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Trichostatin A improved epigenetic modifications of transgenic cells but did not improve subsequent cloned embryo development

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Despite cloning animals from adult cells were produced in various species, cloning remains an extremely inefficient process. Reprogramming impairment of DNA methylation may be partly responsible for the low efficiency in somatic cell nuclear transfer. In this study, bovine fibroblast cells were transfected with enhancer green fluorescence protein (eGFP), and then treated with a histone-deacetylase inhibitor, trichostatin A (TSA). The results showed that the effect of TSA on transgenic cells was in a dose-dependent manner. When the TSA concentration was over 5 ng/mL cell proliferation was significantly inhibited. The majority of the cells died when TSA reached 100 ng/mL ($P<0.01$). Number of cells in S phase was significantly decreased in the 5 to 50 ng/mL TSA-treated groups, while the majority of the cells were at G0/G1 phases. Expression of eGFP were approximately two-fold higher in 25 ng/mL (31.7%) and 50 ng/mL (32.2%) TSA groups when compared to the control (15.0%). Reduced DNA methylation and improved histone acetylation were observed when the cells were treated with 10 to 50 ng/mL TSA. Transfer of the TSA-treated cells to enucleated recipient oocytes resulted in similar cleavage rates among the experimental groups and the control. Cells treated with 50 ng/mL TSA resulted in significantly lower blastocyst development (9.9%) than the other experimental and the control groups (around 20%). Analysis of the putative blastocysts showed that 86.1% of the embryos derived from TSA-treated cells were eGFP positive, which was higher than that from untreated cells (73.4%). In conclusion, transgenic cells were more sensitive to TSA treatment than non-transgenic cells. Treatment of transgenic cells with TSA decreased the genome DNA methylation level, and eGFP gene expression was activated. Donor cells with reduced DNA methylation did not improve subsequent cloned embryo development. However, transfer gene expression was improved in cloned embryos.

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