（学位第３号様式）

学 位 論 文 要 旨

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題　目	Improvement of *in vitro* embryo production by controlling endoplasmic reticulum stress and autophagy in cattle （小胞体ストレスおよびオートファジー制御によるウシ体外胚生産系の改良に関する研究）

The aim of this study was demonstrated the stress-associated developmental events and the effects of stress-modulating agents on *in vitro* development and embryo quality in cattle. We first investigated the regulation of oxidative stress by supplementing sericin, a potential antioxidant during *in vitro* culture (IVC) on embryo developmental competency and quality. The results show that sericin significantly decreased reactive oxygen species (ROS) and apoptosis, and increased the mitochondrial activity and developmental rate of embryos under oxidative stress induced by heat shock treatment. More importantly, apoptosis and transcript abundance for HSPA1A and BAX were significantly decreased but IFNT2 level was increased in blastocysts obtained from sericin than in the control. These findings indicate that sericin supplementation during IVC is useful for production of embryo with high tolerance for oxidative stress. Next, we examined effects of endoplasmic reticulum (ER) stress during IVC on developmental kinetics and cryo-tolerance in embryos. For this purpose, tauroursodeoxycholic acid (TUDCA) and/ tunicamycin (TM), an ER stress inhibitor and inducer respectively were supplemented during IVC. As a result, treatment of TUDCA restored the detrimental effects of TM-induced ER stress impairments in embryos development and quality. In addition, addition of TUDCA significantly suppressed ROS generation, apoptosis and expression of ER stress markers (*GRP78, ATF4, ATF6, IER1, sXBP1, CHOP* and *BAX)*; while it increased anti-apoptotic *BCL2* gene and glutathione levels compared to the control. Moreover, ER stress inhibition via TUDCA enhances embryo cryo-tolerance after vitrification. Based on these findings, we conducted a follow-up study, to examine whether ER stress attenuation via TUDCA during *in vitro* maturation influences oocyte developmental competences. The results show that addition of TUDCA during IVM significantly decreased ROS, apoptosis and ER stress-induced protein/genes level in matured COCs; thereby increases the maturation rate, and subsequent embryos development in vitro. Collectively, these findings suggest that controlling ER stress during IVM or IVC improves *in vitro* embryo development with high cryo-tolerance. Finally, we explore whether the induction of embryo autophagic activity during culture influences preimplantation development and embryo quality at the genomic level. To examine this possibility, rapamycin was used to induce, while wortmannin was used to suppress autophagic activity in embryos during IVC. Surprisingly, we found that autophagy is highly activated in embryos at 4-cell stage by rapamycin treatment, as evidenced by significant upregulation of autophagy triggering molecules (*LC3, ATG5, ATG7*) and suppression of *mTOR* expression, and rapid maternal mRNA degradation. Further, induction of autophagy influences the blastocyst outcomes and activates many development-related genes (*BCL2, MnSOD, SOX2, POU5F1, NANOG, PLAC8, IFN-tau,* and *GLUT5)* in blastocysts that represent embryo quality. In our knowledge, for the first, this research will focus on regulation of autophagy with rapamycin during bovine embryogenesis improves embryo quality at the genomic level. In conclusion, these findings suggest that an appropriate balance between oxidative and ER stress along with regulation of autophagy during culture influences in vitro early embryogenesis and may contribute to the development of strategies for the production of bovine blastocysts with high developmental competence.