

# Identification polymorphism of LHR and FSHR genes in Indonesian Holstein dairy cattle associated with productive and reproductive traits

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**Abstract.** Setyorini YW, Kurnianto E, Sutopo, Sutiyo. 2023. Identification polymorphism of LHR and FSHR genes in Indonesian Holstein dairy cattle associated with productive and reproductive traits. *Biodiversitas* 24: 2898-2905. Luteinizing and follicle-stimulating hormones and their receptors play an important role in the reproductive system's hormonal activity and physiological function. This study aimed to identify the genotype variation of the Luteinizing Hormone Receptor (LHR) gene exon 11 and the Follicle-stimulating Hormone Receptor (FSHR) gene exon 10 and to elucidate the associating between polymorphism with the milk and reproductive traits in dairy cattle. A total of 100 samples of deoxyribonucleic acid (DNA) from Indonesian Holstein dairy cattle were used in this study. Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) was performed for genotyping of the Single Nucleotide Polymorphism (SNP) of the LHR and FSHR genes with the HhaI and AluI restriction enzymes, respectively. The collected phenotype data were milk production, quality, and reproductive trait. In addition, the general linear model procedure in the SAS program was carried out to investigate the association of genotypes with productive and reproductive traits. The results showed that the LHR gene in the population of this study was monomorphic, while the FSHR gene was polymorphic with three genotypes, namely CC, CG, and GG. The sequences of the FSHR gene indicated the presence of a mutation at nucleotide number 2037 that substitutes cytosine for guanine. Cows with the CC genotype showed better parameter service per conception ( $P < 0.05$ ) than the other genotypes but did not correlate with other reproductive parameters and milk traits. Therefore, it was concluded that SNP g.2037C>G polymorphism at FSHR gene exon 10 was identified in the Indonesian Holstein Dairy cattle population and associated with service per conception parameters but not with milk trait.

**Keywords:** FSHR gene, genetic marker, genotype, LHR gene, PCR-RFLP

## INTRODUCTION

Milk production and reproduction are polygenic factors, influenced by numerous genes, and economically determine dairy cattle profitability (Nayeri et al. 2016). Genetic selection can improve these traits in addition to management and nutrition. One way to improve the estimation of genetic progress is by Single Nucleotide Polymorphism (SNP) of specific genes involved in reproductive factors (Cochran et al. 2013). Genetic diversity based on genotype traits can promote better breeding and selection systems in animal breeding (Lestari et al. 2023). Due to their usage in genetic selection, genetic polymorphisms in many genes that affect economic traits have been developed. They evaluate the current biodiversity and differences between cattle concerning utilization for population growth and preservation (Omer et al. 2016).

The Luteinizing Hormone Receptor (LHR) and the Follicle-stimulating Hormone Receptor (FSHR) are transmembrane receptors that are essential for hormonal activity during reproduction and have been recognized as genetic marker candidates for reproduction (Omer et al. 2016; Arslan et al. 2017). Due to its ability to bind to specific receptors present in the plasma membrane of target cells, bovine LHR, which is a member of the G protein-

coupled receptor, provides an important function in mediating the action of LH and chorionic gonadotropins (Abdi et al. 2017; Faraj et al. 2021). The LHR gene synthesizes proteins that allow the action of LH when binding to it and play a fundamental role in folliculogenesis (Cañizares-Martinez et al. 2021), development of the follicle, ovulation, corpus luteum and embryonic preimplantation (Faraj et al. 2021). The gene FSHR, which encodes the transmembrane receptor that interacts with FSH, is found on bovine chromosome 11 (BTA 11). It has 10 exons and 11 introns and is most abundant in the ovaries and testes (Omer et al. 2016; Arslan et al. 2017). In addition, the anterior pituitary organ produces the gonadotropin hormone FSHR, which is involved in steroidogenesis and gametogenesis (Hamny et al. 2017).

Studies related to LHR and FSHR genes have been conducted on *Bubalus bubalis* Linnaeus 1758 (Sosa et al. 2016; Kathiravan et al. 2019), *Bos taurus* Linnaeus 1758, *Bos indicus* Linnaeus 1758, and crossbreed cattle (Andreas et al. 2014; Faraj et al. 2021), *Ovis aries* Linnaeus 1758 breed (Du et al. 2019; He et al. 2021), and human (Meireles et al. 2021; Atoum et al. 2022). Several studies on SNP have reported that there is a polymorphism of the LHR gene in Holstein cattle in Azerbaijan (Abdi et al. 2017), Pasundan cattle (Said et al. 2021), Holstein cattle in Honduras

(Cañizares-Martinez et al. 2021), and one of them at the site of exon 11 (Hastings et al. 2006; Marson et al. 2008). Meanwhile, FSHR polymorphism studies have been reported in studies on Holstein dairy cows (Arslan et al. 2017), Pesisir cattle (Afriani et al. 2022), Madrasin cattle (Utomo et al. 2020), Pasundan cattle (Putra et al. 2020), and Holstein bulls (Nikitkina et al. 2021). In addition, FSH gene polymorphism in *B. taurus* can change the ability to bind to FSH and may affect its reproductive status (Hamny et al. 2017). Dairy cattle now widely kept in Indonesia are derivatives of Friesian Holstein (FH) cattle which have different productivity from imported cattle (Aditya et al. 2015). BSN (2014) states that Indonesian Holstein dairy cows are FH cows born and adapted to Indonesia, and they must be known for their genetic diversity to support genetic selection in producing cows with superior productivity.

Studies on the effect of the LHR gene on milk production and reproduction in Indonesian Holstein dairy cows have not been reported. In contrast, studies on the FSHR gene have been studied in different populations (Pambuko et al. 2021) but have only been limited to genetic diversity. Polymorphism studies of LHR and FSHR genes can explore the potential of these genes to become candidate marker genes for production and reproductive traits in dairy cows. Furthermore, this information can determine breeding strategies, especially in Holstein dairy cows that have adapted to the Indonesian environment. For this reason, this study was conducted to detect genotype variation of LHR and FSHR genes using PCR-RFLP and sequencing methods and to investigate the association with the productive and reproductive traits in Holstein dairy cattle in Indonesia.

## MATERIALS AND METHODS

### Ethical approval

The Animal Ethics Committee of the Faculty of Animal and Agricultural Sciences, Universitas Diponegoro Number 58-06/A-7/KEP-FPP, has approved the experimental procedure in this study.

### Material and data collection

A total of one hundred female Indonesian Holstein dairy cattle from the dairy breeding center in Central Java,

Indonesia, selected by purposive sampling method, were used in this study. The dairy cows selected as material were Holstein dairy cows born and grown in Indonesia, aged 3-7 years, have been given calving at least two times, and have a record of milk production and reproduction. In addition, dairy cattle were reared under the same environmental conditions and uniform maintenance management.

The phenotype data collected were on total milk production, quality, and reproductive traits. The total milk production of 305 days was calculated individually using the test interval method and standardized by correction factors for length of lactation, milking frequency, and mature equivalent. Milk quality data including milk fat, protein, specific gravity, total solid nonfat, and milk lactose, were analyzed using a milk analyzer (Lactoscan MCC, UK) in 48 dairy cows in the lactation period. Reproductive data were service-per-conception in Heifer, gestation period at first pregnancy, age at first calving, the interval from calving to conception, service per conception in parity I, gestation period at second pregnancy, age at second calving, and calving interval. In addition, blood samples were collected from each cattle through coccygeal veins using an 18 G venoject needle and a vacutainer tube containing an anticoagulant Ethylenediamine tetraacetic acid (EDTA), and stored in a refrigerator at 4°C.

### DNA extraction, PCR-RFLP, and sequencing analysis

Whole genome deoxyribonucleic acid (DNA) was extracted from blood samples using a mini genomic DNA extraction kit (Geneaid, Taipei, Taiwan) per the manufacturer's protocol at the Division of Biology, Integrated Laboratory of Universitas Sebelas Maret. DNA extraction is conducted through sample preparation, cell lysis, DNA binding, washing, and DNA elution. Then 0.8% agarose electrophoresis was carried out to check the genomic quality of DNA.

Genomic DNA was analyzed using the Polymerase Chain Reaction Restriction Fragment Length Polymorphism (PCR-RFLP) procedure. Two pairs of primers were used to amplify LHR and FSHR genes based on the GenBank National Center for Biotechnology Information (NCBI) reference sequence with access codes U20504 and L.22319.1, respectively (Table 1).

**Table 1.** Primer sequences, annealing temperature, product size, and restriction enzymes used for the amplification of LHR and FSHR genes

Locus	Primer sequence (5' to 3')	Ann. temp.	Product size (bp)	RE	GenBank acc. no.	Reference
LHR exon 11	F: CAAACTGACAGTCCCCGCTTT R: CCTCCGAGCATGACTGGAATGGC	56°C	303	HhaI	U20504	Marson et al. (2008)
FSHR exon 10	F: CTGCCTCCCTCAAGGTGCCCCCTC R: AGTTCTTGGCTAAATGTCTTAGGGGG	50°C	306	AluI	L.22319.1	Arslan et al. (2017)

Note: LHR: Luteinizing hormone receptor; FSHR: Follicle-stimulating hormone receptor; Ann. Temp.: Annealing temperature; bp: base pair; RE: Restriction enzyme

PCR was carried out in a total volume of 25 µL of reactions consisting of 1 µL of each forward and reverse primer, 12.5 µL of 2xMyTaq HS Red Mix (Bioline, London), 9.5 µL of nuclease-free water, and 1 µL of genomic DNA. Amplification was performed using a thermal cycler machine (Bio-Rad, USA) on 100 DNA samples that had been extracted. The PCR program was initiated with predenaturation at 95°C for 5 minutes, 35 cycles of denaturation for 30 seconds, annealing at 54°C (LHR) and 50°C (FSHR) for 30 seconds, extension at 72°C for 30 seconds, and final extension for 10 minutes. PCR products were electrophoresed using 2% agarose gel. The agarose gel was stained with ethidium bromide, and a 100 bp marker ladder was used as the DNA band size standard. Agarose gel was visualized using a gel documentation system (Gillite UV, Pacific Image Electronics, Taiwan).

RFLP was performed using restriction enzymes for each gene (Table 1). A total of 31 µL of the reaction mixture composed of 1 µL restriction enzyme, 10 µL PCR product, 2 µL tango buffer, and 18 µL nuclease-free water was then incubated at 37°C for 2 hours. The digested fragments were checked with 2% agarose gel using electrophoresis (Mupid-Exu, Advance, Japan). The fragments were visualized under a gel documentation system, and their size was identified by comparing them with DNA markers.

Sequencing by the 1st Base sequencing service (Apical Scientific Laboratory, Malaysia) was performed on PCR product samples representing each genotype type to obtain nucleotide sequences. The study of polymorphism and confirmation of the existence of SNP was carried out by aligning the DNA and comparing it with the GenBank sequence using Basic Local Alignment Search Tool (BLAST) program by the National Center for Biotechnology Information (NCBI) and Mega X program version 11.

### Data analysis

Data calculated by estimation of the genotype frequency and allele distribution of LHR and FSHR genes by referring to Nei and Kumar (2000) with the formula:

$$X_i = \sum n_{ii}/N$$

Where:

$X_{ii}$  : the frequency of  $ii$  genotypes

$n_{ii}$  : the number of genotype  $ii$ ,  $N$  is the total samples

$$X_i = (2n_{ii} + \sum n_{ij})/2N$$

Where:

$X_i$  : the frequency of the  $i$  allele

$n_{ii}$  : the number of genotype  $ii$

$n_{ij}$  : the number of genotype  $ij$

$n$  : the total samples

Chi-square test to analyze whether alleles and genotypes were in the law of Hardy Weinberg Equilibrium, calculated by the formula:

$$X^2 = \sum (O-E)^2/E$$

Where:

$X^2$  : the chi-square test

$O$  : the number of observed genotype  $ii$  or  $ij$

$E$  : the expected number of genotype  $ii$  or  $ij$

The calculation results of  $X^2$  were then compared with the  $X^2$  table at a 5% significance rate. The frequency of alleles and genotypes was in equilibrium if the chi-square value was less than 5.99.

Investigations of the association of the genotype with productive and reproductive traits were conducted on polymorphic genes by Anova testing using General Linear Model (GLM) procedure in the Statistical Analysis Program of on Demand for Academics (SAS 2021). The statistical model used was:

$$Y_{ij} = \mu + \alpha_i + \epsilon_{ij}$$

Where:

$Y_{ij}$  : the observed trait value of the animal (productive and reproductive traits)

$\mu$  : the population's mean,  $\alpha_i$  is the fixed effect of  $i$ -th genotype

$\epsilon_{ij}$  : the random error effect

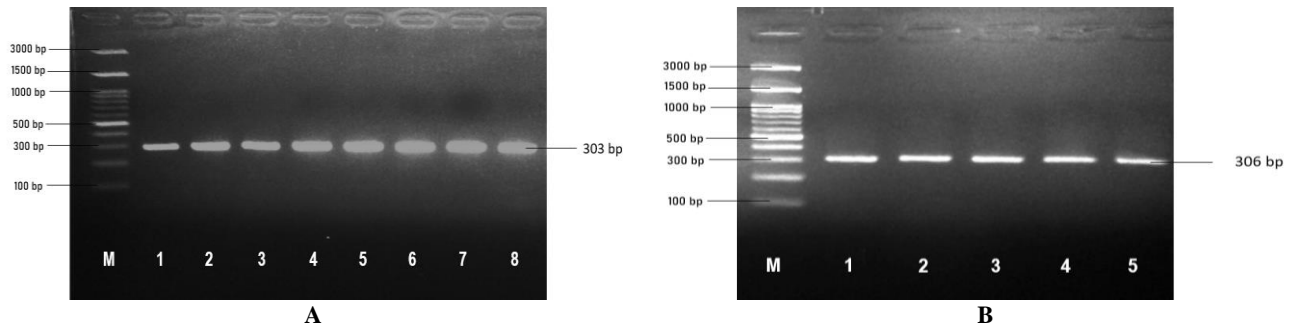
Provided that the value of  $P < 0.05$  is declared significant.

## RESULTS AND DISCUSSION

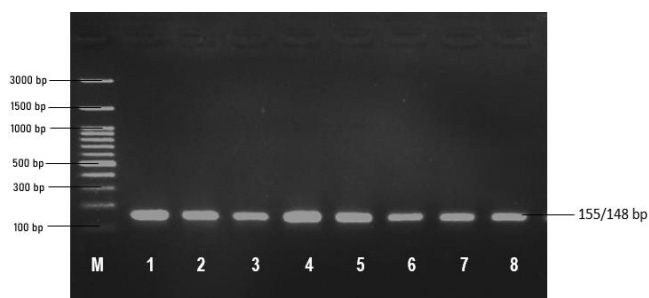
The primer used in this study successfully amplified the LHR gene at the location of exon 11 and FSHR gene exon 10, which was shown by electrophoresis results with sizes 303 and 306 base pairs, respectively (Figure 1). According to previous studies, genotype detection of LHR and FSHR genes was carried out with an RFLP approach (Marson et al. 2008; Arslan et al. 2015). Genotypic determination was based on observations of the length of the fragments formed.

There were no genotype variations in the LHR gene from PCR-RFLP results; namely, only one type of CC genotype was found, indicated by the formation of a 155/148 bp size band (Figure 2). On the other hand, polymorphism was detected in the PCR-RFLP results of the FSHR exon 10 genes which showed the presence of three genotypes for FSHR, as shown in Figure 3, indicated by the formation of bands, namely CC (243, 63/50 bp), CG (243, 193, 63/50 bp) and GG (193, 63/50 bp).

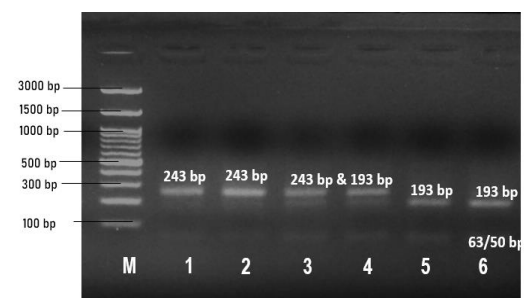
The alignment of nucleotides sequencing FSHR gene results with Gen Bank acc L.22319.1 indicated the presence of SNP located at nucleotide number 2037 of the FSHR gene that substitutes cytosine (C) for guanine (G), called SNP g.2037C>G (Figure 4). The type of SNP in this mutation is missense mutation by changing the amino acid of Threonine to Serine. The enzyme AluI (*Arthrobacter luteus*) works by cutting on the base sequence of AG-CT. In addition, there are also SNPs at other mutation point locations, namely SNPs on nucleotide sequences 2071 and 2119 (Figure 5).



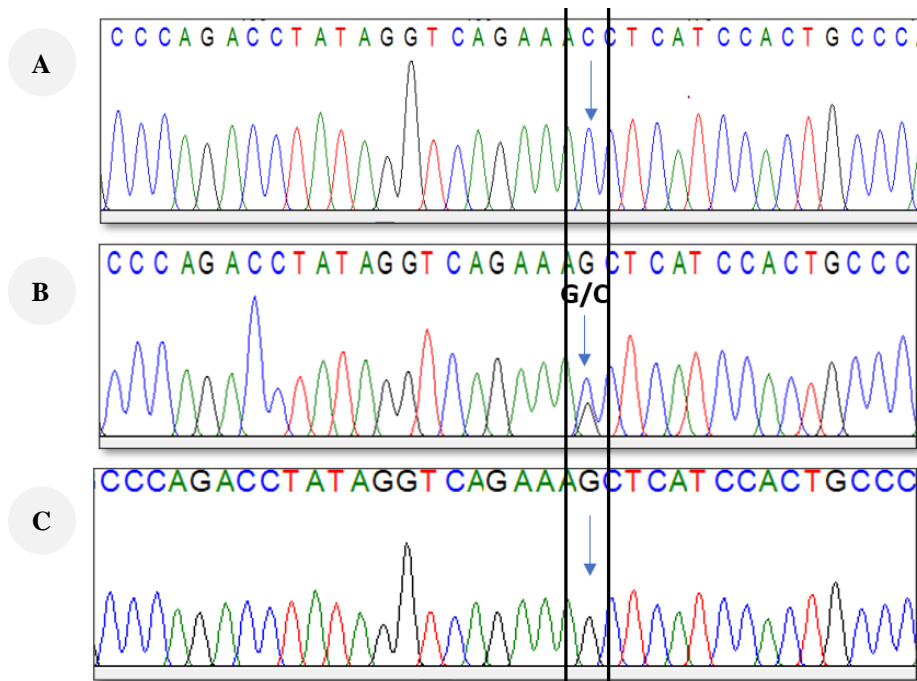
**Figure 1.** A. 303 bp amplification of Luteinizing hormone receptor (LHR) gene fragments in 2%-agarose gel; M: Marker 100 bp; 1-8: samples. B. 306 bp amplification of Follicle-stimulating hormone receptor (FSHR) gene fragments in 2% agarose gel; M: Marker 100 bp; 1-5: samples



**Figure 2.** PCR-RFLP electrophoresis results of Luteinizing hormone receptor (LHR) gene after digesting by enzyme restrictions *Hha*I; M: 100 bp DNA ladder; 1-8: CC genotype (monomorphic)



**Figure 3.** PCR RFLP electrophoresis results of Follicle-stimulating hormone receptor (FSHR) gene after digesting by enzyme restrictions *Alu*I. M: 100 bp DNA ladder; 1-2: CC genotype; 3-4: CG genotype; 5-6: GG genotype



**Figure 4.** Chromatogram of sequencing results of SNP g.2037C>G of Follicle-stimulating hormone receptor (FSHR) gene. A. CC genotype; B. CG genotype; C. GG genotype. The arrows indicate the site of the base mutation

Sequence ID: [L22319.1](#) Length: 2375 Number of Matches: 1

Range 1: 2001 to 2122 [GenBank](#) [Graphics](#)

▼ Next Match ▲

Score	Expect	Identities	Gaps	Strand
209 bits(113)	2e-58	119/122(98%)	0/122(0%)	Plus/Plus
Query 1	GCTATGAAGTGCAAGCCCAGACCTATAGGTCAGAAAGCTCATCCACTGCCCAACTTTC	60		
Sbjct 2001	GCTATGAAGTGCAAGCCCAGACCTATAGGTCAGAAACCTCATCCACTGCCCAACTTTC	2060		
Query 61	ATCCAAGGAATGGCCACTGCCCCCGAGTCCCAGGGTTACTAATGGTTCCAATTACACCC	120		
Sbjct 2061	ATCCAAGGAACGGCCACTGCCCCCGAGTCCCAGGGTTACTAATGGTTCCAATTACACAC	2120		
Query 121	TT	122		
Sbjct 2121	TT	2122		

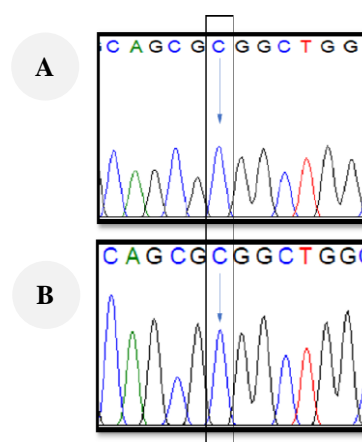
**Figure 5.** Alignment of sequence Follicle-stimulating hormone receptor (FSHR) gene GenBank Accession number L22319.1 with sample sequencing and AluI restriction site on exon 10 gene FSHR (underline)

The LHR gene sequencing results showed the same nucleotide sequence between samples (Figure 6), and there was no variation when aligned with the sequence of GenBank accession numbers U.20504.2 and NM\_174381.1. The nucleotide sequence at the restriction site of the HhaI enzyme (*Haemophilus haemolyticus*) indicates the GCG-C sequence; a restriction occurs at this point and is called the CC genotype. The expected SNP location identified through the enzyme cutting location was in nucleotide number 1334, with the change of cytosine base to thymine (T) called SNP g.1334C>T in GenBank acc No. U.20504.2 or nucleotide number 1337 (SNP g.1337C>T) in GenBank NM\_174381.1 (Figure 7).

The genotype and allele frequencies of the LHR and FSHR genes are presented in Table 2. An SNP is declared polymorphic if it has an allele frequency of less than 0.99 (Nei and Kumar 2000). The FSHR gene is polymorphic with a higher frequency of allele C (0.705) than that of allele G (0.295), and the genotype heterozygote CG (0.490) is more dominant than the genotype homozygote CC (0.460) and GG (0.050). The estimated value of heterozygosity of the observation gene ( $H_o$ ) in the FSHR gene was higher (0.490) than the heterozygosity of expectations ( $H_e$ ) in the study population (0.416). From the results of chi-square testing, it is known that Hardy-Weinberg's equilibrium occurred in the study population, where the  $X^2$  count is smaller than the  $X^2$  table (Table 2). The Hardy-Weinberg equilibrium can be linked to the lack of a specific selection process for this SNP in the parenteral population (Cañizares-Martínez et al. 2021).

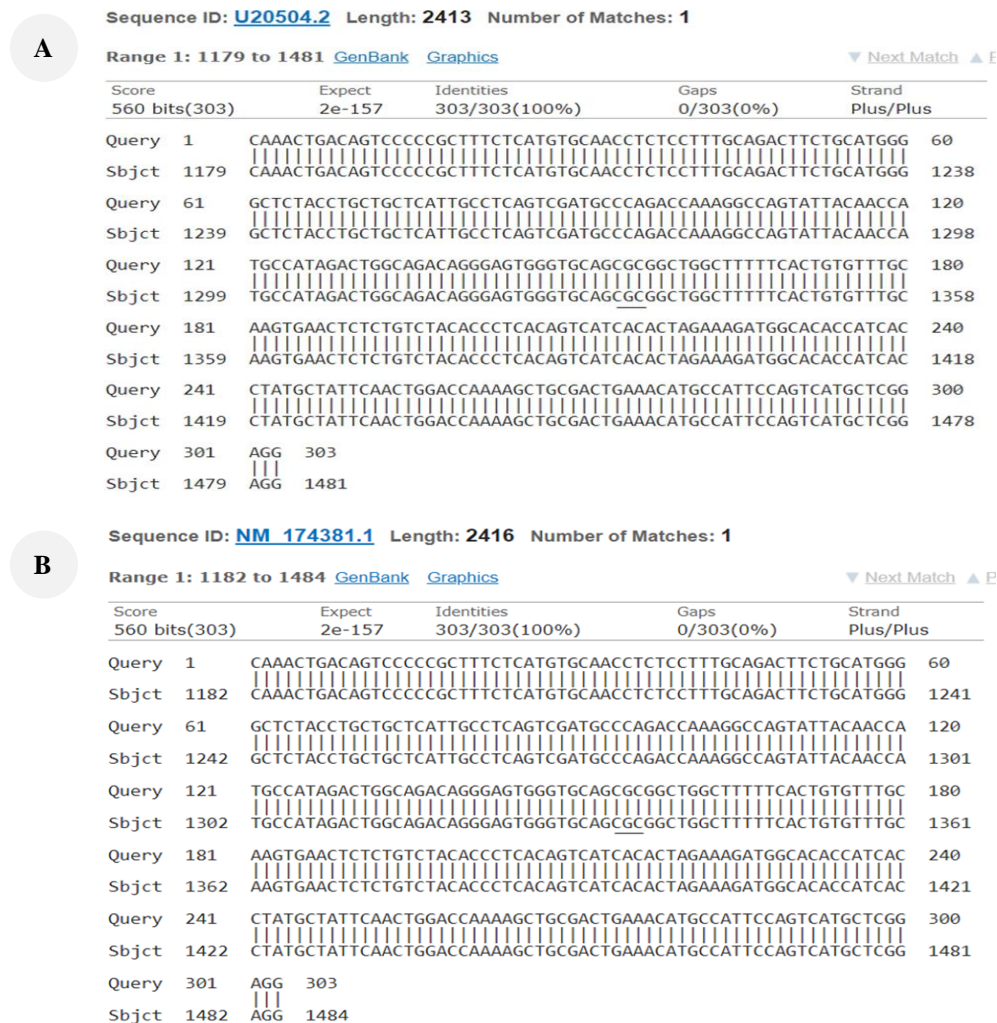
The LHR exon 11 gene in the study population was monomorphic with a CC genotype and C allele frequencies of 100%. So for *LHR/HhaI* gene, heterozygosity calculations cannot be carried out. The monomorphic LHR gene was also found in Egyptian Buffalo (Sosa et al. 2016). The result of this study was different from research on the exon 11 of the LHR gene in the Pasundan cattle population that was reported to be polymorphic in SNP g.1553C>T but not associated with service-per-conception (Said et al. 2021). Arslan et al. (2017) reported that the LHR genotype was

polymorphic with dominant CC (92.86%) in the high insemination number group. Still, the genotype was not associated with the number of inseminations but was found only one genotype (CC) in the Holstein dairy cattle group with a low insemination number. LHR in female cows is expressed in the ovaries; its activation has various endocrinological effects (Hastings et al. 2006), which are necessary for ovulation, luteinization, and the last phases of follicular development and oocyte maturation (Omer et al. 2016). Therefore, it may have an impact on cows' ability to reproduce and their ability to produce milk. LHR expression is necessary for ovulation and luteinization of the dominant follicle in response to LH (Abdi et al. 2017). Although the LHR gene alters the function and is a potential reproductive marker for cattle (Abdi et al. 2017; Arslan et al. 2017), it was shown to be monomorphic in this study population. Further studies are still needed to detect the influence of other SNPs in the Indonesian Holstein dairy cattle population related to production and reproduction traits in larger samples.



**Figure 6.** Chromatogram of sequencing results of Luteinizing hormone receptor (LHR) gene (monomorphic). A and B: CC genotype. The arrows indicate the site of the base mutation





**Figure 7.** Alignment of sequence Luteinizing hormone receptor (LHR) gene GenBank Accession Number U20504.2 (A) and NM 174381.1 (B) with sample sequencing and HhaI restriction site on exon 11 gene LHR (underline)

**Table 2.** Genotype and allele frequencies of FSHR and LHR gene in Indonesian Holstein dairy cattle

Gene	Genotype frequency			Allele frequency		He	Ho	X <sup>2</sup>
LHR	TT	CT	CC	C	T	nc	Nc	Nc
	0	0	1.00	1.00	0			
FSHR	CC	CG	GG	C	G	0.416	0.490	3.2*
	0.460	0.490	0.050	0.705	0.295			

Note: N: The number of samples; Ho: Observed heterozygosity; He: Expected heterozygosity; nc: Not calculable; X<sup>2</sup>: Chi-square test; \*: Hardy-Weinberg equilibrium where X<sup>2</sup> table<sub>2;0.05</sub>: 5.99

The genotype frequency of the *FSHR/AluI* gene obtained in this study differs from previous research on FSHR gene polymorphism by Pambuko et al. (2021), which reported that the frequency of the CC genotype was dominant (0.56) compared to the CG (0.38) and GG (0.06) genotypes. This difference in frequency is likely due to differences in the number, location, and origin of the cows used as samples. These results were in contrast to the study of Putra et al. (2020) in Pasundan Cattle, which reported

that the G allele (0.76) was higher than the C allele (0.24) with the genotypes were CC (0.10), CG (0.27) and GG (0.63). Omer et al. (2016) found that the FSHR gene had no genotypic variation (monomorphic), namely only one AA genotype in the Indigenous Sudanese cattle population. A study on Madura and Madrasin cattle revealed that the FSHR gene exon 10 showed monomorphism with the CG genotype in the population (Utomo et al. 2020). Heterozygosity of the FSHR gene was found to be higher

in the Brahman cattle breed (0.333) than FH (0.125), Simmental (0.250), Limousin (0.200), and monomorphic in Angus (Andreas et al. 2014).

The statistical analysis results in this study on the FSHR gene genotype did not find any significant association between the genotype and the phenotype of milk production, milk protein, total solid nonfat, and milk lactose (Table 3). Likewise, in reproductive properties, genotypes were associated only with service numbers per conception at parity I with a *p*-value of 0.027 ( $P < 0.05$ ) (Table 4). Cows with the CC genotype showed better parameter service-per-conception ( $P < 0.05$ ) than the other genotypes but did not correlate with other reproductive parameters.

The hypothalamic-gonadal axis and its interactions control reproductive function (Sosa et al. 2016). The formation of follicles and the process of steroidogenesis in the ovaries of female cows are significantly influenced by the interaction between FSH and its receptors (Andreas et al. 2014). FSH receptor (FSHR) encodes transmembrane receptors for FSH, and the hormone's function depends on its activity (Arslan et al. 2017). FSH, along with other hormones, plays a crucial part in oogenesis and spermatogenesis. In females, FSH is in charge of recruiting ovarian follicles and promoting their growth; it also stimulates the action of the aromatase in granulosa cells, producing estradiol (Gaviria et al. 2016). The result in this study is similar to those in dairy cows in Iran, indicating that polymorphism at the 5' locus flanking the FSHR gene affects the service period per-conception but has no effect on non-pregnant and calving days until the first service

interval (Sharifiyazdi et al. 2018). This study's results differed from the genotypic variation of SNP A-320T *FSHR/TaqI* gene in Antioquia Holstein cattle, which did not show significant effect on productive and reproductive characteristics (Gaviria et al. 2016). A previous study revealed that the FSHR gene was associated with cattle ovulation rate but not with superovulation response, fertilization, and viable transfer embryo percentage (Andreas et al. 2014). Nikitkina et al. (2021) found that polymorphism at locus 5'-upstream region of the FSHR gene influences double ejaculate volume, concentration, and the total number of spermatozoa on Holstein bull. A study by Marson et al. (2008) found that the FSHR gene has no impact on sexual precocity, defined by the chance of becoming pregnant after the first mating.

Investigations in this study found that the CC genotypes of SNP g.2037C>G at FSHR gene exon 10 may be favorable among Indonesian Holstein dairy cattle for better service-per-conception parameters. This may be related to CC genotypes having better ovulation rates than other genotypes (Andreas et al. 2014). Ovarian conditions affect fertility, where inactive ovaries are the main reason for the appearance of estrus, characterized by inadequate follicular development and not ovulation (Sosa et al. 2016). Therefore, this SNP can be as a genetic marker for the service-per-conception parameters. Using molecular markers to assist genetic selection for superior reproductive traits, especially for service-per-conception parameters, can reduce the time and cost required for conception.

**Table 3.** Association studies of *FSHR/AluI* gene polymorphism with milk trait in Indonesian Holstein dairy cattle

Milk performance	Genotype (N)		
	CC	CG	GG
Total milk yield 305 ME (kg/head/lactation)	4947.98±932.12 (46)	5190.98±1157.32 (49)	4925.79±763.74 (5)
Milk fat (%)	4.13±0.19 (21)	4.12±0.25 (25)	4.11±0.17 (2)
Milk protein (%)	3.05±0.07 (21)	3.07±0.12 (25)	2.98±0.03 (2)
Specific gravity	1.0284±0.0004 (21)	1.0285±0.0004 (25)	1.0285±0.0004 (2)
Solid nonfat (%)	8.17±0.14 (21)	8.23±0.18 (25)	8.16±0.01 (2)
Milk lactose (%)	4.32±0.13 (21)	4.33±0.17 (25)	4.18±0.05 (2)

Note: N: The number of samples; Means in the same row with different superscripts differ significantly ( $P < 0.05$ )

**Table 4.** Association studies of *FSHR/AluI* gene polymorphism with the reproductive trait in dairy cattle

Reproductive trait	Genotype (N)		
	CC	CG	GG
Service-per-conception in Heifer (times)	1.07±0.25 (46)	1.16±0.37 (49)	1.20±0.45 (5)
Gestation period at first pregnancy (days)	276.24±7.76 (46)	276.73±5.78 (49)	273.20±7.05 (5)
Age at first calving (days)	812.20±85.43 (46)	835.47±108.70 (49)	817.00±106.12 (5)
The interval from calving to conception (days)	116.67±63.43 (46)	142.90±58.36 (49)	159.20±58.49 (5)
Service-per-conception in parity I (times)	1.30±0.47 <sup>a</sup> (46)	1.61±0.73 <sup>ab</sup> (49)	1.80±0.45 <sup>b</sup> (5)
Gestation period at second pregnancy (days)	282.07±11.56 (46)	279.90±7.98 (49)	279.80±12.28 (5)
Age at second calving (days)	1210.93±111.52 (46)	1258.27±108.23 (49)	1256.00±76.81 (5)
Calving interval (days)	398.74±61.99 (46)	422.79±58.095 (49)	439.00±64.26 (5)

Note: N: The number of samples; Means in the same row with different superscripts differ significantly ( $P < 0.05$ )

In conclusion, the LHR exon 11 gene was monomorphic with the CC genotype in the Indonesian Holstein population in this study. Meanwhile, the FSHR exon 10 gene was found to be polymorphic with three genotypes, predominantly CG heterozygote genotypes, and indicated the presence of a mutation at nucleotide number 2037 that substitutes cytosine for guanine. Polymorphism in SNP g.2037C>G at FSHR gene exon 10 was identified in the Indonesian Holstein Dairy cattle population and associated with service-per-conception parameters but not associated with other reproductive traits, milk production, and milk quality.

## ACKNOWLEDGEMENTS

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