学 位 論 文 内 容 の 要 旨

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Regulatory Role of the Circadian Clock System in Luteal Regression and Cell Death Pathways in Cattle

（ウシにおける概日時計システムの黄体退行および細胞死経路に対する制御機構）

Against the backdrop of a growing global population and the demand for food resources, beef and dairy products serve as critical sources of high-quality protein amid dietary transitions. Globally, cattle are prized for their genetic versatility, production efficiency, and economic value, and support global food security. However, the cattle's current reproductive inefficiency threatens supply stability. Approximately 30% of global cows fail to reproduce due to abnormal luteal function (such as delayed or incomplete degeneration), causing over $20 billion in annual livestock losses, particularly in intensive breeding systems. Therefore, it is necessary to improve the reproductive efficiency of cattle.

The corpus luteum (CL) is a transient endocrine structure that secretes progesterone (P4) following ovulation and plays a crucial role in regulating the estrous cycle and maintaining pregnancy in mammals. Disruptions in luteal function can lead to irregular estrous cycles, impaired implantation, and reduced fertility, ultimately decreasing reproductive efficiency in herd management systems. Luteolysis, the structural and functional degradation of the CL, is mediated by programmed cell death pathways such as apoptosis and autophagy. The circadian clock (CC) system has been extensively studied as a mechanism for regulating physiological functions in a rhythmic and time-dependent manner. However, its involvement in rapid and acute tissue remodeling processes, such as CL regression, remains poorly understood. In particular, whether and how core clock components such as the nuclear receptor subfamily 1 group D member 1 (*NR1D1*) contribute to the regulation of apoptosis and autophagy during the short timescale of luteolysis remains unclear. This study will explore the mechanism of luteal regression from the perspective of the CC, providing potential solutions for improving the reproductive efficiency in cattle, especially its interaction with the mechanisms related to autophagy and apoptosis.

Firstly, I collected fresh CL samples and analyzed the dynamic expression patterns of P4, steroidogenic enzymes, and CC components during the four stages of the estrus cycle (early, middle, late, and regression). The results showed that P4 secretion followed a distinct unimodal pattern throughout CL development, peaking at the middle stage and subsequently declining, with the lowest content in the regression stage. These changes paralleled the expression profiles of key steroidogenic genes, including *STAR*, *CYP11A1*, and *HSD3β*. Additionally, similar expression patterns were shown from the CC system

genes (*NR1D1*, *BMAL1*, *CLOCK*, *PER2*, *CRY1*, *DBP*). The protein expressions of NR1D1 and BMAL1 are synchronized with their genes.

Then, I established an *ex vivo* model of luteolysis induced by $PGF_{2\alpha}$ to evaluate the functional and molecular markers of CL regression, including apoptotic and autophagy pathways. The significantly reduced P4 confirmed the successful establishment of the *ex vivo* CL regression model. Further results demonstrated increased apoptotic (*CTSB*, *CASPASE8*) and autophagic (*LC3*, *ATG5*) factors, with CTSB showing the most marked upregulation in CL tissues by $PGF_{2\alpha}$ Treatment. In contrast, the expression of CC-related genes and proteins was not statistically significant, although $PGF_{2\alpha}$ treatment tended to suppress *NR1D1* gene expression and BMAL1 protein levels.

To investigate the specific involvement of NR1D1, pharmacological activation or inhibition was carried out using GSK4112 (an agonist of NR1D1) and SR8278 (an antagonist of NR1D1), revealing opposite effects on P4 synthesis and cell death markers. The results show that both treatments significantly reduced P4 levels, and GSK4112 treatment led to downregulation of *CYP11A1* expression. More obvious expression changes of NR1D1, along with upregulation of the autophagy-related gene *ATG5* and the apoptosis-related gene *CTSB* in the GSK4112 treatment group. And produced the opposite trends in these markers after SR8278 treatment.

Finally, the $PGF_{2\alpha}$-induced model was used in combination with SR8278 to evaluate whether the NR1D1 antagonist could counteract luteinization. Importantly, I observed that the inhibition of NR1D1 significantly restored P4 synthesis in $PGF_{2\alpha}$-induced CL tissues, particularly in the culture medium, and increased the expression of the steroidogenic gene *STAR*. Furthermore, the lysosomal protease CTSB showed an opposite trend in the $PGF_{2\alpha}$ + SR8278-6h and $PGF_{2\alpha}$ + SR8278 groups, while LC3 expression increased, suggesting modulation of autophagic activity.

Collectively, these results suggest that the regulatory role of the CC system, particularly NR1D1, plays a critical role in the regulation of CL function and regression. This mechanism may contribute to improving reproductive efficiency and productivity in cattle, thereby promoting a more stable and efficient supply of animal-based food products such as milk and meat. As a result, it could support the sustainability and resilience of livestock production systems in response to increasing global demand.