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Differentiation Status of Donor Genome may Influence the Developmental Competence of Porcine Nuclear Transfer Embryos

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Cellular differentiation influences the epigenetic state of the donor cell nucleus, which in turn, determines the efficiency at which an enucleated oocyte can reprogram a donor cell into a totipotent status. Mesenchymal stem cells (MSCs) are easily collected sources among adult stem cells and have a more flexible potential as donor cells for nuclear transfer (NT). We compared the cloning efficiency for preimplantation development *in vitro* using undifferentiated (MSCs) and differentiated cells in the same lineage (osteocyte, adipocyte and chondrocyte) by assessing the cleavage, blastocyst rate and total cell number. MSCs obtained from the aspirated bone marrow of neonatal pig were cultured in a-DMEM supplemented with 5% FCS. The differentiation potential was demonstrated by culture of MSCs at passage 3 under the conditions that were favorable for adipogenic, osteogenic and chondrogenic development. For NT, cells from passages 3~5 were transferred into the perivitelline space of enucleated MⅡ oocytes that were subjected into *in vitro* matured oocytes collected from slaughterhouse derived ovaries. After fusion with needle-type electrode, eggs were cultured in 7.5 μg/ mL cytochalasin B for 3 h, and further subsequently cultured in PZM-3 medium for 6 d. Statistical significance was tested using ANOVA with Bonferroni and Duncan. NT eggs using differentiated MSCs; osteocyte, adipocyte and chondrocyte revealed significantly ($p<0.05$) lower cleavage (74.5, 63.4 and 74.3%, respectively) and blastocyst development (33.7, 30.1 and 36.5%, respectively) than those of undifferentiated MSCs (92.2

and 47.8%, respectively). Total cell numbers were not significantly different among all groups. The results demonstrate that donor cells of differentiated origin supports varying degrees of embryonic development. As reported that the epigenetic status of the donor genome affects the development of cloned embryos, the cloning efficiency could be improved by investigating and manipulating the altered epigenetic marks of donor cells.

Key words) *Mesenchymal Stem cells, Differentiated mesenchymal stem cells, Nuclear transfer, Porcine*

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