330 TRICHOSTATIN A IMPROVES THE *IN VITRO* DEVELOPMENT OF CLONED BOVINE EMBRYOS RECONSTRUCTED WITH TRANSGENIC DONOR CELLS

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Trichostatin A (TSA), a histone deacetylase inhibitor, has been described as a potential modulator of nuclear reprogramming in bovine zygotes reconstructed by somatic cell nuclear transfer (SCNT), but with controversial results (Lee *et al.* 2011 J. Reprod. Dev. **57**, 34–42; Sangalli *et al.* 2012 Cell Reprogramming **14**, 1–13). The effect of TSA in zygotes reconstructed with transgenic cells cultured for long periods is not known. This study aimed to evaluate the effect of TSA on development of bovine embryos reconstructed with donor cells transfected with a green fluorescent protein (GFP)-reporter transgene. Bovine fibroblasts at second passage were transfected with lentiviral vectors carrying the GFP transgene and cultured at 37.5°C under 5% CO_2 in air. Transfected cells were cultured for additional 10 passages to establish a cell lineage expressing the protein. In the 12th passage, the cells were frozen in 10% dimethyl sulfoxide plus FCS (Nutricell, Campinas, Brazil) and frozen–thawed cells expressing GFP were used as nucleus donors. *In vitro*-matured oocytes were enucleated, fused to GFP positive fibroblasts, and activated with ionomycin. Putative zygotes were randomly distributed into 2 groups: SCNT-CONT ($n = 55$): zygotes were cultured for 4 h in CR_2aa medium plus BSA with 6-DMAP followed by 7 h in CR_2aa medium plus 2.5% FCS; SCNT-TSA ($n = 49$): zygotes were cultured in the same conditions described above, but supplemented with 50 nM TSA (Sigma-Aldrich, St Louis, MO). Then, embryos from all groups were cultured in CR_2aa supplemented with 2.5% FCS under 5% CO_2, 5% O_2, and 90% N_2 at 38.5°C. Evaluations of cleavage and blastocyst percentages were performed at 72 and 168 h post-activation, respectively, and 4 replicates were carried out. Expression of GFP in embryos at blastocyst stage was visualised using an epifluorescence microscope. Statistical analysis was performed by ANOVA and data are shown as mean ± SEM. No difference ($P > 0.05$) on cleavage percentage was found between groups (72.9 ± 11.3% and 66.1 ± 14.4% for SCNT-CONT and SCNT-TSA, respectively). The blastocyst percentage calculated based on putative zygotes tended ($P = 0.077$) to be higher for SCNT-TSA (16.7 ± 4.0%) than for SCNT-CONT (6.8 ± 2.3%). When the blastocyst percentage was calculated based on cleaved embryos, a higher rate ($P < 0.05$) was achieved in SCNT-TSA (26.7 ± 3.8%) than in SCNT-CONT (10.3 ± 3.6%) group. Blastocysts of both groups expressed GFP, with no difference among embryos. In a previous study, we reported that TSA had no positive effect on *in vitro* embryo development or gene expression, despite the reduction on apoptosis index [Camargo *et al.* 2011 Acta Sci. Vet. **39**(Suppl.), S442; Camargo *et al.* 2012 Reprod. Fert. Dev. **24**, 121–122). In the present study, however, the treatment with TSA of zygotes reconstructed with transgenic cells cultured for a long time improved embryo development without impairing GFP expression. This result suggests that TSA may be effective in clones reconstructed with transgenic cells.

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331 OPTIMIZATION OF BRANCHED 25 kDa POLYETHYLENIMINE FOR EFFICIENT GENE DELIVERY IN BOVINE FETAL FIBROBLASTS

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Cost-effective, highly efficient, and noncytotoxic transfection of bovine fetal fibroblasts (BFF) has proven difficult to achieve by regular chemical and physical methods. The aims of this study were to evaluate transient transfection efficiency and toxicity of commercially available branched 25 kDa polyethylenimine (25 kDa PEI, Sigma-Aldrich, St. Louis, MO, USA) and to optimize the transfection conditions leading to high percentages of PEI-transfected fibroblasts with minimum cytotoxic effects. Bovine fetal fibroblast (BFF) cells were seeded a day before transfection in 24-well plates at a density of 3×10^4 cells per well in DMEM with antibiotics and 10% SFB. When 70 to 90% confluence was reached, cells were washed with PBS and incubated in DMEM without antibiotics or SFB. For the transfection-mix preparation, increasing amounts of plasmidic DNA (pZsGreen1; 2 to 6 μg) were added to 50 μL of DMEM without antibiotics or SFB, incubated for 5 min at room temperature, and complexed with 0.5 to 4 μg of PEI (from 1 mg mL^{-1} solution) in 50 μL of PBS for 10 min. This transfection mix was added to the cell cultures and, 2 h later, 500 μL of DMEM with antibiotics and 10% SFB was added to each well. Detection of green fluorescent protein (GFP) expression by flow cytometry (reported as percentage of green fluorescent cells) was performed 48 h after transfection. Results were analysed by ANOVA and Tukey test and expressed as mean ± SEM ($P < 0.05$). We found no significant difference between the percentage of GFP-positive cells transfected with 1 or 2 μg of 25 kDa PEI at 2 μg of DNA/well (15.2 ± 1.3 *v.* 16.9 ± 0.9%, respectively; $P > 0.05$), whereas cells transfected with 1 or 2 μg of low-molecular-weight PEI (2 kDa) showed extremely low transfection efficiencies. Increasing the DNA load up to 6 μg significantly enhanced cell transfection (3.5- and 6-fold comparing 2 μg *v.* 4 μg and 6 μg of DNA, respectively; $P < 0.05$) at 1 and 2 μg of 25 kDa PEI/well. In order to evaluate the cytotoxic effect of PEI, cell viability was determined using the MTT assay in 96-well plates (cells/well), with each condition scaled down to replicate the effect of 2 kDa or 25 kDa PEI in a 24-well plate. The MTT results (expressed as % of the control) indicated that PEI became cytotoxic at concentrations equivalent to 2 and 4 μg/well (54.7 ± 3.4 and 18.5 ± 5.7, respectively), whereas 1 μg/well produced a slight detrimental effect on cell viability (90.0 ± 2.6). No evidence of cytotoxicity was observed when the BFF were incubated with 0.5 μg/well of 25 kDa PEI and 1 or 2 μg/well of 2 kDa PEI. To study if a combination of low- and high-molecular-weight PEI could improve transfection efficiency and reduce toxicity, we tested a mixture (1 : 1) of 2 kDa and 25 kDa PEI. Even though the 1 : 1 mixture was less cytotoxic, the efficiency of gene delivery was not improved. We conclude that, under our experimental conditions, the highest percentage of GFP-expressing cells with good viability was obtained when 1 μg of 25 kDa PEI was added per well. Therefore, branched 25 kDa PEI transfection represents an efficient, simple, and cost-effective alternative for gene delivery in bovine fibroblast cells in culture.

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