Isolation of rabbit ES-like cells with human ES cell features

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World Rabbit Science

World Rabbit Sci. 2008, 16: 51 - 61

ABSTRACTS OF 2nd INTERNATIONAL MEETING ON RABBIT BIOTECHNOLOGY,
JOUY EN JOSAS. FRANCE. JUNE 2007.

Rabbit is more and more used as an experimental animal for the study of biological functions and of human diseases. The reasons of this development are multiple. Rabbits are closer to human than rodents. Rabbits are larger than mice and even rats thus more appropriate for some surgical operations. Cloning in rabbits is now possible and its complete genome is about to be sequenced. Moreover, rabbits are being used to produce pharmaceutical proteins at an industrial scale. A specific domain of rabbit biotechnology has become a reality. Expectedly, the techniques of biotechnology should be implemented in future to solve some specific problems of rabbit breeding and particularly for the struggle against diseases.

This urged Jianglin Fan to organize in Tsukuba the first meeting on rabbit biotechnology in 2005. This first success inclined to pursue the venture. The 2nd meeting thus took place in Jouy en Josas in June 2007. About 20 speakers and 40 participants attended the two days meeting. A major part of the presentations referred to models, transgenic or not, for the study of human diseases. The state of the art in the use of genetic markers, cloning and use of ES cells was depicted. The techniques for breed conservation (semen, embryo and ovary freezing) were shown. The summary of the presentations and posters are reported in this issue.

An important point is that a community involved in the development of rabbit biotechnology emerged during the meeting. It was decided to organize other meetings on rabbit biotechnology in future with the idea of not competing with the World Rabbit Meetings but to be complementary. The next rabbit biotechnology meeting should take place in China in 2009.

ISOLATION OF RABBIT ES-LIKE CELLS WITH HUMAN ES CELL FEATURES.

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Our research project aims at isolating and characterizing embryonic stem (ES) cell lines from rabbit embryos. ES cells are derived from the pluripotent epiblast of the peri-implantation embryo. They can self-renew, *i.e.* proliferate indefinetely *in vitro* while retaining pluripotency. Isolation of ES cell lines relies on the adaptation of epiblast stem cells to *in vitro* conditions supporting self-renewal. This has only been achieved in a limited number of species including mouse, primates and chicken. We have isolated and cultivated inner cell mass cells of rabbit blastocysts using optimized procedures for derivation of primate ES cell lines. Theses cultures are performed in presence of FGF2 and inactivated mouse embryonic fibroblasts. We routinely obtain flat colonies of compact cells with a high nucleus/cytoplasm ratio and proeminent nucleoli. These cells are similar to human ES cells. They are phosphatase alcaline positives and express the pluripotency *Oct4* gene. They proliferate very rapidly and, therefore, require passaging every two days. However, they differentiate spontaneously after six passages, a phenomenon associated with the loss of Oct4 expression. To prevent differentiation, we now aim to overexpress transcription factors involved in sustaining pluripotency in mouse and human ES cells, namely Oct4, Sox2 and Nanog. The first strategy makes use of SIV-derived lentiviral vectors to overexpress the human cDNAs in the inner cell mass cells. The second strategy is based on Tat-mediated protein transduction which allows reversible penetration of transcription factors into blastocyst cells. It makes use of the membrane penetration property of the Tat protein of HIV and has been shown to allow the entry of biologically active proteins into mammalian cells, including mouse ES cells, with high efficiency. Either strategies should prevent spontaneous differentiation of inner cell mass cells in culture and, therefore, increase the derivation of self-renewing ES cells.