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Cloned embryos of different disease genes by somatic cell nuclear transfer

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We reconstructed cloned embryos of different genetic diseases by somatic cell nuclear transfer. We want to establish a system for basic researches and clinical therapies of these diseases. At first, we transferred human fetal fibroblast cells into 58 enucleated human oocytes and 21 cloned embryos were reconstructed by electrofusion. After physical and chemical activation, reconstructed embryos developed in vitro 2-cell embryos (20, 95.2%), 4-cell embryos (19, 90.5%), 8-cell embryos (13. 61.9%), 16-cell embryos (7, 33.3%), morula (6, 28.6%), and blastocysts (4, 19.1%), respectively. The STR loci of reconstructed embryos were the same as donor cells.

Embryos produced by inter-species nuclear transfer were performed by put human somatic cells into perivitelline space of enucleated rabbit oocytes. After electrofusion, we obtained 210 cloned embryos from 310 reconstructed embryos. Cloned embryos developed *in vitro* 2-cell embryos (188, 86.6%), 4-cell embryos (188, 86.6%), 8-cell embryos (188, 86.6%), 16-cell embryos (188, 86.6%), morula (141, 65%), and blastocysts (81, 37. 3%), respectively. We selected 18 reconstructed embryos randomly for amplification by human hu-alpgj and rabbit ra-wap probes. Results showed 16 out of 18 reconstructed embryos were hu-alpgj (+) and the other two were hu-alpgj (-), and all the embryos were ra-wap (-). 10 selected embryos were identified for STR-DNA, and the results showed STR loci of those embryos were consistent with donor cells. Furthermore, we obtained reconstructed embryos of different disease (Alzheimer's Disease, Parkinson's Disease, Spinal muscular atrophy, Diabetes, Tumor, *etc*). STR loci of different disease reconstructed embryos were the same as donor cells.

We also designed studies to explore gene expression associated development, DNA methylation, mitochondria, and telomerase activity.

All above researches were complied with International Convention against the Reproduction Cloning of Human Beings which was formulated by the united nations at 2002 and Guiding Principles on the ethics of human embryonic stem cell research which was formulated by The Ministry of Science and Techonlogy of the People's Republic of China and Ministry of Health of the People's Republic of China strictly. Donor voluntarily donated oocytes, somatic cells with no financial payment. Donors signed comprehensive informed consent documents and were informed that all donated materials were to be used for research and not for reproductive purposes.

Keywords: inter-species; intra-species; nuclear transfer; cloning; embryos

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Li Jianyuan, PhD; Professor; Supervisor of PhD Candidates. Director of Shandong Stem Cell Engineering Technology Research Center. Research Interests: In the field of human embryonic stem cell research, he has set up effective platform of nuclear transfer and reconstructed human embryos of different diseases. As a chief scientist, he has accomplished 2 "863" projects and took part in "973' project during the recent 5 years. He has published more than 20 papers at domestic and abroad and reported 3 patents. In addition, he also paid attention to human epididymal functional gene research.