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Methylation Changes at the First Exon of Bovine *oct-4* Gene in Embryos Produced Either by *In Vitro* Fertilization or Somatic Cell Nuclear Transfer

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The relationship of Oct-4 to pluripotent cells is suggested by its tightly restricted expression pattern during embryonic development. Just prior to implantation it is limited to pluripotent cells of the inner cell mass (ICM) that will form the embryo proper but is not expressed in the trophectoderm, the structure that will form the extraembryonic tissues. In order for early cloned embryos to last their development, the Oct-4 expression should be allowed by donor genome, which may be supported by proper reprogramming of potentially repressive, donor-type methylation pattern. Here we examined a part (exon-1) of bovine *oct-4* sequence in preimplantation embryos for methylation status using bisulfite-sequencing technology. We observed a specific methylation pattern in the 4- to 8-cell embryos derived by in vitro fertilization (IVF); among the 11 CpG sites, two (CpG-7 and 10) were found to be methylated in most of the PCR clones. By blastocyst stage, however, this methylation became neatly erased. The same sequence showed no methylation in the genome of bovine sperm. Next, we examined whether a similar methylation change occurred in embryos reconstructed by nuclear transfer. Fetal fibroblasts (donor cells) have a unique methylation state (CpG-3 and 5) in this region, the pattern of which was different from that of IVF 4-/8-cell embryos. When located in enucleated ooplasm and subsequently cultured *in vitro* to the blastocyst, the donor genome had undergone a loss of the singular methylation pattern. Our results indicate that the donor-type methylation of the *oct-4* gene become modified, resulting in a state similar to that of normally fertilized counterparts, and probably suggest that functional restoration of *oct-4* gene that was inactived in somatic donor cells.

key words) ***oct-4, methylation, reprogramming. cloning.***