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Hypomethylation of DNA in Embryos from Stem Cell Nuclear Transfer in Pigs

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Epigenetic modification including genome-wide DNA demethylation is essential for normal embryonic development. Insufficient demethylation of somatic cell genome may cause various anomalies and prenatal loss in the development of bovine embryos from somatic cell nuclear transfer (SCNT) (Kang et al., Nat Genet 28:173-177, 2001). Species-specific differences in epigenetic status of cloned donor genome, such as a level of methylation in porcine SCNT embryos similar to that of normally fertilized embryos, have also been reported (Kang *et al.*, J Biol Chem 276:39980-39984, 2001). Even in normal preimplantation embryos the variation of epigenetic changes including intensity and temporal regulation of demethylation exists among mammalian species (Young and Beaujean, Anim Reprod Sci 82-83:61-78, 2004). In this study, appropriateness of porcine embryonic germ (EG) cells as karyoplasts for nuclear transfer with respect to epigenetic modification was investigated. These cells follow methylation status of primordial germ cells from which they originated, so that contain less methylated genome than somatic cells. The rates of blastocyst development were similar among embryos from EG cell nuclear transfer (EGCNT), SCNT and intracytoplasmic sperm injection (ICSI) (16/62, 25.8% vs. 56/274, 20.4% vs. 16/74, 21.6%). Genomic DNA samples from EG cells (n=3), fetal fibroblasts (n=4) and blastocysts from EGCNT (n=8), SCNT (n=14) and ICSI (n=6) were isolated and treated with sodium bisulfite. The satellite region

(GenBank Z75640) that involves nine selected CpG sites was amplified by PCR, and the rates of DNA methylation in each sites were measured by pyrosequencing technique (Biotage, Sweden). The average methylation degrees of CpG sites in EG cells, fetal fibroblasts and blastocysts from EGCNT, SCNT and ICSI were 17.9, 37.7, 4.1, 9.8 and 8.9%, respectively. The genome of porcine EG cells were less methylated than that of somatic cells ($p<0.05$), and active DNA demethylation occurred in embryos from both EGCNT ($p<0.05$) and SCNT ($p<0.01$). However, the degree of DNA methylation in EGCNT embryos was only one half of SCNT ($p<0.01$) and ICSI ($p<0.05$) embryos, while SCNT and ICSI embryos contained demethylated genome with similar degree. The present study demonstrates that porcine EG cell nuclear transfer resulted in hypomethylation of DNA in cloned embryos yet leading normal preimplantation development. However, it would be interesting to further investigate whether such modification affects long-term survival of cloned embryos.

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