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20 ALTERED GENE EXPRESSION IN BOVINE SOMATIC CELL NUCLEAR-TRANSFERRED EMBRYOS AFTER TRICHOSTATIN A TREATMENT

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Abstract

Trichostatin A is a histone deacetylase inhibitor that improves histone acetylation and chromatin remodeling of somatic cell nuclear-transferred embryos (Iager *et al.* 2008 *Cloning Stem Cells* **10**, 371–379; Maalouf *et al.* 2009 *BMC Dev. Biol.* **9**, 11). We have previously observed that it also improves quality of bovine cloned embryos, which may increase pregnancy rates. This study aimed to evaluate the effect of trichostatin A treatment of zygotes on relative abundance of 9 transcripts in bovine nuclear-transferred blastocysts. *In vitro* matured oocytes were enucleated, fused to somatic cells and activated with ionomycin (Camargo *et al.* 2011 *Reprod. Fertil. Dev.* **23**, 122). After activation, putative zygotes were randomly separated into 2 groups: NT-TRICHO, zygotes were cultured for 4 h in 6-DMAP followed by 7 h in CR₂ aa medium plus with 2.5% fetal calf serum (FCS; Nutricell, Campinas, Brazil), both supplemented with 50 nM trichostatin A (Sigma); NT-CONT, zygotes were cultured in the same described conditions without trichostatin A supplementation. *In vitro*-fertilized embryos (IVF group) were used as a calibrator for relative transcript quantification. Embryos from the 3 groups were cultured in CR₂ aa supplemented with 2.5% FCS under 5% CO₂, 5% O₂ and 90% N₂ at 38.5°C. At 168 h postactivation, the embryos were rapidly frozen in liquid nitrogen. Pools of 10 blastocysts for each group were subject to RNA extraction and reverse transcription, in which cDNA was amplified by real-time PCR using the β -actin and GAPDH genes as endogenous references. The transcripts analysed encode high mobility group N1 (HMGN1), peroxiredoxin 1 (PRDX1), octamer-binding protein 4 (OCT4), insulin-like growth factor 1 and 2 receptors (IGF1r and IGF2r), glucose transporter 1 and 5 (GLUT1 and GLUT5), histone acetyltransferase (HAT) and heat shock protein 70.1 (HSP70) genes. Results were analysed by a pair-wise fixed reallocation randomization test using the REST software v.2. Data from NT-TRICHO and NT-CONT were compared with the IVF group and between themselves. The relative abundance of HSP70, PRDX1, IGF2r and HMGN1 transcripts was higher ($P < 0.05$) in NT-TRICHO compared with the IVF group and no difference was detected for the other transcripts. In the NT-CONT group, the relative abundance of IGF2r and HAT was higher ($P < 0.05$), whereas IGF1r and OCT4 were lower ($P < 0.05$) compared with IVF embryos. When data from NT-TRICHO and NT-CONT were compared, a higher amount ($P < 0.05$) of stress-associated transcripts (HSP70 and PRDX1) were found in NT-TRICHO blastocysts. These results suggest that although trichostatin A may improve chromatin remodeling, alterations on gene expression still persist in bovine somatic cell nuclear-transferred blastocysts in comparison with IVF embryos.

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