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学位論文題名

The complex roles of autophagy and lysosome-cathepsin on the oocyte maturation and preimplantation embryo development in cattle

（ウシ卵子成熟および初期胚発生に及ぼすオートファジーおよびリソソームカテプシンの複合的影響）

This thesis consists of 126 pages including 6 chapters, 33 figures, 3 tables, and 100 reference lists. Two reference papers are also attached.

Assisted reproductive technologies (ART), such as *in vitro* fertilization (IVF), offers great potential for improving the productivity of domestic animals. However, the overall efficiency of *in vitro* produced (IVP) embryos remains lower than *in vivo* produced embryos. Therefore, it is necessary to assess embryo quality prior to embryo transfer and improving the quality and quantity by understanding the molecular and cellular functions. Many approaches have been achieved to evaluate the oocyte quality and developmental competence of oocytes. Recent studies have revealed the important roles of lysosomal cathepsin B (CTSB) and autophagy in development of preimplantation embryos. However, little is known about the role of lysosome-cathepsin and autophagy on the quality and developmental competence of oocytes. This study, I investigated the roles of lysosome-cathepsin and autophagy on the oocyte maturation and preimplantation embryo development in cattle.

1. Relationship between the quality and autophagic activity in bovine oocytes

Bovine cumulus oocyte complexes (COCs) collected from ovaries were separated to two groups as good and poor quality according to their morphological criteria. Then good and poor COCs were subjected to *in vitro* maturation (IVM), IVF followed by *in vitro* culture (IVC) for 8 days. After confirmation that lower total cell number and higher TENEL positive cells of blastocyst derived from poor quality COCs than good quality COCs, then, autophagy activity of oocytes was detected with fluorescent dye both in poor and good oocytes. Autophagy activity was significantly lower in poor quality oocytes than good quality oocytes. Immunostaining of LC3, an autophagy protein marker representing the autophagosomes also revealed lower number of LC3-stained particles in poor quality of oocytes than good. Autophagy inhibition decreased developmental competence together with increase in CTSB activity and apoptosis-related gene expression. These results suggest that the quality and developmental competence is correlated with autophagy associated with CTSB in bovine oocytes.

2. Effect of regulation of autophagy and CTSB on the developmental competence of good and poor quality bovine oocytes

In the previous study, autophagy and CTSB have revealed to have crucial roles on developmental competence of COCs. Thus, experiment was conducted to investigate the effect of autophagy induction and CTSB inhibition by using rapamycin (Rapa), an autophagy inducer, E-64, a CTSB inhibitor, and combined administration in poor quality COCs during IVM. Rapa significantly increased the autophagy activity of poor quality oocytes. CTSB inhibition by E-64 also increased autophagy activity together with autophagy related gene expression. These results suggest that autophagy induction is regulated not only by Rapa, but also by CTSB mediated pathway. Combined administration of Rapa and E-64 significantly increased blastocyst rate, total cell number, and decreased TUNEL-positive cells both in good and poor quality COCs. These results suggest that autophagy induction has a potential rescue effect on developmental competence of poor quality oocytes, and synergetic effect of CTSB inhibition and autophagy induction during IVM also have promoting effect on developmental competence both in poor and good quality oocytes.

3. Effect of regulation of autophagy and CTSB on developmental competence of bovine oocytes collected from transported ovaries

Recent trend on the increased demands of IVP embryos used for embryo transfer has revealed the lack of supply of oocytes and ovaries. Thus, ovarian retrieve from abattoir of distant place became important. However, quality of COCs from long time preserved ovaries decreased causing low developmental competence. Therefore, improving the developmental competence of preserved COCs is an urgent issue. The present research was conducted to investigate the effect of autophagy and CTSB regulation on developmental competence of bovine oocytes collected from transported ovaries. COCs collected from preserved and transported ovaries at 20°C for 24h were used for IVM with or without Rapa and/or E-64, followed by IVF and IVC. Developmental competence of COCs from transported ovaries showed a low blastocyst rate after IVM, IVF and IVC compared with freshly collected COCs. Single administration of E-64 and Rapa did not affect blastocyst rate, however, mixed supplementation of E-64 and Rapa significantly increased the blastocyst rate with increased number of total cell number. These results indicate the synergetic effect of regulation of CTSB and autophagy can improve embryo development derived from long-transferred ovaries.

In conclusion, autophagy can be a one of potential marker for evaluation oocyte quality, and the synergetic effect of CTSB inhibition and autophagy induction can contribute to improve quality and developmental competence of oocytes for embryo transfer in cattle.

Therefore, we acknowledge that Mr. Jianye LI is qualified to be granted the Degree of Doctor of Philosophy in Agriculture from Hokkaido University.