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Effect of Activation Method and Culture Medium on the Development of Porcine Nuclear Transfer Embryo using Fetal Fibroblast

Gi-Sun Im[1], Byoung-Chul Yang[1], Jin-Ki Park,
Hyun-Ju Kim[1], Won-Kyung Chang[1], R. S. Prather[2] and B. N. Day[2]
[1]Animal Biotechnology Division, NLRI(*gsim@rda.go.kr*),
[2]Department of Animal Science, University of Missouri-Columbia

Since the first birth of pig derived from embryonic cells by nuclear transfer, many researches to produce cloned pig have been carried out. Recently, two reports about the birth of somatic cell cloned pigs using *in vivo* oocytes and also Betthauser et al. (2000) reported the birth of somatic cell cloned pigs using *in vitro* oocytes. So here we investigated the effect of activation method and culture medium on in vitro development of porcine nuclear transfer embryo using fetal fibroblast. Oocytes derived from slaughter house obtained ovaries were matured for 42 to 44 h in TCM 199. Matured oocytes were denuded using 0.1% hyaluronidase and then Oocytes with the first polar body were used for enucleation by aspirating the first polar body and adjacent cytoplasm in TCM 199 supplemented with 7.5 μg cytochalasin B. Fetal fibroblast cells were prepared from 35 days old fetus. To be used as donor cells, fetal fibroblast cells were serum starved for 3 to 5 days and then isolated into single cell by trypsinization. Nuclear transfer embryos were fused using 2 times 1.25kV for 30μs. Fused NT embryos were activated with calcium ionophore (CI) and 6-dimethyl- aminopurine (6-DMAP). Activated oocytes were cultured in NCSU 23 or BECM 3 for 6 days. There was no significant difference between chemical activation and no chemical activation for blastocyst development rate(11.6 vs. 14.8%). However, cell number was significantly higher when NT embryos were activated with CI and 6-DMAP (31.2 vs. 22.6). When NT embryos were cultured in NCSU 23 or BECM 3, blastocyst development rate was 16.4 and 13.2%, respectively, and cell number was 31.5 and 24.1, respectively. These results suggest that chemical activation after fusion and culture in NCSU 23 could increase cell number of porcine NT embryos.

(Key words) ***Somatic cell, Nuclear transfer, Activation method, Culture medium***