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Theriogenology
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Theriogenology 65 (2006) 1704–1715
www.journals.elsevierhealth.com/periodicals/the

Anomalous mRNA levels of chromatin remodeling genes in swamp buffalo (*Bubalus bubalis*) cloned embryos

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Received 10 July 2005; accepted 25 September 2005

Abstract

The swamp buffalo (*Bubalus bubalis*) is a multi-purpose animal in agriculture that is challenged by extinction due to low reproductive efficiency. Nuclear transfer (NT) has been used to preserve special breeds of buffalo, as well as to increase the number of animals. However, cloned buffalo embryos have impaired development, as in other species. To understand the chromatin remodeling activities in cloned embryos and to improve NT technology, we examined the expression profiles of five genes involved in DNA and histone modifications, *DNMT1*, *DNMT3A*, *DNMT3B*, *HAT1* and *HDAC1*, in single swamp buffalo metaphase II oocytes, NT and in vitro fertilized (IVF) embryos from the two-cell to the blastocyst stage, by quantitative real time RT-PCR. We observed similar expression dynamics for all genes studied in the NT and IVF embryos: relatively constant levels of expression for all genes were found from the MII oocyte up to the eight-cell stage; the levels of mRNA for *HAT1* and *DNMT3B* continued to be stably expressed up to the blastocyst stage; while dramatic increases were seen for *DNMT3A* and *HDAC1*. Alternatively, the levels of *DNMT1* started to decrease at the eight-cell stage. Despite the similarity in the dynamics of gene expression, dramatic differences in the relative levels of these genes between NT and IVF embryos were observed. The expression levels of all DNA modifying genes were higher in the NT embryos than in the IVF

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doi:10.1016/j.theriogenology.2005.09.015

embryos at the eight-cell and blastocyst stages. The genes *HDAC1* and *HAT1* were also expressed significantly higher at the blastocyst stage in the NT embryos. Our results suggested differences in chromatin remodeling between NT and IVF embryos and that lower levels of DNA passive demethylation and higher levels of DNA de novo methylation occurred in the NT embryos. These observations are novel in the species of buffalo, and may be associated with developmental failure of cloned buffalo embryos due to the transcriptional repression effect of most genes studied here.

Keywords: DNMT; Embryo development; Epigenetic; Histone acetylases; Preimplantation