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SINGLE NUCLEOTIDE POLYMORPHIMS (snps) IDENTIFICATION OF INHIBIN SUB UNIT-α (inha) GENE ON MADURA BULLS

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ABSTRACT

INHA gene is a gene that is suggested to have role in reproductive system. Single Nucleotide Polymorphisms (SNPs) of Madura Bulls were identified in this study. Polymerase Chain Reactions (PCR) was used to amplify INHA gene region and MEGA 7 program was utilized to align the amplified region sequences with sequence from Ensembl database. Four SNPs found in INHA and they are located at the first exon. Two SNPs were misssense mutations that causing the substitution of amino acid leucine^{21} by proline, and amino acid valine^{63} by methionine and the other two SNPs were synonymous mutation. One of the synonymous SNPs was a novel mutation. Based on those identified SNPs, they could be suggested as potential candidate markers of reproduction traits for Madura bulls. Moreover, through heterozygosity value from the observed bulls, it was indicated that the genotype was varied in population. Therefore a molecular selection program could be designed to determine the Madura superior bull.

***Keywords**: INHA, polymorhphisms, heterozygosity, Madura bulls*

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INTRODUCTION

Madura is one of Indonesian indigenous cattle. They has known with their good adaptabilities to poor environment, and their immunity with various typical wet-tropical parasite and disease. Therefore, efforts to improve productivity of Madura cattle are necessary. One way to enhance the productivity of Madura cattle is by select bull with good reproduction traits.

Inhibin is a transforming growth factor β (TGFβ) superfamily member that have role in reproduction by affecting the modulation of pituitary FSH, the proliferation of spermatogonia and Leydig cell testosterone production (Hiendleder et.al., 2000; Stenvers and Findlay, 2010). Inhibin contains an α-subunit (INHA) and a βA (INHBA) or βB (INHBB) subunit to form inhibin A and inhibin B, respectively. In cattle, INHA located on chromosome 2 (at 107,501,784-107,504,801). Some studies had observed the INHA polymorphisms in bull (Chandra et al., 2020; Sang et al., 2011). However, there are no study on the polymorphisms in Madura bull. Considering the effect of INHA on bull reproduction traits, it should be important to conduct study on identifying polymorphisms of INHA gene. Study on inhibin α subunit (INHA) reported that silencing the transcription and translation of this gene may result on increased number Sertoli Cells (SC) in the G phase stage and decreased the number of SCs in S-phase due to inhibition of the cell-cycle promoter Cyclin D1and Cyclin E and promote the cell cycle-inhibitor P21 (Cai et. al, 2011).

MATERIAL AND METHODS

Materials used for this study were blood from 58 Madura bulls at Pamekasan with the age varies between one to more than four years old.

DNA extraction

DNA was isolated from the blood sample. The blood was collected in vacuntainer tube that contain EDTA to stabilize and preserve the blood. Once, the blood has been collected, DNA isolation or extraction will be accomplished. DNA extraction was carried out using a mini kit for blood (Geneaid).

DNA amplification

Before the DNA is amplified, PCR primers need to be designed first. The primers of INHA gene were designed based on study by Sang et.al. (2011) and it is located only at the first exon. The primers sequence are 5'-CTATGTGGCTTCAGCTGCTC-3' (forward) and 5'-GGTCTGGGATTCAACCCAAC-3' (reverse). PCR product is 358 bp.

The Amplification was conducted using BIO RAD T100 Thermal Cycler PCR machine. Sample volume The DNA used is 1 µl. Total volume of reagent amplification of 30 µl consisting of 15 µl of go taq green master mix, primary labels forward and reverse respectively 0.3 µL and 14.4 nuclease free water (NFW) and sample DNA. The PCR condition used for amplication is: initial denaturation of double strand band at 95°C for 5 minutes, followed of 35 cycles of :denaturation at 95°C for 10 seconds; primer annealing at; extension at °C for 30 seconds and final extension at °C for 10 minutes. Once after the amplification is completed, the amplidies product need to be confirmed by gel electrophoresis using 1.5% agarose gel electrophoresis in 0.5x TBE buffer for 40-45 minutes at 110 V. Gels are stained using *Nucleic Acid Dye* 0.5 µg/ml for 20-30 minutes and the product were visualized under UV illumination.

Sequencing and polymorphisms identification

Genes fragment sequencing was carried out using a sequencer machine (ABI Prims 3100-Avant Genetic Analyzer) at 1st Base sequencing company, Selangor, Malaysia. The sequencing results then be interpreted using *BioEdit p*rogram and confirmed by *Molecular Evolutionary Genetic Analysis* 5 (MEGA 5) in order to be able to identify the polymorphism

RESULTS AND DISCUSSION

Four polymorphisms of INHA gene were identified in this study and all of them were in the form of Single Nucleotide Polymorphisms (SNPs). Two of the SNPs (INHA SNP1 and INHA SNP3) were missense mutation which cause changes in amino acids; and the other two SNPs were synonymous mutation (Table 1). There was substitution of amino acid leucine[21] by proline for INHA SNP1 and substitution of amino acid valine[63] by methionine for INHA SNP3. Most of the SNP were not novel SNPs since they had been recorded in the Ensembl data base, only 1 SNP (INHA SNP4) was a novel mutation.

Table 1. Identified INHA SNPs

Gene	DNA variant name	Sequence context	Genomic location	Type of variant
INHA	INHA SNP1	GGGC(T/C)GGAG	107501905	Missense variant ($L^{21}\rightarrow P$)
	INHA SNP2	CACCG(G/A)AGGCA	107502020	Synonymous variant
	INHA SNP3	ATGCC(G/A)TGGG	107502030	Missense variant ($Val^{63}\rightarrow Met$)
	INHA SNP4	AGCC(C/G)GAGGA	107502062	Synonymous variant

Among those four SNPs, only one SNP of INHA gene (INHA SNP2) has been identified in bulls, and Antioquia Holstein cows (Sang *et al.*, 2011; Madrid et al, 2015). Sang et al., (2011) had indicated that INHA SNP2 associated with bull semen quality. However the other SNPs had not been observed their association with reproduction traits. After the SNPs had been identified, the genotype and allele frequency of each SNP can be calculated. Most of the identified SNP has only two genotype, except for INHA SNP3 that has three genotype (Table 2). There are only two bulls carry GA genotype for INHA SNP2. The G allele frequency for INHA SNP2 is much lower than A allele frequency and it is due no GG genotype found for this SNP. This trend also occurred for INHA SNP3 which only had few heterozygote bulls and had low A allele frequency because there are one two bulls carried AA genotype.

Table 2. Allele frequency and Hardy-Weinberg Equilibrium of INHA SNPs

SNPs	Genotype			Allele frequency	
INHA SNP1	TT	TC	CC	T	C
	37	21	0	0.82	0.18
INHA SNP2	AA	GA	GG	A	G
	56	2	0	0.98	0.02
INHA SNP3	AA	GA	GG	A	G
	2	8	48	0.10	0.90
INHA SNP4	CC	CG	GG	C	G
	36	22	0	0.81	0.19

Table 3. Heterozigosity and Hardy-Weinberg equilibrium of INHA SNPs

SNPs	Heterozigosity		Hardy-Weinberg	
	Observation	Expectation	x^2	x^2table
INHA SNP1	0.362	0.297	2.834127	3.841
INHA SNP2	0.034	0.034	0.017852	
INHA SNP3	0.138	0.185	3.813281	
INHA SNP4	0.379	0.307	3.177003	

Heterozygosity and Hardy-Weinberg Equilibrium

In this study, heterozygosity value was used to determine the level of genetic diversity based on the allele frequencies in Madura bull population observed. This analysis compared the heterozygosity observation with the heterozygosity expectation. The information could be used to support the effectiveness of bull selection program in SNAIC. The heterozygosity observation (Ho) and heterozygosity expectation (He) of the SNPs were showed in Table 3. The Ho and He values were found below 0.500 in recent study. For the SNP3, the Ho value was lower than the He value. Otherwise, the Ho of SNP1 and SNP4 were higher than the He values. This result could be indicated the bull population had a good level of INHA gene polymorphism. The higher Ho than He value indicated that the genotype was varied in population (Sharma *et al*. 2016). Based on this variation of INHA gene, a molecular selection program could be designed to determine the Madura superior bull.

Genetic diversity has an important role in a selection program. The good animals could be selected from the diverse population. Chi-square analysis could be used to determine genotype and allele frequencies and to indicate the population equilibrium known as Hardy-Weinberg Equilibrium (Anggraeni *et al*. 2017). The result showed chi-square value of SNP1, SNP2, SNP3, and SNP4 were 2.834; 0.018; 3.813; and 3.177 respevtively. All chi-square values were not significantly different ($\chi^2 < \chi^2$table) in Madura bull population. It could be stated the Madura bull population was in Hardy-Weinberg equilibrium. This means that the random mating was probably occurred and the allele frequencies was constant from generation to generation in Madura bull population. Moreover, the population in Hardy-Weinberg equilibrium showed there were no selection, mutation, migration, and genetic drift,

CONCLUSION

In conclusion, there were four SNPs identified for INHA gene at exon 1. Two of the SNPs (INHA SNP1 and INHA SNP3) were missense variant which alterned the amino acids, while the other two SNP were synonymous variant. Madura population was in Hardy-Weinberg equilibrium due to the random mating. Based on the measured heterozygosity value, it could be suggested that the genotypes were varied in population. Therefore a molecular selection program could be designed to determine the Madura superior bull.

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