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Production of Transgenic Rabbit Embryos by Reconstitution with Primordial Germ Cells Carrying Exogeneous Gene

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The aim of present study was to use primordial germ cells (PGCs) as a karyoplast carrying exogenous DNA in nuclear transplantation in order to produce transgenic embryos in rabbit.

New Zealand White rabbits were used throughout the study. Rabbit gonads were surgically collected from 20-d of fetuses, and incubated in trypsin solution. After 30 min of incubation, the gonads were transferred to 0.5 ml of DMEM + 15% FBS for dispersion by repeated pipetting with a micropipetter. The resulting cell suspensions were mixed with 12% Percoll in DMEM for separation on discontinuous gradients (12, 25, 30, and 45% Percoll). The cell suspensions were placed onto the top of 12% layer and centrifuged at 1,000 xg for 30 min. PGCs-rich fractions from 30% Percoll gradient was collected and washed by centrifugation in DMEM. Transfection of DNA fragment of mWAP/hGH (2.5 kb murine whey acidic protein promoter ligated to 2.1 kb human growth hormone gene, provided from Dr. M. Nishihara) into PGCs was made by liposome mediation. PGCs were frozen and kept in liquid nitrogen for storage until use. Donar does were superovulated with eCG and hCG treatments. Oocytes surgically collected from oviducts at 14 h post-hCG injection were stripped of cumulus cells by re-pipetting in a 3% sodium citrate solution. Oocytes with an extruded first polar body and dense cytoplasm were enucleated by micromanipulation in Hams F-10 medium supplemented with 7.5 g/ml cytochalasin B. Enucleation was confirmed under a fluorescence microscope after staining with 5 g/ml bisbenzimide for 2 min. Each enucleated oocyte was injected with a PGC transfected with the gene into ooplasm. All reconstructed eggs were activated with 5 M ionomycin, followed by incubation in a solution of cdc2 kinase inhibitor (1.9 mM sodium pyrophosphate, 3 h), and co-cultured with rabbit oviductal epithelial cells in

TCM199 + 10% FBS at 39C in 5% CO2 in air.

The rates of cleavage and of development into blastocysts were 65% (51/78) and 23% (18/78), respectively. Out of 11 blastocysts, 4 (36%) were analyzed positive by assessment of hGH gene expression with RT-PCR and Northern dot-blotting. Chromosome analysis showed that all 6 blastocysts analyzed were diploid.

The results indicate that PGCs is useful as a karyoplast for producing transgenic embryos. However, the viability in vivo of the resulting embryos remains to be established.

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