Determination of STAT5A gene SNPs and Their Association with Reproductive Traits in Ongole Grade Cow of Rembang

Indahwati A*, Kurnianto E, Setiatin ET, Samsudewa D, Lestari DA

Faculty of Animal and Agricultural Sciences, Diponegoro University, Tembalang Campus, Semarang 50275, Indonesia
**Corresponding Author: ahyuniindahwati@gmail.com*

(received 31-12-2024; revised 15-02-2025; accepted 04-03-2025)

ABSTRAK

Indahwati A, Kurnianto E, Setiatin ET, Samsudewa D, Lestari DA. 2025. Penentuan SNP gen STAT5A dan hubungannya dengan sifat reproduksi pada sapi Peranakan Ongole di Rembang. JITV 30(1):8-18. DOI:http://dx.doi org/jitv.v30i1.3484.

Sapi Peranakan Ongole (PO) dikenal memiliki efisiensi reproduksi yang baik. Hingga saat ini belum dikonfirmasi melalui penelitian yang lebih mendalam terkait gen yang memiliki kontribusi besar dan dapat dijadikan sebagai penciri genetik sifat reproduksi. Gen Signal Transducer and Activator of Transcription 5A (STAT5A) diduga berperan sebagai mediator dalam jalur sinyal hormon reproduksi yang berpengaruh besar terhadap sifat reproduksi. Penelitian ini bertujuan mengetahui asosiasi antara gen STAT5A sebagai penanda seleksi sifat reproduksi *service/conception* (S/C), *calving interval* (CI), *days open* (DO), dan *estrus post partus* (EPP). Sampel penelitian adalah 80 sapi PO betina berdasarkan rangking sesuai SNI Sapi PO Bibit Betina Tahun 2015. Metode penelitian meliputi isolasi *deoksiribonukleat acid* (DNA), *polymerase chain reaction* (PCR) dan sekuensing DNA menggunakan metode *Sanger* serta menggunakan *primer* GAGAAGTTGGCGGAGATTATC (*Forward*) dan CCGTGTGTCCTCATCACCTG (*Reverse*). Hasil sekuensing didapatkan pita DNA sepanjang 820 base pairs (bp) sesuai dengan hasil *blasting primer* di NCBI pada ekson ke delapan. Analisis data menggunakan metode multivariat principal component analysis (PCA) dan non-parametrik kontingensi lamda. Hasil penelitian menunjukkan terdapat asosiasi antara gen STAT5A dengan S/C, CI, DO dan EPP pada mutasi g.482 G>A. Temuan ini menunjukkan bahwa gen STAT5A memiliki peran penting dalam meningkatkan kualitas reproduksi sebagai penanda untuk dasar seleksi sapi PO unggul di Kabupaten Rembang.

Kata Kunci: Asosiasi, Ekspresi Gen, Sapi PO Betina, Kabupaten Rembang, Parameter Reproduksi, Gen STAT5A

ABSTRACT

Indahwati A, Kurnianto E, Setiatin ET, Samsudewa D, Lestari DA. 2025. Determination of STAT5A gene SNPs and their association with reproductive traits in Ongole grade cow Rembang. JITV 30(1):8-18. DOI:http://dx.doi org/jitv.v30i1.3484.

The Ongole Grade cows are known for their good reproductive efficiency. However, it has not been confirmed through more in-depth research regarding the genes that have significant contributions and can serve as genetic markers for reproductive traits. The Signal Transducer and Activator of the Transcription 5A (STAT5A) gene is suspected to mediate in the reproductive hormone signaling pathway, significantly influencing reproductive traits. This study aims to determine the association between the STAT5A gene as a selection marker for reproductive traits service/conception (S/C), calving interval (CI), days open (DO), and estrus post partus (EPP). The sample used was 79, based on the rank that was determined according to the SNI Ongole Grade cow in 2015. The research method was carried out by isolating DNA, PCR, and DNA sequencing using the Sanger method and with primers GAGAAGTTGGCGGAGATTATC (Forward) and CCGTGTGTCCTCATCACCTG (Reverse). The sequencing results showed an 820 bp DNA band according to the results of primary blasting in NCBI in the eighth exon. Data analysis used multivariate principal component analysis (PCA) and non-parametric contingency lambda analysis. The results showed a significant association between the STAT5A gene and reproductive parameters in the g.482 G>A mutation; this indicates that the STAT5A gene is important in improving reproductive quality and can be used as a marker for selecting superior Ongole Grade cows in the Rembang Regency.

Key Words: Association, Gene Expression, Ongole Grade Cow, Rembang Regency, Reproduction Paramaters, STAT5A Gene

INTRODUCTION

Ongole Grade is widespread in Indonesia and is developing in almost every district with a high livestock population (Subiharta et al. 2012). According to the Ministry of Agriculture Decree, Ongole Grade Strain cattle in Kebumen Regency have been designated Kebumen Ongole Grade cattle (358/Kpts/PK.040/6/ 2015). Rembang Regency has just received the determination of the Ongole Grade Breeding Area. In Rembang Regency, the Ongole Grade population over eight years (2013–2021) decreased from 117180 heads, representing a decline from 94.03% to 91.10%. In the last two years (2021-2023), 152721 heads decreased to 33.78%. However, Ongole Grade is still in great demand as selected livestock. The population decline occurred due to suboptimal development strategies and a lack of research on practical breeding and selection programs.

Sudaryanto et al. (2018) and Lestari et al. (2023) have researched genetic diversity and phylogenetic studies of Ongole-grade cattle in Rembang Regency. The community highly values the Ongole Grade in Rembang Regency due to its good reproductive efficiency (Indahwati et al. 2021). However, this has not been thoroughly validated through in-depth research, so it is necessary to have targeted selection in qualitative, quantitative, reproductive, and genetic aspects. Information on reproductive efficiency has been carried out through research on Ongole Grade in Rembang Regency (Pratiwi et al. 2022; Panjono et al. 2022).

Reproductive efficiency is closely related to gene expression that affects reproductive potential (Pratiwi et al. 2022). The gene influencing reproductive efficiency, including conception rate (CR) and service per conception (S/C), is the Signal Transducer and Activator of Transcription 5A (STAT5A) (Cochran et al. 2013). This gene has been identified in dairy cattle (Michel et al. 2020) and beef cattle (Paramitasari et al. 2015). Observation of genes related to reproductive traits has not been carried out in Ongole Grade in Rembang Regency, so further research is needed to determine the genetic characteristics related to reproduction that are expressed phenotypically.

The main objective of the selection program in cattle farming is to enhance productivity by improving genetic quality. Utilizing molecular biology technology as a selection marker greatly assists livestock selection and development. The method to determine the molecular markers is to identify polymorphism or gene diversity. Genetic quality improvement through the selection of production and reproductive traits has traditionally been carried out using conventional methods (Prihandini et al. 2020), which require a long time to produce high quality breeding livestock. Genomic technology in developed countries has been used in cattle selection programs. It has succeeded in increasing selection accuracy, reducing the cost of lineage testing, shortening generation intervals, and making it possible to identify early undesirable recessive traits in livestock (Sudrajad et al. 2021). The discovery of genetic markers can potentially increase selection accuracy, especially economically (Sutiyono et al. 2018).

The application of molecular genetic technology to improve the genetic quality of cattle is through a selection approach based on phenotypic data information paired with genetic markers related to economic traits according to desired productivity and will be inherited (Archana 2013). Genomic selection can use genetic markers (marker assisted selection), which are significantly associated with highly economic-value livestock traits. This technology can increase accuracy, shorten generation intervals, and allow selection to be carried out earlier (Putri et al. 2021; Sudrajad et al. 2021). The expected novelty in the research is to determine the genetic characteristics expressed phenotypically (qualitatively and quantitatively), such as the STAT5A gene related to reproductive traits with qualitative and quantitative class grouping based on the Indonesian National Standard (SNI). Therefore, this study aims to determine the association between the STAT5A gene as a selection marker for reproductive traits in Ongole Grade cattle.

MATERIALS AND METHODS

Materials

Samples

The samples used in this study were 200 Ongole-grade cows for reproductive traits and whole blood samples of 80 Ongole-grade cows in Rembang Regency. The materials used were DNA isolated from blood and DNA isolation kits in the form of GeneJet from Thermo Scientific, 70% alcohol, agarose gel, tris acetate EDTA (TAE), loading dye, floro-safe DNA stain, absolute ethanol, a pair of primers, Taq polymerase Bioline fast ready mix + dye, PCR water, ddH2O, DNA ladder, and phosphate buffer saline (PBS).

Equipment

The equipment used was a syringe, cotton, EDTA tube, ice box, ice gel, centrifuge, micropipette, pipette tips, microcentrifuge tube, parafilm, label, marker, hot plate, Vortex, spin-down, polymerase chain reaction (PCR) machine, PCR tube, Erlenmeyer flask, analytical balance, plastic wrap, aluminum foil, bubble wrap, microwave, gel mold, comb, stopwatch, electrophoresis machine, and gel doc.

Methodes

Reproductive traits

A total of 200 Ongole Grade cows that have been identified have given birth. The research unit is classified into 4 SNI classes based on morphometric measurements in the study's second phase. Samples with Grade I, Grade II, Grade III, and non-grade each consist of 80, 40, 30, and 50 Ongole Grade cows. Secondary data surveys were conducted from farmers and officers, and then the rankings were ranked according to the order of S/C, CI, DO, EPP, and estrus duration (ED).

The data were analyzed using descriptive statistics and principal component analysis (PCA). The percentage of variance criteria were determined by looking at the cumulative percentage of variance, where the component with the more significant percentage of

variance would be taken. The analysis showed a relationship between the studied variables and their interrelatedness.

Blood samples

The sampling technique was carried out by selecting 20 cows from each class based on the ranking (Class I, II, III, and non-class) based on Service per Conception (S/C), Calving Interval (CI), Days open (DO) and Estrus Post Partum (EPP). Blood sampling was done by cleaning the neck of the cow using cotton with 70% alcohol. Blood was taken through the jugular vein as much as 3 ml using a vacuum tube with EDTA and put into an ice box containing ice gel. The blood sample was used to obtain genomic DNA.

DNA isolation

For mammalian DNA blood samples, isolation was based on the GeneJET™ isolation kit protocol (Thermofisher ScientificTM). Blood samples were taken with a tube containing EDTA. Mixing 200 µl of blood, inserting an Eppendorf tab with 20 µl of proteinase and 400 µl of K solution using a micropipette, then vortexing/pipetting at a speed of 1500 rpm. The mixture was incubated at 56^0 C, then Vortex occasionally for 10 minutes or a shaking water bath, rocking platform, or thermomixer was used until the cells were completely lysed. Then 200 µl Ethanol (96–100%) was added and mixed by pipetting or vortexing. Transfer the prepared lysate to a GeneJET™ Genomic DNA Purification Column inserted in a collection tube. The column was centrifuged at 6,000 × *g* for 1 minute. Discard the collection tube containing the flow-through solution, and place the GeneJET™ Genomic DNA Purification Column into a new 2 mL collection tube. Then 500 µl of wash buffer I and 30 ml of ethanol were added and centrifuged at 12,000 rpm for 1 minute. The liquid was discarded, and the purification column was put back into the collection tube. Wash buffer II 500 and 30 ml ethanol were added to the GeneJET™ Genomic DNA Purification Column, centrifuged at 12,000 rpm for 3 minutes, and discarded to a sterile 1.5 mL microcentrifuge tube. Elution buffer 200 was added to the center of the GeneJET™ Genomic DNA Purification Column membrane to elute genomic DNA, incubated for 2 minutes at room temperature, and centrifuged for 1 minute. The filter was removed and stored on a shelf at an optimum temperature of 56ºC. The results of DNA isolation are stored at -20ºC and can be known by conducting qualitative tests using the electrophoresis method with agarose gel.

Polymerase Chain Reaction

The primers used for the STAT5A gene sequencing stage (Katib et al. 2008) were in the sequence GAGAAGTTGGCGGAGATTATC (Forward) and CCGTGTGTCCTCATCACCTG (Reverse) in the eighth exon with a base pair size of 820 base pairs (bp). Primer genes in bovines were designed based on sequences from the gene bank using BLAST. PCR mixture including 3µl DNA template, 25µl PCR Master Bioline MyTaq HS Red Mix, 1µl each forward and reverse primers, and 20µl PCR water was put into a PCR tube. Samples were inserted into the PCR machine with the following conditions: initial denaturation stage at 95ºC for 1 minute, denaturation stage at 95ºC for 15 seconds, annealing stage at 51.8ºC for 15 seconds, and extension stage at 72ºC for 55 seconds. The results of PCR analysis were stored in a freezer at -20ºC.

Elektrophoresis

Electrophoresis was performed after DNA isolation and PCR analysis. The results of DNA isolation, as much as 4 µL were added with 1 µl of loading dye and inserted into the wells on a 1% agarose gel using a micropipette. Electrophoresis was performed at a voltage of 100 volts for 30 minutes. Electrophoresis of the PCR analysis results was performed with a DNA ladder. The DNA ladder was inserted into the end well, while the results of the PCR analysis were inserted into the well after it. Electrophoresis was performed at a voltage of 100 volts for 30 minutes. The electrophoresis results were visualized using UV light on the Gel Documentation System (GDS).

DNA sequencing

Sample sequencing was conducted at PT using the Sanger method. Sanger sequencing uses oligonucleotide primers to amplify specific DNA regions. The sequencing stage begins with the denaturation of double-stranded DNA. Then, the oligonucleotide primers attach to the single-stranded DNA and are extended using a mixture of deoxynucleotide triphosphates (dNTPs).

Data analysis

The data analysis to identify mutations from the sequencing results used MEGA11 (Molecular Evolutionary Genetics Analysis version 11) from https://www.megasoftware.net/ (Tamura et al. 2021). Association data analysis between S/C, CI, DO, and EPP with the STAT5A gene was performed using lambda contingency analysis. The diversity of each individual's genotype was determined from the DNA bands of the genes found. Allele frequency (Xi) is the ratio of an allele to all alleles at a locus in the sample population. Gel electrophoresis or sequencing-based methods were used to determine genotype diversity, and chi-square

software tools were applied for band pattern analysis. Allele and genotype frequencies are described and used to calculate Hardy-Weinberg equilibrium using chromatography from sequencing results. Each sample was compared based on the same size (marker), and the allele frequency was calculated based on the formula of Nei & Kumar (2000):

$$\text{Alele frequency} \rightarrow \text{Xi} = \frac{(2n_{ii} + \sum n_{ij})}{2N}$$

where Xi is alele of frequency i, nii is number of genotyped individuals ii (homozygote), nij is number of genotyped individuals ij (heterozygote), and N is number of individuals.

$$\text{Genotype frequency} \rightarrow \text{Xii} = \frac{n_{ii}}{N}$$

where Xii is frequency of genotype ii, nii is number of genotyped individuals ii, and N is number of individuals.

Statistical analysis

Statistical analysis used principal component analysis (PCA) and non-parametric Contingency Lambda. The PCA is a statistical procedure used to observe a variable that may be correlated into an uncorrelated value.

RESULTS AND DISCUSSION

Average reproductive parameters of Ongole Grade cow in Rembang Regency based on class of Indonesian nasional standard is showed in the Table 1. From these five parameters, PCA analysis was carried out to determine the relationship between the variables being studied and their interrelationships, and the results are presented in Table 2. The best reproductive parameters of Ongole Grade cows in Class I and Class II and the differentiating factors of the reproductive parameters are S/C, CI, DO, and EPP. These parameters are used in further analysis to determine their association with the STAT5A marker gene.

Identification of DNA quality

The formation of bands near the wells depicts the results of DNA isolation and PCR electrophoresis. The bright and thick bands indicate a sufficient concentration of isolated DNA. The image of the results of electrophoresis of DNA isolation is shown in Figure 1, and the electrophoresis of PCR results in Figure 2. The sequencing results show that a DNA band of 820 bp is obtained according to the results of the primary blasting at NCBI..

Table 1. The average reproductive parameters of Ongole Grade cows in Rembang Regency are based on the class of Indonesian national standards

Reproductive Traits	Class I	Class II	Class III	Non Class
S/C	1.85	1.89	1.90	1.94
CI (day)	395.57	392.04	401.56	395.69
DO (day)	115.69	114.11	122.95	124.43
EPP (day)	97.78	95.20	106.89	107.39
ED (hour)	19.49	19.11	21.19	19.85

S/C= service/conception; CI= calving interval CI; DO= days open; and EPP= estrus post partus; ED= estrus duration

Table 2. Principal componen analysis of reproductive performance of Ongol grade cow in Rembang Regency

	Component	
Reproductive Traits	1	2
S/C	0.831	-0.300
CI	0.964	0.031
DO	0.989	0.052
EPP	0.827	0.340
ED	-0.121	0.946

S/C= service/conception; CI= calving interval CI; DO= days open; and EPP= estrus post partus; ED= estrus duration

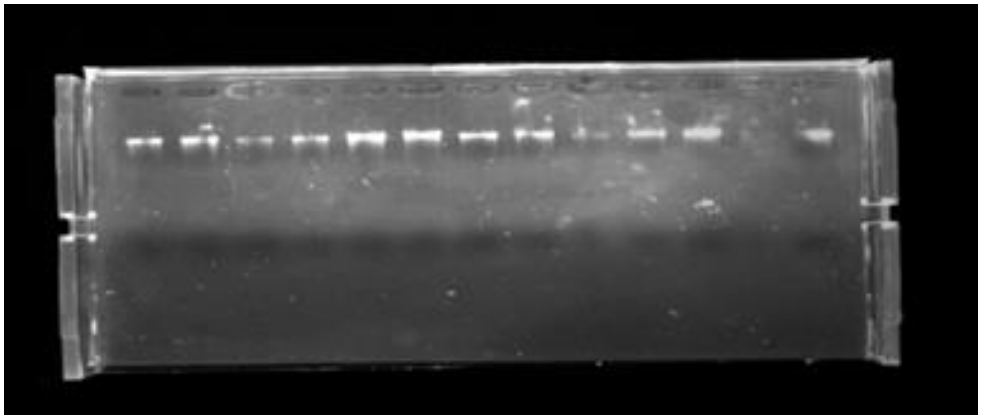


Figure 1. Electrophoresis Image of STAT5A Gene DNA Isolation Results

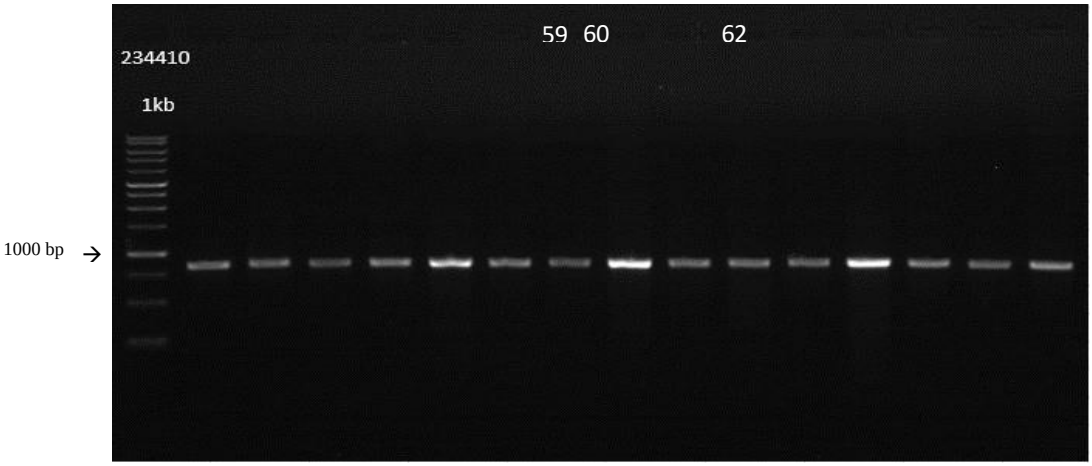


Figure 2. Electrophoresis Image of STAT5A gene PCR Results

SNPs of STAT5A gene in Ongole Grade cow in Rembang Regency

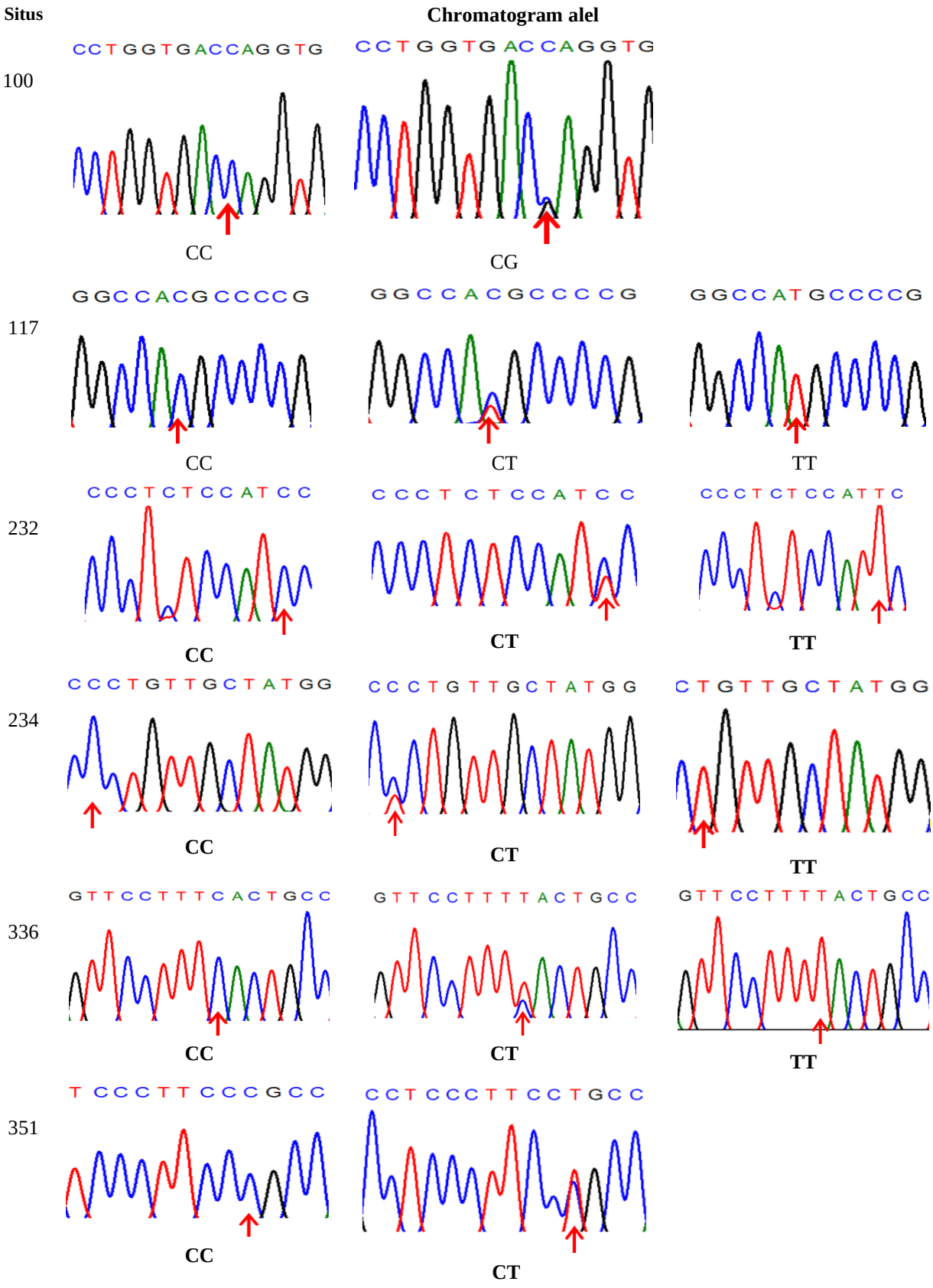
The 80 samples taken were 20 of the best from each class and analyzed for molecular test. One sample was unsuitable for further sequencing, so the sequencing data obtained in this study were 79 samples of the best Ongole Grade cow reproduction in Rembang. The results of DNA sequencing along 820 bp were alligated to 713 bp. After examining the chromatogram waves, SNP points were found at nine sites (100, 117, 232, 234, 336, 351, 372, 482, and 589) dominated by transition mutation. The mutation revealed in this study was forming 9 SNPs (g.100 C>G; g.117 C>T; g.232 C>T; g.234 C>T; g.336 C>T; g.351 C>T; g.372 T>G; g.482 G>A; g.589 C>T). At each SNP point, there are 2-3 genotypes (CC, CG, CT, GG, TG, TT, AG, and AA). Data of SNP found in the STAT5A gene is presented in Figure 3. The SNP that is associated with reproductive traits is site 482.

Genetic diversity of STAT5A gene in Ongole grade cow in Rembang Regency

The data from the observation of the STAT5A gene SNP points were tabulated for genetic diversity analysis and to determine their association with reproductive traits. The genotype and allele frequencies of the STAT5A gene are presented in Table 3.

Table 3 shows the diversity of STAT5A genes in Ongole Grade cows in Rembang Regency. Then, each allele in the analysis results has a frequency value of less than 0.95, so it can be said to be polymorphic, except the C allele in SNP g.100 C>G. This is in accordance with the opinion of Nei & Kumar (2000), which states that if one allele has a frequency of less than 99%, then the gene is said to be polymorphic or diverse; otherwise, it is said to be monomorphic or uniform.

The data in the table above showed that x^2 count < x^2 table, so there is no deviation or show balance based on the Hardy-Weinberg law, except the C allele in SNP g.336 C>T. Most of the SNPs were not significantly different in the Chi-square (X^2) results, which means that the population was in HWE conditions. The HWE condition assumes that random mating occurs in the population and that no migration, mutation, or new alleles exist. The heterozygosity value in a population ranges from zero to one, indicating genetic diversity in a population. The heterozygosity value is influenced by the number of samples, alleles, and the frequency of alleles (Asmarasari et al. 2014).


Situs
Chromatogram alel
100
CCTGGTGACCAGGTG
CCTGGTGACCAGGTG
CC
CG
117
GGCCACGCCCCG
GGCCACGCCCCG
GGCCATGCCCCG
CC
CT
TT
232
CCCTCTCCATCC
CCCTCTCCATCC
CCCTCTCCATTC
CC
CT
TT
234
CCCTGTTGCTATGG
CCCTGTTGCTATGG
CTGTTGCTATGG
CC
CT
TT
336
GTTCCTTTCACTGCC
GTTCCTTTTACTGCC
GTTCCTTTTACTGCC
CC
CT
TT
351
TCCCTTCCCGCC
CCTCCCTTCCTGCC
CC
CT

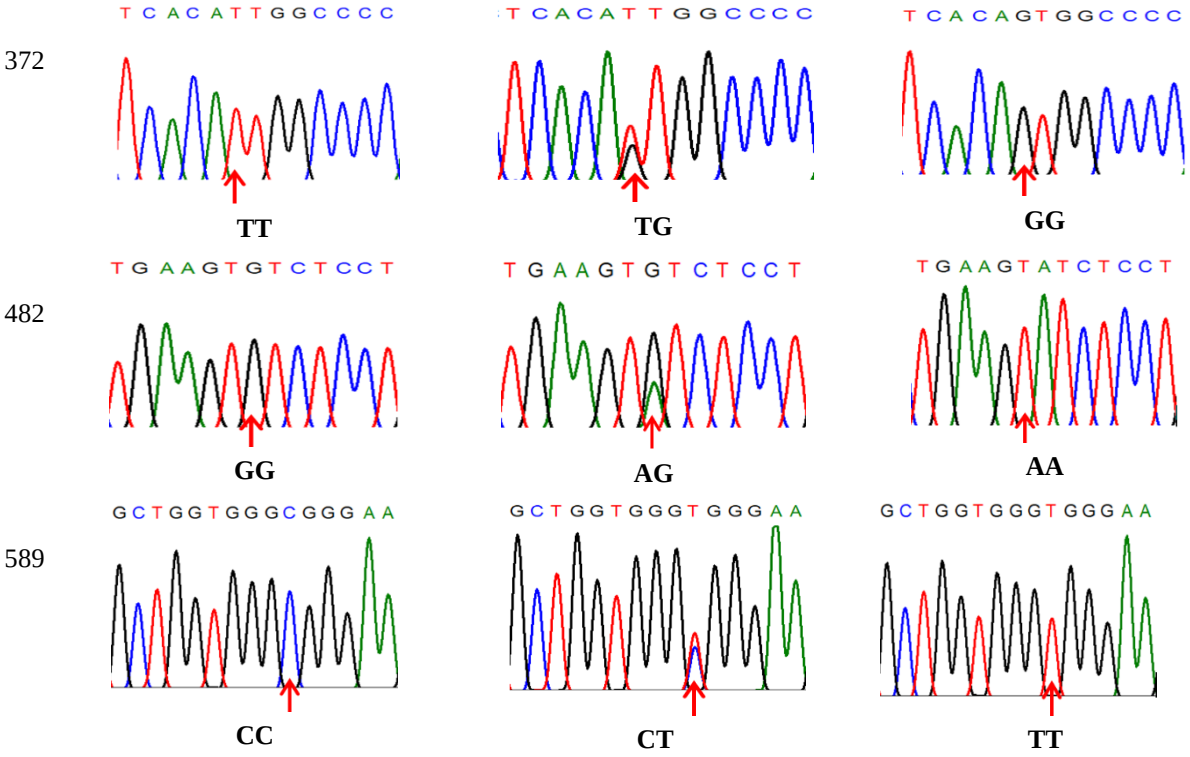


Figure 3. Chromatogram results and SNPs found in the STAT5A gene in PO cattle in Rembang Regency

Table 3. Genotype and allele frequencies of STAT5A gene SNP

SNP	Freq Genotipe (n = 79)			Freq Allele (n = 79)		Ho	He	X^2 Result	X^2 Table 0.05
	CC	CG		C	G				
g.100 C>G	0.987	0.013	0.000	0.994	0.006	0.013	0.013	0.003	5.99
	CC	CT	TT	C	T				
g.117 C>T	0.747	0.228	0.025	0.861	0.139	0.228	0.240	0.193	5.99
	CC	CT	TT	C	T				
g.232 C>T	0.671	0.304	0.025	0.823	0.177	0.304	0.292	0.138	5.99
	CC	CT	TT	C	T				
g.234 C>T	0.671	0.304	0.025	0.823	0.177	0.304	0.292	0.138	5.99
	CC	CT	TT	C	T				
g.336 C>T	0.873	0.101	0.025	0.924	0.076	0.101	0.140	6.129*	5.99
	CC	CT		C	T				
g.351 C>T	0.873	0.127	0.000	0.937	0.063	0.127	0.119	0.361	5.99
	TT	TG	GG	T	G				
g.372 T>G	0.620	0.291	0.089	0.766	0.234	0.291	0.359	2.801	5.99
	GG	AG	AA	G	A				
g.482 G>A	0.785	0.190	0.025	0.880	0.120	0.190	0.212	0.832	5.99
	CC	CT	TT	C	T				
g.589 C>T	0.785	0.190	0.025	0.880	0.120	0.190	0.212	0.831	5.99

SNP= single nucleotide polymorphism; n= number of samples. Degree of freedom is 2; X2r > X2t (0.05)= significant (*)

Most of the SNPs in the population were in HWE conditions; this may be because there is no accumulation of genotypes, selection, mutation, migration, and the same mating in the population studied so that in the population, there is a balance in the frequency of genotypes or alleles. If selection, migration, mutation, and genetic drift are not found, then the genotype frequency of a large enough population will always be balanced. The occurrence of random gamete fusion causes the genotype and allele frequencies to remain constant from the previous generation to the next generation, so it can be said that the population is in Hardy-Weinberg's law equilibrium (Yurnalis & Sarbaini 2014).

Association of STAT5A gene with reproductive traits

Based on the PCA analysis of five reproductive parameters (S/C, CI, DO EPP, and estrus duration), the results showed that the differentiating factors were S/C, CI, DO, and EPP, while the duration of estrus is not a differentiating factor. The four parameters were subjected to lambda contingency analysis to find associations between reproductive parameters in the genotype SNP sequencing results (Table 4). Lambda contingency analysis would be beneficial for finding the association or relationship between two device attributes. Each SNP can form 2-3 genotypes consisting of homozygotes and heterozygotes. For example, at site 100, there are 2 homozygous and heterozygous genotypes, while at site 117, there are 3 genotypes with 2 homozygotes and 1 heterozygous.

Based on the results of the analysis, it was found that there was an association between the STAT5A gene and the reproductive traits S/C, CI, DO, and EPP at SNP g.482 G>A with the strongest associations in GG, AG, and AA respectively; this is by the results of the study by Khatib et al. (2009), that STAT5A affects embryo survival at the development stage. The reproductive traits S/C determine the development stage and affect the value of CI, DO, and EPP. The study's results stated that mutation 153137 (G/C) in exon 8 of the STAT5A gene was significantly associated with the fertilization rate and embryo survival in embryonic compared to other SNPs tested. In other hand, no significant associations were found between reproduction traits and any of the studied polymorphisms, apart from age at first calving, for which STAT5A polymorphism (Oikonomu et al. 2011)

According to Liu et al. (2020), STAT5A is expressed in adipose tissue (lipids). Lipids play a role in synthesizing steroid hormones, including hormones that regulate reproduction, namely androgen, estrogen, and progesterone. These hormones, especially progesterone, function to maintain pregnancy. So, the association of the STAT5A gene with reproductive parameters is related to hormonal mechanisms.

The study by Paramitasari et al. (2015) showed that the STAT5A AvaI locus has a monomorphic C allele. The heterozygosity value in the STAT5A gene can be ascertained by the presence of the AvaI restriction site (C|CCGAG). SNP analysis showed that mutations in the STAT5A gene promoter region could be an early candidate for selection markers for reproductive traits in Bali cattle. Further validation studies are needed to prove the role of the STAT5A gene in genomic selection (Juniarti 2015). Selvaggi et al. (2013) stated that STAT5A is an important mediator in the lactogenic hormone response and a candidate marker for lactation traits in cattle.

Table 4. Association analysis of SNPs STAT5A gene with reproductive traits S/C, CI, DO and EPP

Mutation	Traits	P Value	Mean±SEM		
			CC (n=78)	CG (n=1)	
g.100 C>G	S/C	0.996	1.35±0.47	1.50±0	
	CI (day)	1	379.36±25.16	370.00±0	
	DO (day)	1	100.60±22.42	90.00±0	
	EPP (day)	0.991	95.77±22.19	90.00±0	
			CC (n=59)	CT (n=18)	TT (n=2)
g.117 C>T	S/C	0.968	1.36±0.49	1.36±0.49	1.00±0
	CI (day)	0.979	380.92±22.47	374.78±15.99	370.00±0
	DO (day)	0.905	102.56±24.00	94.78±15.99	90±0
	EPP (day)	0.901	98.16±24.17	88.33±12.49	90±0
			CC (n=53)	CT (n=24)	TT (n=2)

Mutation	Traits	P Value	Mean±SEM		
g.232 C>T	S/C	0.991	1.37±0.50	1.34±0.43	1.00±0
	CI (day)	0.999	379.68±25.66	379.04±25.03	370.00±0
	DO (day)	0.996	100.70±22.41	100.83±23.29	90±0
	EPP (day)	0.648	95.66±22.06	96.25±23.37	90±0
			CC (n=53)	CT (n=24)	TT (n=2)
g.234 C>T	S/C	0.991	1.31±0.50	1.34±0.43	1.00±0
	CI (day)	0.999	379.68±25.66	379.04±25.03	370.00±0
	DO (day)	0.996	100.70±22.41	100.83±23.29	90±0
	EPP (day)	0.648	95.66±22.06	96.25±23.37	90±0
			CC (n=69)	CT (n=8)	TT (n=2)
g.336 C>T	S/C	0.981	1.39±0.49	1.06±0.18	1.00± 0
	CI (day)	0.993	381.07±25.19	365.75±23.30	370.00±0
	DO (day)	0.993	102.28±25.19	87.50±19.82	90±0
	EPP (day)	0.576	96.96±22.51	86.25±19.23	90±0
			CC (n=69)	CT (n=10)	
g.351 C>T	S/C	0.435	1.36±0.48	1.32±0.42	
	CI (day)	0.502	379.80±25.82	375.40±19.20	
	DO (day)	0.391	100.87±23.39	97.70±12.99	
	EPP (day)	0.759	96.07±23.34	93.99±9.49	
			TT (n=49)	TG (n=23)	GG (n=7)
g.372 T>G	S/C	0.347	1.39±0.53	1.36±0.53	1.07±0.19
	CI (day)	0.902	381.76±28.26	376.22±20.71	371.57±4.16
	DO (day)	0.588	102.88±25.00	98.09±18.75	91.43±3.78
	EPP (day)	0.832	98.57±25.25	91.30±16.87	90±0
			GG (n=62)	AG (n=15)	AA (n=2)
g.482 G>A	S/C	<0.001	1.37±0.49 [a]	1.29±0.43 [b]	1.45±0.07 [c]
	CI (day)	<0.001	382.75±25.86 [a]	364.80±17.31 [b]	385.00±21.21 [c]
	DO (day)	<0.001	100.03±22.30 [a]	100.40±23.38 [b]	114.50±20.51 [c]
	EPP (day)	0.316	95.39±22.11 [a]	106.00±25.01 [b]	90.00±0 [c]
			CC (n=62)	CT (n=15)	TT (n=2)
g.589 C>T	S/C	0.993	1.39±0.49	1.23±0.42	1.25±0.35
	CI (day)	0.79	379.65±23.43	378.07±32.96	375.50±7.78
	DO (day)	0.375	100.27±20.97	102.00±20.08	95.00±7.07
	EPP (day)	0.186	94.84±20.47	100.00±29.28	90.00±0

S/C= service/conception; CI= calving interval CI; DO= days open; and EPP= estrus post partus; ED= estrus duration. Different superscript letters between Mean ± SEM indicate a significant difference (P<0.05)

The research of Nestor at al. (2020) stated that STAT5A polymorphism 19:42407732 was not associated with the reproductive parameters evaluated in this study of a Holstein cow herd in Mexico. Genotype AG of COQ9 polymorphism 18:25527339 was associated with lower SPC and is suggested as a molecular marker to improve Holstein cow reproductive performance in dairy herds in Mexico. A Chi-square test by Basant et al. (2022) showed that the Egyptian water buffalo population was in Hardy-Weinberg equilibrium. STAT5A gene might be a potential molecular marker for effective animal selection and breeding programs.

Sudhakar (2021) said that Significant associations were not observed in support of *STAT5A* as a marker for milk production traits in either Ongole or crossbred cattle of indicine admixture, and no reason could be found to consider this locus as a universal marker for milk production traits in indicine cattle and buffaloes. Considering the monomorphic nature of the gene in buffaloes and their higher milk fat content than bovine milk much remains to be explored regarding the underlying differences across the bovine and the bubaline species. Tina et al. (2022) stated that it is evident from this investigation that STAT5A polymorphism could be a promising indirect marker to improve milk production in crossbred cattle of Kerala.

CONCLUSION

There are 9 SNPs identified in this study, which are g.100 C>G; g.117 C>T; g.232 C>T; g.234 C>T; g.336 C>T; g.351 C>T; g.372 T>G; g.482 G>A and g.589 C>T). Most SNPs (eight of the nine) were not significantly different in the Chi-square (X2) results, meaning the population was in HWE conditions. There is an association of the STAT5A gene with the reproductive parameters S/C, CI, DO, and EPP at SNP g.482 G>A so that it can be used as a marker in the implementation of the selection of superior Ongole Grade Cow of Rembang.

ACKNOWLEDGEMENT

The Author is very grateful to the institution and all parties for assistance in terms of materials and locations to carry out this research. The Universitas Diponegoro Grant has supported this research no. 4/UN7.F5/HK/III/2023.

REFERENCES

Asmarasari SA, Sumantri C, Mathius IW, Anggraeni A. 2014. Diacylglycerol Acyltransferase1 gene polymorphism and its association with milk fatty acid components in Holstein Friesian dairy cattle. JITV. 19:159-167.DOI: 10.14334/jitv.v19i3.1078.

Basant M, Sherif I, Ramadan , Mohamed E, Eman M, Abd EF. 2022. Polymorphism of the Signal Transducer and Activator of Transcription 5A (STAT5A) gene in Egyptian water buffaloes using the SSCP technique. BJAS. 7:173-177. DO:10.21608/bjas.2022.244768.

[BSN] Badan Standarisasi Nasional. 2015. SNI 7651.5:2015. Bibit sapi potong. Bagian 5: Peranakan ongole. Jakarta (Indones): Badan Standarisasi Nasional.

Busyro, Nurhantari Y. 2020. Data genetik dari empat lokus Short Tandem Repeat (STR): TPOX, D3S1358, D7S820, dan CFS1PO pada populasi Indonesia. J Indones Forensic Legal Med. 2:150-155.

Indahwati A, Kurnianto E, Setiatin ET, Samsudewa D. 2022. Evaluation of breeder preferences on reproduction aspects of Ongole Grade in the breeding area of Rembang Regency. IOP Conf. Ser.:Earth Environ. Sci. 1246: 012073. DOI:10.1088/1755-1315/1246/1/012073.

Juniarti R. 2015. Identifikasi Keragaman Gen Signal Transducer And Activator of Transcription 5a (STAT5A) Promoter p ada Sapi Bali [Thesis]. Bogor (Indones): Faculty of Animal Science, IPB Unversity.

Khatib H, Maltecca C, Monson RL, Schutzkus V, Rutledge JJ. 2009. Monoallelic maternal expression of STAT5A affects embryonic survival in cattle. BMC Genet. 10. DOI:10.1186/1471-2156-10-13.

Lestari DA, Sutopo, Setiaji A, Kurnianto E. 2023. Genetic diversity and phylogenetic study of Ongole grade cattle population in Central Java based on blood protein polymorphism. Biodiv. 24:617-624. DOI:10.13057/biodiv/d240170.

Liu W, Ji Y, Xhang B, Chu H, Yin C, Xiao Y. 2020. STAT5A promotes brown adipocyte differentiation and thermogenic program through binding and transactivating the Kdm6a promote. National Library of Medicine. 19: 895-905. DOI:10.1080/15384101.2020.1731644.

Michel RNG, Ayala VMÁ, Galindo GJ, Duifhuis RT, Sánchez CDR and Valencia PM. 2020. Effect of COQ9 and STAT5A polymorphisms on reproductive performance in a Holstein cow herd in Mexico. Animal Reproduction 17:e20200039. DOI:10.1590/1984-3143-AR2020-0039.

Michel-Regalado NG, Ayala-Valdovios MÁ, Galindo-García J, Duifhuis-Rivera T, Sánchez-Chiprés DR, Valencia-Posadas M. 2020. Effect of COQ9 and STAT5A polymorphisms on reproductive performance in a Holstein cow herd in Mexico. J Anim Reprod. 4:e20200039. DOI:10.1590/1984-3143-AR2020-0039.

Nei M, Kumar S. 2000. Molecular evolution and phylogenetics. New York (USA): Oxford University Pr.

Oikonomu G, G Michailidis, A Kougioumtzis, M. Afdi, G Banos. 2011. Effect of polymorphisms at the STAT5A and FGF2 gene loci on reproduction, milk yield and lameness of Holstein cows. Research in Veteribary Science 9 (2):235-239.

Panjono, Priyanti A, Aryogi, Putra ARS, Atmoko BA, Maulanan H, Prabowo BW. 2022. Kinerja induk sapi peranakan ongole di Kecamatan Kragan Kabupaten

Rembang. J Ilmiah Fillia Cendekia. 7:66-71. DOI:10.32503/fillia.v7i1.2344.

Paramitasari KA, Sumantri C. 2015. The genetic variability of prolactin and signal transducers and activators of transcription 5A (STAT5A) genes in Bali Cattle. MedPet.38:1-11. DOI:10.5398/medpet.2015.38.1.1.

Pratiwi WR, Panjono, Bintara S. 2022. Kinerja induk sapi PO, Simpo dan Limpo di Kabupaten Rembang, Jawa Tengah. [Thesis]. Yogyakarta (Indones): Gadjah Mada University.

Prihandini PW, Primasari A, Luthfi M, Pamungkas D. 2020. Identifikasi keragaman gen follicle stimulating hormone sub-unit beta (FSH-β) pada tiga sumber daya genetik sapi lokal Indonesia. In Prosiding Seminar Nasional Teknologi Peternakan dan Veteriner.Bogor (Indones): ICARD. pp. 183-191

Putri RF, Nugraha CD, Furqon A, Septian WA, Suyadi S. 2021. Identifikasi keragaman genetik gen inhibin subunit alpha (INHA) dan inhibin subunit beta A (INHBA) pada sapi peranakan Ongole (PO). JTRAPO. 22:69-76. DOI:10.21776/ub.jtapro.2021.022.01.9.

Selvaggi M, Tufarelli V, Pinto F, Centoducati G, Dambrosio A, Santacroce MP, Dario C. 2013. Bovine STAT5A gene polymorphism analysis and its association with milk composition traits in Jersey cows. Int J Biosci Biochemist Bioinform. 3:341-344.

Sudhakar K, Aranganoor TK, Ramasamy S, Nagarajan M. 2021. Single nucleotide polymorphism in STAT5A could not endorse variation in milk production traits in Indian bovine population. Acta Vet Hungarica. 60:324–333. DOI:10.1556/004.2021.00046.

Sudaryanto AT, Sutopo, Kurnianto E. 2018. Keragaman fenotipe sapi peranakan Ongole di wilayah sumber bibit di Jawa Tengah. J Vet. 19:478-487. DOI:10.19087/jveteriner.2018.19.4.478.

Sudrajad P, Volkandari SD, Cahyadi M, Prasetyo A, Komalawati, Wibowo S, Subiharta. 2021. Pemanfaatan informasi genom untuk eksplorasi struktur genetik dan asosiasinya dengan Performansn ternak di Indonesia. Livest Anim Res. 19:1-12. DOI:10.20961/lar.v19i1.47658.

Sutiyono, Sutopo, Ondho YS, Setiatin ET, Samsudewa D, Suryawijaya A, Lestari DA, Kurnianto E. 2018. Short communication : genetic diversity of Ongole grade cattle of Rembang District, Central Java, Indonesia, based on blood protein polymorphism. Biodiversitas. 19:1429-1433. DOI:10.13057/biodiv/d190432.

Tina S, Jamuna V, Kulangara, Anilkumar, Aravindakshan. 2022. Polymorphism of bovine STAT5A gene and its association with milk production traits in crossbred cattle of Kerala, India. IJANS. 92: 746–750.

Yurnalis, Sarbaini. 2014. Keragaman sekuen gen reseptor hormone pertumbuhan Exon 10 sebagai informasi dasar seleksi pada sapi Pesisir plasma nuftah Sumatera Barat. J Peternak Indones. 16:63-70. DOI:10.25077/jpi.16.1.63-70.2014.