Trichostatin on production of genetically modified bovine embryos by somatic cell nuclear transfer

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Somatic cell nuclear transfer (SCNT) has been described as an alternative to generate genetically modified livestock animals once this method allows that cells be cultured for several passages, a requirement to establish lineages of genetically modified cells, can be used as nuclear donors. Nevertheless, cellular and molecular mechanisms involved on nuclear reprogramming are still unknown, reflecting in the low efficiency of SCNT in producing viable offspring. Moreover, long-term cell culture, required for establishment of genetically modified cell lineages, induces senescence and can impair nuclear reprogramming (Banito *et al*., 2009. Genes Dev, 23:2134-2139; Tat *et al*., 2011. Cell Reprogram, 13:331-344) and reduce embryo quality (Jang *et al*. 2004. Theriogenology, 62:512-521), despite viable bovine clones have already been born from cell cultured for several passages (Kubota *et al*. 2000. Proc Nat Acad Sci, 97:990-995). The modulation of nuclear reprogramming by chemical agents, like inhibitors of histone deacetylase and methylation, has been studied and can be useful for donor cells difficult to reprogram. One of histone deacetylase inhibitors is the trichostatin A, but studies with it produced controversial results. Some studies showed that exposing zygotes clones to 50 nM Trichostatin can increase bovine blastocyst production (Lee *et al*., 2011. J Reprod Dev, 57:34-42; Sawai *et al*., 2012. J Reprod Dev, in press), but such results were not repeated by others, which also did not find improvement on pregnancy and birth rates (Cui *et al*., 2011. Cell Reprogram, 13:179-189; Sangalli *et al*., 2012. Cell Reprogram 14:1-13). In previous study, we also observed that trichostatin treatment did not increase blastoscyst rate but reduced the index of apoptotic cells and end out with the birth of one animal (Camargo *et al*., 2011. Acta Sci Vet, 39(supl):S442); however, alteration on expression of genes important for development, like IGF2r and HMGN1, were found in blastocysts, suggesting that the reprogramming was not completely successful (Camargo *et al*., 2012. Reprod Fertil Dev, 24:121-122). Recently, we observed that the trichostatin treatment of zygotes reconstructed with genetically modified somatic cells after long-term culture (12 passages) increased the blastocyst rate (10.3±3.6% vs 26.7±3.8% for control [untreated zygotes] and zygotes treated with 50 nM trichostatin, respectively; unpublished data), contrasting with our previous results with zygotes reconstructed with non-transgenic cells with low-passage number (4-6 passages). The trichostatin treatment did not interfere on expression of reporter gene (GFP). This positive result with genetically modified donor cells may be due to the trichostatin effect on zygote reconstructed with cells cultured by long periods, as required for the establishment of the transgenic lineage. Therefore, the effect of trichostatin may be effective in zygotes clones reconstructed with cells difficult to reprogram, like those transgenic ones cultured for long-term and possibly close to senescence. Nevertheless, it is necessary to evaluate whether the increase on blastocyst production reflects on improvement of quality and pregnancy rate of transgenic clone embryos.

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