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Quantitative PCR Analysis of the CYP11A1 Gene in Cattle with Persistent Corpus Luteum

Uji Kuantitatif PCR Gen CYP11A1 pada Sapi yang Mengalami Corpus Luteum Persisten

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Naskah diterima:

Abstrak

Corpus luteum adalah kelenjar endokrin sementara yang terbentuk dari pecahnya folikel setelah ovulasi, yang sangat penting untuk memelihara kebuntingan pada mamalia. Corpus luteum persistent (CLP) adalah keadaan dimana corpus luteum tidak mengalami regresi dan tetap tinggal pada ovarium dalam waktu yang lama sehingga menjadi salah satu penyebab penurunan tingkat produktivitas sapi betina karena dapat menyebabkan gangguan pada reproduksinya. Gejala klinis dari CLP yang sering terlihat adalah sapi mengalami anestrus dalam waktu yang lama sehingga proses reproduksi menjadi terhenti. Gen CYP11A1 diketahui memiliki peran dalam regulasi hormon prostaglandin yang berperan penting untuk regresi corpus luteum (CL). Penelitian ini bertujuan untuk melihat perbedaan secara kuantitatif gen CYP11A1 pada sapi normal dan CLP. Hasil penelitian menunjukkan terdapat peningkatan ekspresi gen CYP11A1 pada sapi yang mengalami CLP dibandingkan normal. Hal ini diharapkan dapat memberikan informasi tentang potensi CYP11A1 sebagai marker untuk diagnosa sapi yang mengalami CLP sehingga dapat digunakan sebagai marker seleksi sapi betina produktif atau unggul.

Kata Kunci: Corpus Luteum Persisten; CYP11A1; Gangguan Reproduksi; PCR Kuantitatif; Sapi

Abstract

The corpus luteum is a temporary endocrine gland formed from the ruptured follicle after ovulation, which plays an essential role in maintaining pregnancy in mammals. Persistent corpus luteum (PCL) is a condition in which the corpus luteum fails to regress and remains in the ovary for a prolonged period, thereby becoming one of the causes of reduced productivity in female cattle due to reproductive disorders. The clinical symptom commonly observed in PCL is prolonged anestrus, causing the reproductive process to cease. The CYP11A1 gene is known to play a role in the regulation of prostaglandin hormones, which are important for corpus luteum (CL) regression. This study aimed to quantitatively evaluate the CYP11A1 gene in cattle affected by PCL. DNA band analysis was performed to determine the presence of this gene in PCL cattle. The results showed an increased expression of the CYP11A1 gene in cattle with PCL compared to normal cattle. These findings are expected to provide information regarding the potential of CYP11A1 as a marker for the diagnosis of cattle with PCL, so that it may be used as a selection marker for productive or superior female cattle.

Keywords: Cattle; CYP11A1; Persistent Corpus Luteum; Reproduction Disorder; Quantitative-PCR

Introduction

Fertility is a key factor in determining the reproductive performance of female cattle. Fertility is defined as the ability of the reproductive organs to function optimally to support the reproductive process (Priyo et al., 2020). Reproductive disorders can lead to population decline in cattle. One of the most common reproductive disorders in cattle in Indonesia is persistent corpus luteum (PCL). Persistent corpus luteum is a condition in which cattle retain the corpus luteum for a long time due to failure to regress. This condition is caused by several factors, such as postpartum infection, hormonal imbalance, and drug use. It is also caused by inadequate postpartum cattle care, which makes cattle susceptible to uterine infections (Hagman, 2022; Ratnani, 2020). This disorder is also associated with the failure of the pituitary gland to release sufficient luteinizing hormone to trigger ovulation (Dirja, 2024).

In cases of PCL, failure of CL regression by the hormone PGF2α can be caused by inflammation of the endometrial wall (Magata et al., 2012). Persistent corpus luteum is also caused by the failure of the pituitary gland to release sufficient amounts of luteinizing hormone to cause ovulation (Dirja, 2024). Hormonal imbalances, such as increased estrogen or progesterone levels, also disrupt the normal mechanism of corpus luteum regression, thus prolonging the luteal phase (Hagman, 2022). Several genes are known to play a role in the hormonal regulation of inflammatory responses and CL degeneration during the reproductive cycle of cattle. One such gene is the CYP11A1 gene, which encodes the enzyme P450scc, an enzyme involved in the biosynthesis of progesterone from cholesterol (Miller, 2017). This enzyme plays a crucial role in regulating progesterone levels, which influence the estrous cycle, luteal cycle, and fertility in cattle (Miller, 2017).

The molecular mechanisms involved in PCL are still not fully understood. Some examples of genes that have been studied in association with PCL in cattle include genes related to the proliferation and differentiation of endometrial cells, such as the cyclin D1 gene and the Bcl-2 gene. However, no studies have investigated the molecular mechanisms of the CYP11A1 gene. Therefore, this study aimed to quantitatively measure the CYP11A1 gene in cattle with normal cycles and cattle experiencing luteal phase disorders, specifically PCL, using qPCR techniques. This study is expected to provide basic information regarding the molecular role of CYP11A1 in the occurrence of PCL in cattle and evaluate its potential as a candidate biomarker for PCL cattle.

Materials and Method

This research was conducted from April to August 2025 at the Biotechnology Laboratory of PAU UGM, the Reproduction and Obstetrics Laboratory of FKH UGM, the Mancasan Slaughterhouse (RPH), UP2KH, and the Faculty of Animal Husbandry UGM. The main materials for this study were ten blood samples (5 normal and 5 PCL) from female cattle obtained from the slaughterhouse that had a normal pregnancy cycle or PCL based on the Reproductive Status Certificate. The research stages carried out were ultrasound examination by a veterinarian, sample collection, RNA extraction, electrophoresis, quantitative PCR testing, and data analysis.

RNA from blood samples was extracted using the Zymo Quick-RNA Mini Prep Kit (Zymo Research, USA) according to the manufacturer's protocol. Blood samples were mixed with DNA/RNA Shield and Proteinase K, then incubated and centrifuged to obtain the supernatant. RNA was purified using a Zymo-Spin™ column through binding, washing, and DNase I treatment to remove DNA contamination. RNA was then eluted using DNase/RNase-Free Water and stored at −80°C. The next step is cDNA synthesis from RNA, which is carried out according to the Excel RT™ Reverse Transcription Kit II manufacturer's protocol. The quality of the obtained cDNA is then confirmed using conventional PCR techniques with an internal control primer for β-actin (ACTB) housekeeping gene. This was done by mixing 5 µl Go-Taq PCR Master Mix, 0.5 µL ACTB forward primer, 0.5 µL ACTB reverse primer, 1 µL cDNA template and the reaction was made up to 10 µL with nuclease-free water in a 0.2 ml PCR tube. The reaction was run for 30 cycles with the following conditions: pre-denaturation at 94°C for 1 minute, denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 1 minute and final extension for 5 minutes. The PCR results were then checked by 1.5% agarose gel electrophoresis.

The next stage, the quantitative PCR (qPCR) test, was carried out according to the manufacturer's protocol for the Excel-Taq™ 2X Q-PCR Master Mix (SYBR, NO-ROX) SMOBIO kit. The relative gene

expression obtained was evaluated using the 2^-ΔΔct method (Livak method) (Livak & Schmittgen, 2001). Data normality was tested using the Shapiro-Wilk test. Furthermore, the Mann-Whitney test was performed to determine the significance of the differences in expression between normal and PCL cattle. A p value <0.05 was considered statistically significant. All statistical tests were performed using SPSS software version 22.

Results and Discussion

mRNA quantity testing using qPCR technique is a very effective approach to measure the quantity of RNA in certain cells or tissues (Ilmi *et al.,* 2025). This method plays a crucial role in the study of the CYP11A1 gene, which is known to influence the luteolysis process in CL in this study. To measure gene expression from mRNA, the RNA needs to be reverse transcribed into complementary DNA (cDNA) through an enzymatic reaction called Reverse-Transcriptase (Artika, 2022). The cDNA to be used also needs to be quality verified first using conventional PCR and agarose gel electrophoresis. This test is an important step to ensure primer specificity and template quality before proceeding with RT-qPCR or other gene analysis. Furthermore, initial primer validation with conventional PCR also serves to ensure there is no non-specific amplification, especially when working with heterogeneous tissues or low-quality RNA (Kumar et al., 2025).

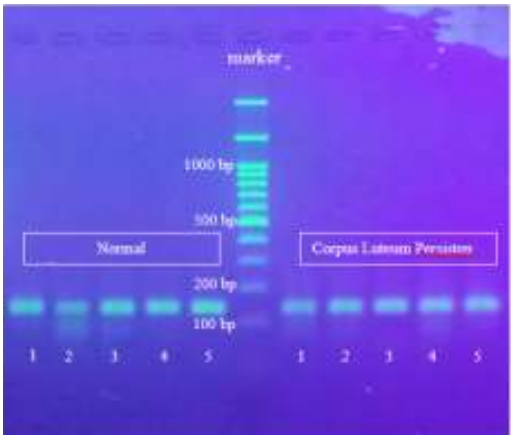


Figure 1. Visualization of clear and sharp cDNA band position on agarose gel.

The agarose gel electrophoresis results in Figure 1 show a clear, sharp DNA band at a position corresponding to the size of the gene amplification product, approximately 136 bp. This indicates successful cDNA synthesis from RNA. It also confirms that the resulting cDNA product is of sufficient quality for use in further gene expression analyses such as qPCR.

The results presented in Table 1 show that CYP11A1 gene expression in cattle with persistent corpus luteum increased 3.12-fold compared to normal cattle. CYP11A1 is a mitochondrial enzyme that catalyze the conversion of cholesterol to pregnenolone, the first and essential step in steroid hormone biosynthesis (Miller, 2017). CYP11A1 activity significantly determines the capacity of luteal cells to produce progesterone, so increased expression is often associated with increased corpus luteum steroidogenic function (Hernandez et al., 2022; Miller, 2017). Physiologically, CYP11A1 expression is stable during the early to mid-luteal phase to maintain progesterone production, and decreases significantly during the luteal regression phase in response to prostaglandin ($F_2\alpha$) (Kfir et al., 2018). Increased CYP11A1 expression in PCL cattle may be indicated as one of the causes of luteolysis failure and the continued steroidogenic activity in the corpus luteum. This finding is in line with reports that corpus luteum that maintains the expression of steroidogenic genes, such as CYP11A1, STAR, and HSD3B1, tends to maintain progesterone production

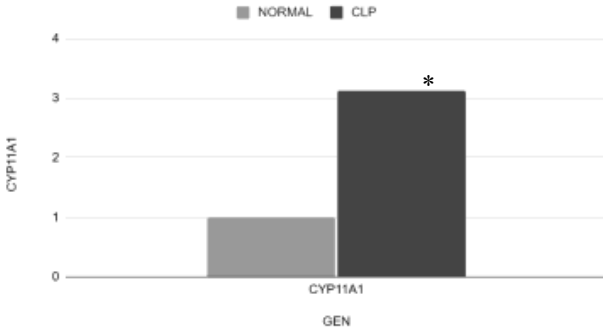


Figure 2. Relative expression of the CYP11A1 gene in corpus luteum persistent (PCL) cattle compared to normal cattle. (*) results are statistically significant ($p < 0.05$).

Table 1. Relative Expression of CYP11A1 on Normal and cattle with persistent corpus luteum (PCL)

Sample	Ct Ref	Ct Target	ΔCt	ΔΔCt	2^-ΔΔCt ± SEM
Normal (n=5)	28.89	28.96	0.07	0	1.00 ± 0.10
PCL (n=5)	30.38	28.81	-1.57	-1.64	3.12 ± 1.68

*Note: n = number of samples

and experience delayed luteal regression (Pate & Hughes, 2023). This is also supported by the findings of Atli et al (2012), who said that CYP11A1 expression began to show a clear decrease in the final phase of luteal regression in normal cattle, however in PCL cattle in this study, CYP11A1 gene expression was found to be even higher than in normal cattle.

Figure 2 shows significant differences between PCL cattle and normal cattle. Under normal conditions, functional luteolysis is characterized by decreased progesterone synthesis followed by decreased steroidogenic gene expression in response to prostaglandin F2α (PGF2α) (Pate & Hughes, 2023). PGF2α induces molecular changes that lead to a loss of steroidogenic capacity and activate apoptosis and luteal tissue remodeling pathways (Miyamoto et al., 2010; Pate & Hughes, 2023). Therefore, the high expression of CYP11A1 in PCL cattle indicates that the luteolysis mechanism may not be functioning optimally.

In addition to regulation by PGF2α, corpus luteum steroidogenesis activity is also influenced by various metabolic and endocrine factors. Adiponectin is known to suppress the expression of steroidogenic proteins, including CYP11A1, through activation of the AdipoR2–AMPK pathway, thereby decreasing progesterone production (Pei et al*.,* 2023). Adiponectin has also been reported to induce autophagy and apoptosis of luteal cells which play a role in the process of corpus luteum regression (Li et al.., 2024). Disruption of this pathway can cause luteal cells to persist and maintain their steroidogenic activity. The continued function of the corpus luteum in PCL cattle is also supported by histological results showing a predominance of intact luteal cells. According to Skarzynski (2020), corpus luteum that fails to regress generally still maintains luteal cell integrity and the expression of genes that support progesterone synthesis. Thus, the increased CYP11A1 expression found in this study further strengthens the hypothesis that corpus luteum persistence occurs due to the failure to reduce steroidogenic activity and disruption of the luteolysis mechanism.

Conclusion

Quantitative PCR test showed that CYP11A1 gene expression increased in cattle with PCL reproductive disorders compared to normal cattle, this explains that this gene contributes to maintaining the corpus luteum in PCL cases and this gene has the potential to be used as a biomarker for non-invasive detection of PCL cases from blood samples but further verification using CL organs is still needed.

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