Improved in vitro development of cloned bovine embryos using S-adenosylhomocysteine, a non-toxic epigenetic modifying reagent.

ABSTRACT

In this study, fibroblast cells were stably transfected with mouse POU5F1 promoter-driven enhanced green fluorescent protein (EGFP) to investigate the effect of S-adenosylhomocysteine (SAH), the reversible non-toxic inhibitor of DNA-methyltransferases (DNMTs), at different intervals post-fusion on in vitro development of cloned bovine embryos. Treatment with SAH for 12 hr resulted in 54.6 ± 7.7% blastocyst production, which was significantly greater than in vitro fertilized embryos (IVF: 37.2 ± 2.7%), cloned embryos treated with SAH for 72 hr (31.0 ± 7.6%), and control cloned embryos (34.6 ± 3.6%). The fluorescence intensities of the EGFP-POU5F1 reporter gene at all intervals of SAH treatment, except of 72 hr, were significantly higher than control somatic cell nuclear transfers (SCNT) embryos. The intensity of DNA-methylation in cloned embryos treated with SAH for 48 hr was similar to that of IVF embryos, and was significantly lower than the other SCNT groups. The levels of H3K9 acetylation in all SCNT groups were significantly lower than IVF embryos. Real-time PCR analysis of gene expression revealed significantly higher expression of POU5F1 in cloned versus IVF blastocysts. Neither embryo production method (SCNT vs. IVF) nor the SAH treatment interval affected expression of the BCL2 gene. Cloned embryos at all intervals of SAH treatment, except for 24 hr, had significantly increased VEGF transcript compared to IVF and control SCNT embryos. It was suggested that the time interval of DNMT inhibition may have important consequences on different in vitro features of bovine SCNT, and the improving effects of DNMT inhibition on developmental competency of cloned embryos are restricted to a specific period of time preceding de novo methylation. Mol. Reprod. Dev. 78:576–584, 2011.

Keyword: S-adenosylhomocysteine; Cloned bovine embryos; DNA-methyltransferases; Green Fluorescent Proteins.