Tech-Trends

Volume 3 , Series 8

Nuclear reprogramming of cloned rabbit embryos

Trichostatin A and nuclear reprogramming of cloned rabbit embryos

Abstract: To investigate the influence of histone deacetylases on nuclear reprogramming after nuclear transfer, we treated the cloned embryos with a histone deacetylase inhibitor, Trichostatin A (TSA). In the present study, global changes in acetylation of histone H3-lysine 14, histone H4-lysine 12, and histone H4-lysine 5 were studied in rabbit in vivo fertilized embryos, somatic cell nuclear transfer (SCNT) embryos, and TSA treated SCNT embryos. From the pronuclear to the morula stage, the deacetylation-reacetylation changes in acetylation of histone H3-lysine 14 and histone H4-lysine 12 occurred in both fertilized embryos and TSA-treated cloned embryos; however, the distribution pattern in untreated cloned embryos failed to display such changes. More interesting, the signal of acetylation of histone H4-lysine 12 in cloned embryos was detected in both the inner cell mass and the trophectoderm, whereas TSA-treated cloned embryos showed the same staining pattern as fertilized embryos and the staining was limited to the inner cell mass. The histone acetylation pattern of TSA-treated SCNT embryos appeared to be more similar to that of normal embryos, indicating that TSA could improve nuclear reprogramming after nuclear transfer.

Electroporation Procedure:

Briefly, the zona pellucida of the cumulus-free oocyte was dissected by introducing a slit near the first polar body, and the cytoplasm containing the metaphase II spindle was squeezed out. Next, a donor cell was transferred into the perivitelline space of the enucleated oocyte. The couplets were then transferred to the fusion chamber containing fusion medium (0.25 *M* sorbitol, 0.5 m*M* HEPES, 0.1 m*M* $Ca(CH_3COO)_2$, 0.5 m*M* $Mg(CH_3COO)_2$, and 1 mg/mL of BSA), and fusion was induced by 2 direct-current pulses (1.4 kV/cm, 80 s each, 1 s apart). The fusion results were examined 30 min later, and fused couplets were activated by double DC pulses of 1.2 kV/cm for 20 s at 3 h after fusion. The activated embryos were then washed 3 times with M199 supplemented with 10% FBS and were used for further experimentation.

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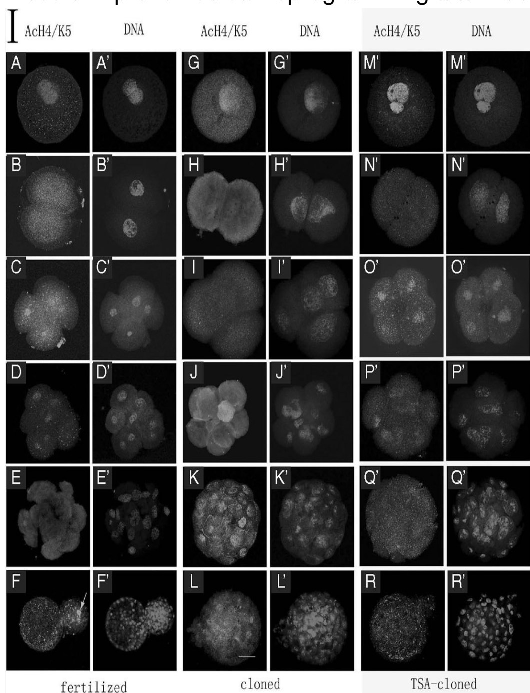

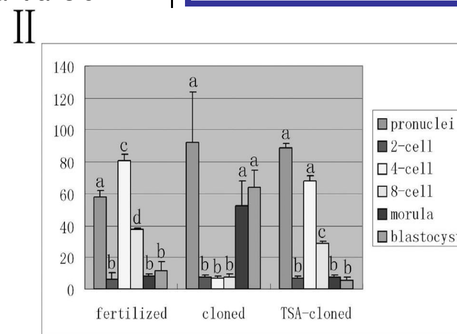

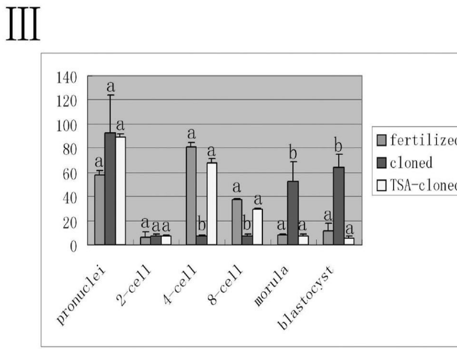

Figure 3. (I) Acetylation of lysine 5 on histone 4 (AcH4/K5) in rabbit fertilized (A, A′ to F, F′), cloned (G, G′ to L, L′), and Trichostatin A (TSA)-cloned (M, M′ to R, R′) preimplantation embryos. Embryos were immunostained with anti-AcH4/K5 (green) and DNA was stained with propidium iodide (red). The staining patterns in a one-cell embryo at the pronuclear stage (A, A′), at 6 h postactivation (G, G′), and at 6 h after TSA treatment during activation (M, M′); 2-cell stage (B, B′; H, H′; and N, N′); 4-cell stage (C, C′; I, I′; and O, O′); 8-cell stage (D, D′; J, J′; and P, P′); morula stage (E, E′; K, K′; and Q, Q′); and blastocyst stage (F, F′; L, L′; and R, R′) are shown, including the metaphase stage blastomere (arrow in F). Scale bar represents 20 m. (II) Total nuclear acetylation intensities in fertilized, cloned, and TSA-cloned embryos as quantified by using Image software (http://rsb.info.nih.gov/ij/). Each column represents the mean value of these intensities averaged on a per embryo basis. Different letters (a, b, and c) depict differences ($P < 0.05$) in the relative levels of histone acetylation across developmental stages within each embryo type. (III) Total nuclear acetylation intensities in fertilized, cloned, and TSA-cloned embryos as quantified by using ImageJ software. Each column represents the mean value of these intensities averaged on a per embryo basis. Different letters (a, b, and c) depict differences ($P < 0.05$) in the relative levels of global histone acetylation between embryo types at the same developmental stage.

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J Anim Sci 2008.86:1106-1113. doi: 10.2527/jas.2007-0718

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