2025, Scienceline Publication
World's Veterinary Journal
World Vet J, 15(2): 224-228.
ISSN 2322-4568

DOI: https://dx.doi.org/10.54203/scil.2025.wvj24
PII: S232245682500024-15

Detection of Luteinizing Hormone Receptor in Holstein Friesian Dairy Cows Undergoing Repeat Breeding

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SHORT COMMUNICATION
Received: March 19, 2025
Revised: April 22, 2025
Accepted: May 21, 2025
Published: June 25, 2025

ABSTRACT

The tropical climate in Indonesia will cause heat stress in imported cattle, such as Holstein Friesian dairy cows, which farmers in East Java Province widely keep. Heat stress can cause repeat breeding cases caused by failure in ovulation, which is greatly influenced by luteinizing hormones, whose receptors are found in the blood. This study aimed to detect luteinizing hormone (LH) gene receptors in the blood of dairy cows undergoing repeat breeding. This research was conducted from April to August 2023 using 4 randomly drawn 30 Holstein Friesian dairy cows with repeat breeder cases at the Wilis Farmer Village Unit cooperative. East Java. A ten-milliliter blood sample was taken from the jugular vein of each Holstein Friesian Dairy Cow and placed into a sterile tube. Deoxyribonucleic acid (DNA) extraction of luteinizing hormone gene receptors from the blood of Holstein Frisian dairy cows was done using a DNA extraction kit. DNA amplification of the receptor gene of the luteinizing hormone of Holstein Frisian dairy cows' blood was done using the PCR method, and DNA amplification was checked by 1% agarose electrophoresis. The results of the luteinizing hormone gene receptor from the blood of four Holstein Frisian dairy cows undergoing repeat breeding at the Tani Wilis Village Unit cooperative, East Java, were at 600 bp on gel electrophoresis. The LHR gene profile is potentially used as a marker to detect dairy cows with repeat breeding under heat stress.

Keywords: Blood, Cow, Luteinizing hormone, Receptor gene, Repeat breeding

INTRODUCTION

Indonesia has a tropical climate; in summer, the environmental temperature will rise to around 38-45°C, which will cause heat stress. Heat stress has long been known as an environmental condition that threatens livestock fertility (Oke et al., 2021). Heat stress has also been shown to cause a marked reduction in oocyte developmental ability and spermatozoa fertilization capacity, leading to decreased reproductive rates and losses for the cattle industry (Khan et al., 2023). The case of decreased reproductive rates in dairy cattle farms in Indonesia, which is often encountered, is a repeat breeder. The case of a repeat breeder in question is a condition where a female cow did not become pregnant after having mated 3 times or more with the semen of a bull or inseminated without symptoms (Amiridis et al., 2009).

The incidence of repeat breeding in dairy cattle in Sinjai Regency, Indonesia, was 62% of the 82 dairy cattle observed (Yusuf et al., 2012). Cases of repeat breeding were also found in dairy cows in Batu City, East Java, with a prevalence of 20.64% of the 281 dairy cows (Arningdiah et al., 2024). Cases of repeat breeding have been reported in several tropical and subtropical countries, including Bangladesh and Japan. In Bangladesh, the repeat breeding of dairy cattle farms was 122 out of 1524 dairy cattle examined (Asaduzzaman et al., 2016). The repeat breeding cases reported in Japan were 14%, ranging from 5% to 24% among the nine studied dairy herds (Yusuf et al., 2010).

The level of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) of cattle blood occurrence of repeat breeding in cattle will be related to repeat breeding in cattle because both hormones are involved in reproduction. Follicle growth in ovaries is significantly influenced by FSH and LH (Crowe and Mullen, 2011). In developing ovarian follicles, LH will attach to the G protein-coupled receptor (GPCR). The luteinizing hormone receptor (LHR) is crucial to gonadal maturation and function in both men's and women's reproductive physiology (Dufau et al., 2010). The luteinizing hormone receptor (LHR) in the blood sample of an Indonesian Holstein Friesian dairy cattle was found at 303 base pairs (bp) on gel electrophoresis, according to a previous study that used polymerase chain reaction fragment length polymorphism (PCR-RFLP) (Setyorini et al., 2023). The study by Setyorini et al. (2023) found LHR in Indonesian

To cite this paper: Madyawati SP, Prastiya RA, Srianto P, Pradana M, Yansri AA, and Kholik K (2025). Detection of Luteinizing Hormone Receptor in Holstein Friesian Dairy Cows Undergoing Repeat Breeding. *World Vet. J.*, 15(2): 224-228. DOI: https://dx.doi.org/10.54203/scil.2025.wvj24

Holstein Friesian and showed that Holstein Friesian dairy cattle under heat stress conditions in Indonesia can be detected through the presence of luteinizing hormone receptors in the blood.

The Tani Wilis Village Unit Cooperative is a center for the dairy cattle breeding of Holstein Friesian dairy cow descendants in East Java, Indonesia. Holstein Friesian dairy cows are widely raised in Indonesia and have undergone adaptation (Aditya et al., 2015). The studies on the detection of luteinizing hormone (LHR) receptors in cattle using a molecular approach in Indonesia are still minimal, especially in East Java, so it is necessary to conduct LHR detection in the blood of Holstein Friesian cattle at the Wilis Farmer Village Unit Cooperative at the Wilis Farmer Cooperative, as LHR gene profile data under heat stress conditions and those undergoing repeat breeding that can be used as markers. The present study also aimed to increase insight into genetic knowledge and reproductive status in dairy cattle that have undergone adaptation in the selection of Friseian Holstein dairy cow seeds.

MATERIALS AND METHODS

Ethical approval

The Animal Care and Use Committee (ACUC) of the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, Number 1.KEH.058.05.2022 has approved the procedure in this research.

Study design

This descriptive study was conducted from April to August 2023 using 4 of 30 Holstein Friesian dairy cows with an average age of 3.5 years that had repeat breeder cases at the Willis Farmer Village Unit cooperative. East Java. The criteria for choosing cows suffering from breeding cases were Holstein Frisian dairy cows with normal estrous cycles that have been mated 2 or more times with fertile bulls or inseminated with the semen of bulls but are not pregnant (Amiridis et al., 2009).

Preparation of blood samples

The 10 ml blood sample was taken from the jugular vein using a sterile 18 G needle and placed into a sterile tube. The blood samples of cows were tested at the Professor Nidom Foundation Laboratory in Surabaya, Indonesia.

DNA extraction

DNA extraction of the luteinizing hormone gene in dairy cows' blood was done using reagents and procedures from GENEzol™ (Geneaid, Taipei, Taiwan). The DNA extraction carried out in this study, which included preparation of samples, lysis of cells, binding of DNA, washing, and elution of DNA, was also adopted from the study conducted by Setyorini et al. (2023).

The 300 µl of blood sample from cow blood was combined with 900 µl of GENEzol, mixed thoroughly with a vortex, and allowed to incubate at room temperature for five minutes for DNA extraction from cow blood. Subsequently, 200 µl of Chloroform was introduced to the sample for every 1 mL of GENEzol and centrifuged at 12,000 x g for 15 minutes at 4°C. The top aqueous layer was gently removed, and the interphase, along with the organic phase, was retained for DNA extraction. Three hundred microliters of absolute ethanol were added, mixed by inverting multiple times, incubated at room temperature for five minutes, and centrifuged at 2,000xg for five minutes at 4°C, after which the supernatant was removed**.** Subsequently, 1 mL of sodium citrate solution (0.1 M sodium citrate in 10% ethanol) was introduced, allowed to incubate at room temperature for 30 minutes, and then centrifuged at 2000xg for 5 minutes at 4°C. Next, 1.5 mL of 70% ethanol was introduced, incubated at ambient temperature for 10 minutes, and centrifuged at 2000xg for 5 minutes at 4°C. The supernatant was removed, and the DNA pellet was allowed to air-dry for 10 minutes at room temperature. A total of 300 µl of 8 mM NaOH was added to the DNA pellet, incubated at 55-60°C for 10 minutes, and centrifuged at 12,000xg for 10 minutes. The supernatant was removed, and the DNA pellet was allowed to air-dry for 10 minutes at room temperature. A total of 300 µl of 8 mM NaOH was added to the DNA pellet, incubated at 55-60°C for 10 minutes, and centrifuged at 12,000xg for 10 minutes. The supernatant was moved to a fresh 1.5 mL tube as the DNA sample proceeded with the Polymerase chain reaction (PCR) process.

DNA amplification by PCR

In this study, a total PCR reagent volume of 25 µL was utilized, comprising 12.5 µL of PCR mix (Gotaq green), 1 µL of forward primer, 1 µL of reverse primer, 5 µL of dNTPs, 0.5 µL of KOD DNA polymerase, and 5 µL of extracted LH receptor gene DNA as the template. The PCR machine from Biorad, known as the i-cycler (Biorad system, USA), was utilized to handle the mixture. In the present study, specific primers were utilized for the LH receptor

(LHR) gene, with the forward primer being 5'-TTT ACC AAC CTC CTG GAT GC-'3 and the reverse primer as 3'-GTT AGG CAC ATC AGG CAA AAA-'5, generating an amplicon of 373 bp, based on Prasetyo et al. (2021).

PCR tubes containing PCR mix and DNA were inserted into the thermocycler machine, after which the thermocycler machine was run with denaturation conditions of 94^{0}C for 30 seconds, annealing for 30 seconds at 55^{0}C, and extension at 72^{0}C for 1 minute for 35 cycles. Furthermore, the LH receptor PCR results were electrophoresed to see the amplification band (Li et al., 2011). Then, DNA amplification was checked by 1% agarose gel electrophoresis. The data from the results of this study were analyzed descriptively in the form of images, including the electrophoresis of luteinizing hormone gene receptors from the blood of Holstein Frisian dairy cows.

RESULTS AND DISCUSSION

Based on the PCR results of the LH receptor gene (LHR) from the blood of 4 of 30 Holstein Friesian dairy cows with repeat breeder cases at the Wilis Farmer Village Unit cooperative, East Java, it was found that LHR was located at 600bp on 1% agarose electrophoresis gel (Figure 1).

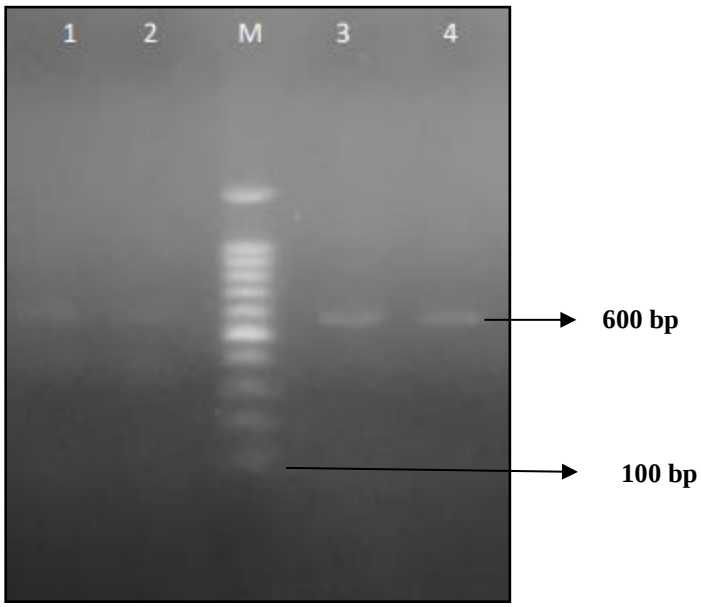

Figure 1. 600 bp of Luteinizing hormone receptor (LHR) of Adult Holstein Friesian dairy cows in 1% agarose electrophoresis gel. M = Marker; 1-4 = Samples

The results of the study indicated that LHR was at 600bp on the electrophoresis gel, showing a difference with the primer used as a reference, which had a position of 373bp. The results of this study are also different from the study conducted by Setyorini et al. (2023), who documented that LHR from the blood of Holstein Friesian dairy cows in Central Java was found at 303 bp in 2% agarose electrophoresis gel.

The variation in LHR within the electrophoresis band can be attributed to differences in LH receptors and their interactions. Research on LHR characterization has shown that over 400 clones and 50 variants of bovine LHR have been identified when compared with the published bovine LHR cDNA sequence via NCBI Blast, and cDNA from bovine Leydig cells examined through RT-PCR showed at least 5 bands detected at 700 bp, 800 bp, 1000 bp, and 1500 bp, suggesting variants (Ma et al., 2012). As an analogy, in humans, several LHR isoforms have been identified in reproductive tissues by RT-PCR, located at approximately 100 and 200 bp on gel electrophoresis, which were confirmed as LHR by direct sequencing (Juel Mortensen et al., 2021).

LHR variants in PCR products and on gel electrophoresis may be due to the large amount of LH secretion that will bind to blood receptors, so the reproductive cycle phase plays a very important role in detecting LHR in cow blood. LHR has been stated to be associated with ovary cells of theca and granulosa cells, promoting follicle development and regulating the production of sex steroids in response to LH. LH levels occur in bovines in the middle of the estrus cycle, triggering ovulation (Kawate et al., 2001). After ovulation, LHR is associated with the corpus luteum for progesterone production, which is important for maintaining pregnancy (Narayan and Puett, 2003). Human studies have shown that serum LHR levels are associated with the pubertal process and function of the gonads; LHR was temporarily suppressed by high levels of serum hCG (Juel Mortensen et al., 2021). The LHR is associated with the reproductive cycle phase, indicating that the changes in LHR are heavily influenced by the cow's age and its reproductive cycle

status. For instance, the estrus phase would be different from the metestrus phase in the LHR fragments obtained in the electrophoresis gel.

LHR variants in PCR products and in gel electrophoresis can be caused by the extraction process and annealing temperature (Nakamura et al., 2004). A previous study reported that LHR in gel electrophoresis was at 303 bp, but after the addition of enzyme restrictions, it became 155 bp in gel electrophoresis (Setyorini et al., 2023). The difference in LHR visualization in PCR products and in gel electrophoresis is shown at an annealing temperature of 62 ^{0}C at positions 801 bp and 615 bp (Nakamura et al., 2004). In another study, visualization of LHR on gel electrophoresis was found at 303 bp at an annealing temperature of 54^{0}C (Setyorini et al., 2023). Astari et al. (2021) have indicated that the use of different temperatures at different points in the PCR reaction can obtain PCR products with different band variations.

Based on the results of this study, future research is needed on the characteristics of the LHR gene in the age and reproductive cycle of dairy cattle, as well as on the PCR optimization method and various DNA extraction methods to avoid variations in electrophoresis bands.

CONCLUSION

The luteinizing hormone receptor (LHR) gene has been detected in the blood of Holstein Frisian dairy cows with repeat breeder cases at the Wilis Farmer Village Unit cooperative, East Java, by the PCR method at position 600bp on agarose electrophoresis gel. This LHR gene profile can be used as a marker in dairy cows experiencing heat stress and those undergoing repeat breeding for the selection of breeding cows.

DECLARATIONS

Funding

This research was financially supported by the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, under the contract number 1290/UN3.1.6/PT/2023.

Acknowledgments

The authors greatly acknowledge the Faculty of Veterinary Medicine, Universitas Airlangga, for permitting and facilitating this research through the RKAT budget. The authors also thank Universitas Pendidikan Mandalika, Indonesia.

Availability of data and materials

All data and materials are available upon reasonable request from the corresponding author.

Authors' contributions

Sri Pantja Madyawati conceptualized the study. Ragil Angga Prastiya designed the research methodology, and Pudji Srianto edited and directed the research. Kholik Kholik submitted the manuscript and analyzed the data. Munawer Pradana prepared the manuscript. All authors checked and approved the last edition of the article.

Competing interests

The authors have not declared any conflict of interest.

Ethical considerations

The authors have checked the ethical issues, including plagiarism, consent to publish, misconduct, double publication and/or submission, and redundancy.

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