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Roles of autophagic and lysosomal cathepsin status on preimplantation development of bovine embryos

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1. Background and Objective

In vitro embryo production, offers great potential for improving the productivity of domestic animals. However the mechanism of development and differentiation of preimplantation embryos is still unclear. Recent research has revealed the important roles of lysosomal cathepsin and autophagy in preimplantation embryo. Therefore, the aim of my study is to investigate the roles of autophagic and lysosomal cathepsin status during oocyte maturation and preimplantation development of bovine embryos.

2. Methods

Full grown germinal vesicle (GV) oocytes, Metaphase II (MII) eggs and IVP preimplantation embryos at different developmental stages (1-, 2-, 8-cell, morulae and blastocyst) as well as separated trophectoderm (TE) and inner cell mass (ICM) were sampled. In addition, trophectoderm (TE) and inner cell mass (ICM+TE) samples were sampled by dissection of blastocyst. Experiment 1; qRT-PCR was employed to compare mRNA transcript abundance of *CTSB* and *Beclin 1* among different stages. Experiment 2; Enzymatic activity and protein localization of CTSB and lysosomal distribution were evaluated using fluorescent substrate and immunocytochemistry. Experiment 3; Autophagy activity was also evaluated using fluorescent staining.

3. Results and Discussion

1)The levels of *CTSB* and *Beclin 1* transcript were significantly higher in MⅡ followed by a significant decrease after 8-cell. Moreover, within the blastocyst, *CTSB* transcript showed significantly higher expression in TE than ICM.

2) Consistent with the qRT-PCR profile of CTSB, CTSB activity supported by lysosomal distribution showed the highest activity just after fertilization (1-cell embryo) and at the morula among different stages. These findings were supported by immunocytochemistry where CTSB protein was clearly localized and significantly increased in morulae and blastocysts stages. However, interestingly in contrast to qRT-PCR result, CTSB activity and protein expression were significantly higher in ICM than TE both in intact and separated blastocysts.

3)Autophagic activity followed the same pattern of CTSB and exhibited the highest activity just after fertilization (1-cell embryo). Again, similar to CTSB activity, autophagic activity was significantly higher in ICM when compared to the corresponding TE. These findings suggest that maternal CTSB and autophagy might have essential roles at the start point of both embryonic development and differentiation.

4. Conclusion

Taken together, these results suggest that autophagy-lysosomal CTSs may have a pivotal role during embryonic developmentand differentiation.