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論　文　内　容　要　旨

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学位論文題目	Studies on preservation of porcine zygotes for embryo production by interspecies somatic cell nuclear transfer (異種体細胞クローン胚作出のための豚配偶子の保存に関する研究)		

内容要旨

Reproduction is a characteristic of all living things and is a mechanism to maintain the species. In animal husbandry, reproduction plays an extremely significant role in many ways. For example, without reproduction, there is no proliferation of a herd, and thus no commercial cattle industry. Reproduction is also a mechanism to improve genetics and enhance livestock breeds. Therefore, the topic of reproduction has attracted significant attention from scientific researchers who are studying many technologies in this field.

Mammals are impressive organisms that have different morphologies with important characteristics allowing scientists to create animal model systems. The establishment of these animal systems is especially important for further investigations because it is exceedingly difficult to establish an appropriate model for research in complex animal species, including humans. In recent years, the major challenge for the field of reproductive biotechnology has been to explore the molecular and cellular mechanisms that are involved in controlling the quality of oocytes. The mammalian oocyte is a specific structure consisting of cytoplasmic organelles that communicate among themselves and are spatially associated. Thus, this thesis proposes the development of technologies for generating genetically modified pigs, specifically, and animals in general.

Chapter 3 reported the effects of 100 % fetal bovine serum (FBS) and 100 % porcine follicular fluid (pFF) as a storage medium for the development of porcine zygotes stored at 25 °C for 24 hours. Moreover, the study evaluated the additive effects of chlorogenic acid (CGA) in the storage medium. Results showed that 100 % of FBS was superior to BSA-containing TCM 199 as a storage medium for the storage of porcine zygotes at 25 °C for 24 hours. Moreover, the supplementation of 50 μM CGA to FBS has favorable outcomes on the post-storage development of zygotes, but the quality of embryos developed from stored zygotes decreased.

Chapter 4 investigated whether the removal of the ZP affects the development of porcine zygotes after their vitrification and warming and determined the appropriate volume of the corresponding medium for the individual culture of ZP-intact and ZP-free embryos and evaluated the protective effect of ZP during cryopreservation on the resulting development of

the vitrified-warmed zygotes. Results show that the volume of culture medium influenced the development of ZP-intact zygotes, and a volume of 15 μL was most suitable for their development. However, the volume of the culture medium did not modify the development of ZP-free zygotes. The removal of the ZP before vitrification did not adversely affect embryonic development or quality of the resulting blastocysts.

Further, chapter 5 purposed to examine the feasibility of using domestic elephant fibroblast cell injection electrofusion for interspecies somatic cell nuclear transfer (iSCNT) to produce elephant embryos. Interspecies somatic cell nuclear transfer (iSCNT) is an invaluable tool for studying nucleus-cytoplasm interactions and may provide an alternative for cloning endangered animals whose oocytes are challenging to obtain. The development ability of iSCNT embryos decreases with increases in taxonomic distance between the donor cell and recipient species. In this study, more than 69 % of the domestic elephant fibroblast cells successfully fused with the porcine oocytes following electrofusion, and 0.6 % of embryos were able to reach the blastocyst stage. This is the first reported demonstration of using SCNT to reprogram elephant somatic cell nuclei that can develop to the blastocyst stage.

In conclusion, this study showed that porcine zygote could be stored by TCM-199, pFF, or FBS medium at 25 °C for 24 hours, in which the supplementation of 50 μM CGA to FBS has favorable outcomes on the post-storage development of zygotes. The porcine zygote could develop with the removal of the ZP before vitrification. The pig oocytes used in this study supported the remodeling and reprogramming of the elephant somatic cell nuclei. The SCNT technique developed in this study may soon be used for the mass production of cloned elephant embryos.