCSU #23 medium supplemented with 10% porcine follicular fluid, 0.57 mM cysteine, 0.5 g/mL LH, 0.5 g/mL FSH and 10 ng/mL EGF. The COCs were further cultured for an additional 22 h in the same medium at 39℃ in an atmosphere of 5% CO_2 in air, without hormonal supplements.

Primary cultures of fibroblasts isolated from a female fetus on day 40 of gestation were established in DMEM + 15% FCS. For nuclear donation, cells at the 5th-6th passage were cultured in DMEM + 0.5% FCS for 5 days in order to arrest the cells in G0/G1. After enucleation, oocytes were reconstructed by transfer of donor cells and fusion with three DC pulses (1.4 KV/cm, 30 sec) in 0.28 M mannitol containing 0.01 mM $CaCl_2$ and 0.01 mM $MgCl_2$. Eggs were then divided into three treatment groups; control (without further treatment, Group 1), eggs cultured in 10 g/ml cycloheximide (CHX) for 5 h (Group 2), and eggs cultured in 1.9 mM 6-dimethylaminopurine (6-DMAP) for 5 h (Group 3). The eggs were then cultured in sets of 30 in 60 l drops of NCSU#23 supplemented with 4mg/ml BSA (essentially fatty acid free) until day 7 at 39℃ in a humidified atmosphere of 5% CO_2. On day 4 the culture were fed by adding 20 l NCSU #23 supplemented with 10% FBS.

Development rates into blastocysts were significantly higher ($P<0.05$) in Group 3 embryos compared to Group 1 controls (27.6±2.7% vs. 20.1±4.1%, respectively), but rates did not differ in Group 2 compared to control (23.8±5.7%). Total cell number in Group 3 blastocysts was however significantly higher ($P<0.05$) than in Groups 1 and 2 (44.6±2.4 vs. 19.9±1.9 and 21.9±2.1, respectively).
These results suggest that 6-DMAP is more efficient than cycloheximide in the activation of electrically fused NT oocytes during *in vitro* production of cloned porcine embryos.

Key words) ***oocyte activation, cloning, pig***