学 位 論 文 審 査 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)

Amid a growing global population and increasing demand for food resources, beef and dairy products remain vital sources of high-quality protein, particularly during transitions in dietary habits. Globally, cattle are valued for their genetic versatility, production efficiency, and economic importance, playing a crucial role in ensuring food security. However, the current low reproductive efficiency of cattle poses a significant threat to supply stability. Approximately 30% of cows worldwide fail to conceive due to abnormalities in luteal function—such as delayed or incomplete luteolysis—resulting in estimated annual economic losses exceeding $20 billion, particularly in intensive breeding systems. Therefore, enhancing reproductive efficiency in cattle has become an urgent priority.

The corpus luteum (CL) is a transient endocrine structure that secretes progesterone (P4) following ovulation and is essential for regulating the estrous cycle and maintaining pregnancy in mammals. Dysfunction of the CL leads to irregular estrous cycles, implantation failure, and reduced fertility, ultimately diminishing reproductive efficiency in herd management. CL regression, or luteolysis, is characterized by both structural and functional degradation of the CL and is mediated by programmed cell death mechanisms, including apoptosis and autophagy.

The circadian clock (CC) system has been extensively studied for its role in regulating rhythmic physiological functions across various tissues. However, its involvement in rapid and acute tissue remodeling processes such as CL regression remains poorly understood. In particular, the regulatory roles of core clock components—such as nuclear receptor subfamily 1, group D, member 1 (NR1D1)—in modulating apoptosis and autophagy during the short time frame of luteolysis remain unclear.

I investigated the mechanisms underlying CL regression from the perspective of the circadian clock, with a particular focus on NR1D1 and its potential role in regulating apoptosis and autophagy. The aim is to provide new insights into improving reproductive efficiency in cattle through molecular regulation of CL function.

First, fresh CL samples were collected across the four stages of the estrous cycle (early, mid, late, and regressed). The dynamic expression patterns of P4, steroidogenic enzymes, and CC components were analyzed. Results showed a clear unimodal pattern of P4 secretion, peaking at the mid-luteal

stage and declining significantly during the regression phase. This trend was mirrored by the expression levels of key steroidogenic genes, including STAR, CYP11A1, and HSD3β. Similarly, core circadian clock genes such as NR1D1, BMAL1, CLOCK, PER2, CRY1, and DBP displayed parallel expression dynamics, with NR1D1 and BMAL1 proteins synchronizing with their respective gene expression profiles.

Next, an *ex vivo* model of CL regression was established using PGF2α to induce luteolysis, and both functional and molecular markers were evaluated. Significant reductions in P4 confirmed the successful induction of regression. Additionally, expression levels of apoptotic markers (CTSB, CASPASE8) and autophagy-related factors (LC3, ATG5) were upregulated, with CTSB exhibiting the most prominent increase. In contrast, expression of CC-related genes and proteins was not statistically significant; however, PGF2 α treatment showed a tendency to suppress NR1D1 gene expression and BMAL1 protein levels.

To further explore the role of NR1D1, pharmacological activation and inhibition were performed using GSK4112 (NR1D1 agonist) and SR8278 (NR1D1 antagonist). Both treatments significantly reduced P4 levels. GSK4112 treatment led to downregulation of CYP11A1 and more pronounced expression changes in NR1D1, along with upregulation of ATG5 (autophagy) and CTSB (apoptosis). In contrast, SR8278 treatment induced opposing trends in these markers, indicating that NR1D1 activity modulates cell death-related pathways.

Finally, the PGF2α-induced CL regression model was used in combination with SR8278 to examine whether NR1D1 inhibition could counteract luteolysis. Notably, NR1D1 inhibition significantly restored P4 production in PGF2α-treated CL tissues, especially in the culture medium, and enhanced the expression of the steroidogenic gene STAR. Furthermore, CTSB expression was suppressed, while LC3 expression increased, suggesting a shift in autophagic activity.

Collectively, these findings demonstrate that the circadian clock, particularly the function of NR1D1, plays a critical role in regulating CL function and regression. Modulating NR1D1 activity could provide a novel approach to understanding the control of CL function and manipulating reproductive efficiency and fertility in cattle. This insight may contribute to the development of advanced strategies for enhancing the stability and productivity of livestock systems, thereby supporting sustainable animal protein supply chains in the face of growing global food demand.

Therefore, we acknowledge that the author is qualified to be granted the Degree of Doctor of Philosophy in Food Resources from Hokkaido University