



PW1258 - Global methylation of bovine cells derived from amniotic fluid, adipose tissue and skin fibroblasts and its use in nuclear transfer in the presence or absence of Trichostatin A

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Trichostatin A (TSA) is an histone deacetylase inhibitor that increases the amount of acetylated histones as well as the demethylation of DNA [1], that can be used in cells less methylated to increase the production efficiency of embryos by nuclear transfer (NT). The aim of this study was to assess the status of overall methylation of three cell types and their role in bovine cloning in the presence or absence of TSA. Amniotic fluid cells were obtained from pregnant bovine Gyr and cultured in Amniomax Complete II medium (Gibco). Biopsies of adipose tissue and ear skin were collected from the same calf after birth. The cells were isolated by explant and cultured in Dulbeccos Modified Eagle Medium (Gibco). For NT, matured cumulus-oocyte complexes were enucleated and reconstructed using cells derived from adipose tissue (ATC) and amniotic fluid (AFC). Then, the cytoplasts were subjected to culture with 50 nM TSA for 20 h (TSA20), 25 h (TSA25) or in medium without trichostatin (WTSA). The control treatments were carried out with fibroblasts (FIB) without TSA and beyond the control parthenogenetic activation (PTN). The cleavage and production of blastocysts rates were compared by Tukey test ($p < 0.05$). Genomic DNA of all the cell types was extracted by salting out method according to [2] The global DNA methylation status was determined using the colorimetric MethylFlash methylated DNA quantification kit (Epigentek), and a descriptive analysis was done with the results. The global DNA methylation of AFC, ATC and FIB was 1.59, 2.09 and 0.43%, respectively. There was no statistical difference in cleavage rate when using AFC (91.18 ± 2.70 , 81.23 ± 15.65 , 92.36 ± 8.42) or ATC (87.24 ± 8.41 , 85.54 ± 3.88 , 83.18 ± 4.94) for TSA20, TSA25 or WTSA, respectively, when compared to FIB (71.97 ± 6.21) and PTN (93.89 ± 7.86). Similarly, blastocyst production rate on the seventh day of cultivation did not differ among treatments: AFC (38.06 ± 9.66 , 33.82 ± 14.23 , 45.39 ± 12.95) and ATC (36.20 ± 10.14 , 33.66 ± 12.84 , 32.70 ± 9.11) for TSA20, TSA25 and WTSA, respectively, and FIB (19.78 ± 18.30). It can be concluded that despite the bovine fibroblasts present a lower methylation rate than other cell types, embryonic production was similar regardless of the origin of the donor cell. And, the use of TSA for 20 h or 25 h did not contribute to increase the efficiency of production of cloned embryos. However, further studies with quality assessment and methylation and acetylation of embryos should be performed for a better understanding of the effects of TSA on clones.

[1] Lee M-J, Kim S-W, Lee H-G, Im G-S, Yang B-C, Kim N-H, et al. Trichostatin A promotes the development of bovine somatic cell nuclear transfer embryos. *J Reprod Develop* 2011; 57: 34-42.

[2] Wu H-C, Delgado-Cruzata L, Flom JD, Kappil M, Ferris JS, Liao Y, et al. Global methylation profiles in DNA from different blood cell types. *Epigenetics* 2011; 6: 76-85.