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Effect of Defined KSOM Medium on the Development of 1-antitrypsin Transgenic Nuclear Transfer Bovine Embryos

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Production of α_1-antitrypsin (αAT) in transgenic cows has a great value in the field of medicine. The present study was conducted to determine the effect of chemically defined KSOM media on in vitro development of bovine transgenic nuclear transfer (NT) embryos. An expression plasmid for human αAT was constructed by inserting a bovine beta-casein promoter, a green fluorescent protein (GFP) marker gene, and a human αAT target gene into a pcDNA3 plasmid. Cumulus cells as donor nuclei in NT were collected from a Holstein cow and transfected by lipid-mediated method using FuGene6 (Roche Molecular Biochemicals, USA) as reagent. GFP expressed cumulus cells were introduced into recipient oocytes under DIC microscopy equipped with FITC filter set. After electrical fusion and chemical activation, reconstructed embryos were cultured in 1) SOF + 0.8% BSA, 2) KSOM + 0.8% BSA, 3) KSOM + 10% FBS and 4) KSOM + 0.01% PVA for 192 h at 39℃ with 5% CO_2, 5% O_2 and 90% N_2 in humidified condition. The development of the embryos was recorded and the GFP expression in blastocyst was determined under FITC filter. The average fusion rate was 73.8% (251/340; n=8). The development rates to 2-4 cells, morula, blastocysts and expression rates in blastocysts varied from 70.3 to 76.5%, 30.2 to 33.8%, 25.4 to 33.8% and 11.8 to 15.6%, respectively. The difference in development and expression rates of embryos among 4 culture groups was not significant ($P>0.05$). This study indicates that chemically defined KSOM medium is also able to support development of bovine transgenic NT embryos at similar rate of SOF or KSOM supplemented with BSA or serum.

Key words) ***nuclear transfer, transgenic, bovine, KSOM, human α-antitrypsin,***