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Mitochondrial DNA Heteroplasmy in Cloned Bovine Embryos following Somatic Cell Nuclear Transfer

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Nuclear transfer (NT) has the potential to produce large number of identical progeny and would greatly benefit ongoing research efforts. Cloned animals produced by NT, however, may not be genetically identical to the donor cell. In NT procedures, nucleus genes originate from donor cell, and mitochondrial genes originate from recipient oocytes.

In this study, the fate of donor mitochondrial DNA were examined during preimplantation development after nuclear transfer in cattle. Isolated cumulus cells were used as donor cells in nuclear transfer. To evaluate the fate of donor mitochondrial DNA, nuclear transfer embryos were analyzed by Allele-specific PCR (AS-PCR), direct DNA sequencing, and DNA chromatography. AS-PCR analysis for detection of donor mitochondrial DNA was performed at the 1-, 2-, 4-, 8-, 16-cell, morula and blastocyst stages of the embryos. The mitochondrial DNA from donor cells was detected at all developmental stages of nuclear transfer embryos. However, mitochondrial DNA heteroplasmy was not observed in direct DNA sequencing of displacement-loop sequence from nuclear-transfer-derived blastocyst embryos. To confirm the mtDNA heteroplasmy in cloned embryos, the AS-PCR product from NT-derived blastocyst was analysed by DNA sequencing and DNA chromatography. The nucleotides of NT-derived blastocysts were in accordance with the nucleotides from donor cells. These results indicate that foreign cytoplasmic genome from donor cells was not destroyed by cytoplasmic event during nuclear transfer.

Key words) ***cumulus cells, mitochondrial DNA, nuclear transfer, cattle***