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The Current Status in Rat and Horse Reproductive Biotechnology

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Importance of the Rat in Biomedical Research

Among laboratory mammals, the rat was the first domesticated species to be used in scientific research (Lindsey, 1979) and the species of rats that has been for most experimental research is the Norway rat (*Rattus norvegicus*). In 1903, William Bateson used the rat to show the concepts of Mendel's laws. In 1909, King established the first inbred rat strain, PA, and the first inbred mouse, DBA1, was also set up at the same year (see review, Jacob and Kwitek, 2002). Currently, according to the Rat Genome Database, 538 established inbred strains for complex traits are available (Lazar *et al.*, 2005).

In humans, to recapitulate the clinical outcome of diseases, rats serve as an important animal model, although species-specific differences exist. Further, they can provide access to clinically appropriate pathways, especially when little is known about the basis of a disease (Jacob and Kwitek, 2002). During the last 14 years there has been a constant increase in the use of the rat for genomic and genetic studies and nearly every drug has been tested in the rat before human application (Lazar *et al.*, 2005). Thus, most rat research is ultimately aimed at improving human health through the understanding of key genetic and physiological factors in common disease pathways.

The size of the rat provides better access for microsurgery (intravenous cannulation, vascularized organ transplantation), enables tissue and organ sampling (pituitary, area of the central nervous system), multiple sampling and *in vivo* function analyses. So far, most rat models have phenotypic characteristics that are relevant to a particular human condition (Jacob and Kwitek, 2002). These were initially induced surgically or pharmacologically, but eventually they were developed by phenotypic selection for certain traits, such as. hypertension (Rapp, 2000), and generating inbred strains; isolation of spontaneous mutants for human disease model, such as type I diabetes mellitus (Colle *et al.*, 1983; Mordes *et al.*, 1987), and transgenesis (Mullins *et al.*, 1990). In general, these models give a chance to advance biomedical research, but they do not always recapitulate

the clinical outcomes of human disease due to species-specific differences.

Nuclear-cytoplasmic Interactions in Rat Oocytes and Reconstructed Eggs Derived by Somatic Cell Nuclear Transfer

Somatic cell nuclear transfer (SCNT) enables the generation of transgenic animal models from genetically modified cells and it is a potential alternative to the ES cell technology, which is still not established yet in rats. However, there are many problems to resolve to establish stable protocols for SCNT in rats.

Soon after exposure to an *in vitro* environment, ovulated rat oocytes are activated spontaneously; this spontaneous oocyte activation is characterized by resumption of meiotic division followed by the cytoplasmic scattering of chromosomes. Neither *in vivo* aging in oviducts after ovulation nor hyaluronidase treatment affected spontaneous oocyte activation. L-type calcium channel blocker, IP3R inhibitor and inhibitor of calcium/calmodulin- dependent kinase II (CaMKII) prevented spontaneous oocyte activation in calcium-containing medium. The activity of CaMKII increased at 20 min and remained high for 30 min followed by decreased activity by 60 min after oocyte recovery. Constitutively active CaMKII was localized close to the meiotic spindle after oocyte recovery. Our findings indicate that rat oocytes are very sensitive to extracellular calcium *in vitro* conditions and CaMKII is one of the upstream signals that activate rat oocytes spontaneously after recovery.

Oocyte activationis an essential step in successful cloning by SCNT. In our study, oocytes were activated by electrical stimulation (EST) alone or in combination with 6-dimethylaminopurine (DMAP), cycloheximide (CHX)/cytochalashin B (CB), and roscovitine (ROS)/CB. All combination groups effectively induced inactivation of MPF activity. The patterns of MAP kinase varied in different treatment groups. DMAP induced faster inactivation of MAP kinase than CHX/CB and ROS/CB treatment groups. CHX/CB-treated oocytes showed synchronous nuclear breakdown and cleavage after activation treatment, whereas DMAP and ROS/CB treated groups showed asynchronous patterns. Although in vitrodevelopment to the blastocyst stage was efficient after parthenogenesis, development of SCNT-derived embryos was arrested at 2-cell stage in all regimens examined.

The procedure of micromanipulation, coordination of cell cycle between donor nuclei and recipient oocytes, and artificial oocyte activation are very important steps in the procedure for cloning animals. Metaphase II (MII) stage and pre-activated telophase II (TII) stage oocytes were used as a recipient cytoplasm with G0/G1, M, and S/G2-phases donor cells. Moreover, pronuclear and 2-cell stage blastomeres derived from SCNT were

used as donor cells with enucleated zygotic and parthenogenetic ooplasts for serial cloning. No significant difference in cleavage rate was observed among activation groups after SCNT. M-phase donor cells had a significantly higher cleavage rate than G0/G1-phase donor cells with MII oocytes and G2-phase donor cells with TII oocytes. However, no reconstructed embryo was able to develop beyond the 2-cell stage during in vitroculture. Moreover, reconstructed embryos cultured *in vivo*, i.e. after transfer to the oviduct of surrogate females, were also unable to develop further. To better understand the causes of developmental arrest, reconstructed 2-cell stage embryos were analyzed to examine the distribution of cytoskeletal proteins and transcription of mRNAs. Abnormal microtubule distribution and downregulated expression of several cytoskeletal transcripts were shown in 2-cell stage reconstructed embryos. These results indicate that the developmental arrest of rat SCNT embryos is associated with improper transcription of cytoskeleton genes, presumably resulting in abnormal microtubule distribution.

Reproduction in Horse

Measurement of Size of Ovaries, Follicles, and Coprora Lutea by Ultrasonography with Jeju Horse

The purpose of the present study was to set up basic information of size and status of ovaries by using ultrasonography to retrieve *in vivo* matured oocytes with ovum pick-up method. Ovaries were collected from the abattoir in Jeju in May and June which is breeding season. When the size of ovaries on ultrasonography was compared with real size measured by caliper, no significant difference was shown ($p<0.05$). The number of preovulatory follicles (>21 mm) was investigated withultrasonography and naked eyes. Ultrasonography group had 0.83 preovulatory follicles per ovary and naked-eye group had 0.75 preovulatory follicle per ovary and their average size was 2.86 cm and 2.34 cm, respectively. The average number of follicle was 4.25 with ultrasonography and 4.38 with naked eyes. There was no significant difference considering the size of follicle and number of follicle between ultrasonography and actual size except for the size of preovulatory follicle, suggesting that information of ultrasonography is able to use for OPU or other reproductive technology of mare.

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