P-81

Generation of the Recloned Puppy by Nuclear Transfer of Somatic Cell derived from a Transgenic Cloned Dog

So Gun Hong[1], Hyun Ju Oh[1,2], Jung Eun Park[1,2], Min Jung Kim[1,2], Ji Eun Kim[1,2], Goo Jang[1], and Byeong Chun Lee[1,2]

[1]Department of Theriogenology and Biotechnology,
[2]Brain Korean 21 Program for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul, Korea.

Introduction: The recloning method has been useful for multiple genetic modification or gene targeting in livestock animals. However, to our knowledge, no studies have been done on recloning in canine species. Moreover, the efficiency of recloning is still controversial. Recently, transgenic dogs with red fluorescence protein (*RFP*) were born by somatic cell nuclear transfer (SCNT), but one of two male dogs died after birth at eleven weeks due to bronchopneumonia. The objective of this study was to reclone the transgenic male dog with roscovitine-treated cells (the cycline-dependent kinase 2 inhibitor) for cell cycle synchronization.

Materials and Methods: During postmortem examination, muscle tissue was isolated from the *RFP* transgenic dog and donor fibroblasts were established from that tissue. Donor cells were cultured and divided into non-treated (control) and roscovitine treated groups. Each donor cell was placed into enucleated *in vivo* matured oocytes, fused by electrical stimulation, and activated with calcium ionophore and 6-dimethylaminopurine. One hundred seventy five re-cloned embryos from control (73) or roscovitine (102) group were transferred into oviducts of ten estrus synchronized surrogate dogs (six for control group and four for roscovitine group).

Results: Each one recipient from both groups was pregnant. One recipient from the control group aborted at around one month while the recipient in the roscovitine group maintained full term development. However, the recloned puppy was delivered dead on Day 62 after embryo transfer. Birth weight of the puppy was 530g and expressed ubiquitously *RFP* gene in the whole body. Microsatellite and mitochondrial (mt) DNA analyses indicated that genome of the puppy was from donor transgenic dog and the mtDNA was from the oocyte dog.

Conclusions: In summary, for the first time, this study demonstrated that a transgenic dog could be recloned from the donor cell treated with roscovitine. Further study will be needed for the generation of viable recloned transgenic dogs.

References

1. This study was financially supported by the Korean MEST, through KOSEF (grant # M10625030005-09N250300510), RNL Bio Co., Ltd., Nestlé Purina PetCare, and the BK21 program for Veterinary Science.