

Single nucleotide polymorphisms (SNPs) in bovine follicle stimulating hormone receptor (bFSHR) gene in Pasundan cattle

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Bovine follicle stimulating hormone receptor (bFSHR) gene is one of the candidate genes that can be used as a molecular selection for reproductive traits in cattle (Yang *et al.* 2010). The bFSHR gene plays an important role in follicular development or folliculogenesis (Dierich *et al.* 1998, Hamny *et al.* 2017). Activation of FSHR is necessary for the hormonal functioning of FSH and is found at high levels in the ovaries and testes of mammals (Houde *et al.* 1994). The bFSHR gene of *Bos taurus* is located in chromosome 11 consisting 10 exons and 11 introns (Simoni *et al.* 1997, Rahal *et al.* 1999). Allelic variants in bFSHR gene were reported in several breeds cattle such as Holstein Friesian (Shirasuna *et al.* 2011, Cory *et al.* 2012, Arslan *et al.* 2017), Chinese Holstein (Yang *et al.* 2010) composite beef cattle (Marson *et al.* 2008, Hernandez-Cruz *et al.* 2009) and indigenous Turkish breeds (Arslan *et al.* 2015).

Previous studies reported that SNP in bFSHR gene located in exon 10 of bFSHR gene (Marson *et al.* 2008, Hernandez-Cruz *et al.* 2009). This SNP can be genotyped using *AluI* restriction enzyme (ag*ct) through PCR-RFLP analysis. Several studies reported that this locus (bFSHR/*AluI*) gene was associated with first ovulation post partum (Shirasuna *et al.* 2011) and number inseminations per pregnancy (Arslan *et al.* 2017). Therefore, the polymorphism of bFSHR/*AluI* gene was occurred in indigenous Turkish cattle (Arslan *et al.* 2015), European-Zebu composite (Marson *et al.* 2008), Holstein cattle (Shirasuna *et al.* 2011), Sudanese cattle (Omer *et al.* 2016) and buffalo (Sosa *et al.* 2015).

There are few literature study that explain the relationship between polymorphism in bFSHR/*AluI* gene and reproductive traits, included in indigenous Indonesian breeds cattle (Arifin *et al.* 2019). Pasundan cattle is one of Indonesian native cattle from West Java province having limited population. This cattle population was decided to

Indonesian native cattle based on Ministerial Decree No: 1051/Kpts/SR.120/10/2014. Phenotypic characterization of Pasundan cattle revealed it to be a combination between *Bos javanicus* (Bali cattle) and *Bos indicus* (Ongole and Madura cattle) and developed by crossbreeding from both subspecies in last hundred years (Said *et al.* 2017).

Pasundan cows (77) from the breeding center at Ciamis Regency, West Java (BPPIBT-SP Ciamis) were used for polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis. 306 bp of PCR products were amplified using primer pairs of Forward: 5'-CTG CCT CCC TCA AGG TGC CCC TC-3' and Reverse: 5'-AGT TCT TGG CTA AAT GTC TTA GGG GG-3' (Houde *et al.* 1994). The thermal cycling conditions consisted of one cycle of 95°C for 1 min, followed by 35 cycles of 55°C for 15 sec and 72°C for 10 sec with a final extension at 72°C for 5 min. The PCR reaction was performed in 10 µl volume consisting of 5 µl PCR mix (MyTag™ HS Red Mix, USA), 0.2 µl of each primer and 4.0 µl of DNA. The PCR products were digested with 0.28 µl of *AluI* (10 U/µl, 1000 U) restriction enzyme at 37°C for 1 h and separated in 2% agarose gel in 1 × TBE (Tris-Borate-EDTA) buffer and stained with GelRed (Biotium, USA) for visualization under UV light using G-BOX documentation system (Syngene, UK). Sequencing analysis was performed by commercial laboratory service (First BASE Laboratory, Malaysia) to obtain the bFSHR gene in Pasundan cattle.

bFSHR locus in Pasundan cattle is polymorphic with moderate polymorphic informative content (PIC) value (0.30<PIC<0.50) as presented in Table 1. Moreover, three genotypes namely CC (243 and 63 bp), GG (193 and 63 bp) and CG (243, 193 and 63 bp) were detected in this study as presented in Fig. 1. The frequency of G allele in the bFSHR/*AluI* of Pasundan cattle was higher than C allele and reveals that GG genotype as the dominant genotype. The moderate PIC value of bFSHR/*AluI* has been reported in several breeds cattle, i.e. Zebu × British composite breeds (0.37), and 0.34 for indigenous Turkish breeds (Marson *et al.* 2008, Arslan *et al.* 2017). Frequency of G allele of bFSHR/*AluI* locus was higher in *Bos taurus*, *Bos*

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Table 1. Genetic diversity of three SNPs in the bFSHR gene of Pasundan cattle

SNP	Genotypic frequency	Allelic frequency	H _e	H _o	n _e	PIC	χ ²
CC (0.10)	C (0.24)	0.36	0.27	1.57	0.30	52.21	
g.2037C/G	CG (0.27)	G (0.76)					
	GG (0.63)						
g.2071C/T	CC (0.02)	C (0.17)	0.28	0.29	1.40	0.24	0.05*
	CT (0.29)	T (0.83)					
	TT (0.68)						
g.2119A/C	AA (0.10)	A (0.29)	0.41	0.39	1.71	0.33	0.14*
	AC (0.39)	C (0.71)					
	CC (0.51)						

SNP, single nucleotide polymorphism; H_o, observed heterozygosity; n_e, number of effective allele; PIC, polymorphic informative content; *under Hardy-Weinberg equilibrium ($\chi^2 < 5.991$).

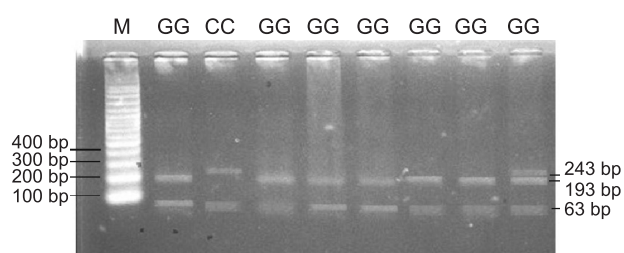


Fig. 1. Detection SNP of g.2037C/G in the bFSHR gene of Pasundan cattle using PCR-RFLP analysis with *AluI* restriction enzyme showed three genotype namely CC (243 and 63 bp), GG (193 and 63 bp) and CG (243, 193 and 63 bp), M, 100 bp DNA ladder.

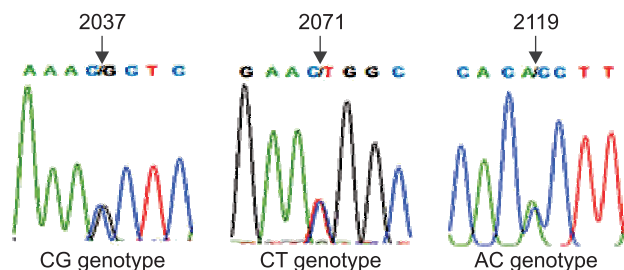


Fig. 2. Partial sequence chromatograms in the exon 10 of bFSHR gene (GenBank: NM_174061) in Pasundan cattle.

indicus and their crossbreds (Marson *et al.* 2008, Hernandez-Cruz *et al.* 2009). bFSHR/*AluI* locus was monomorphic (CC) in buffaloes (Sosa *et al.* 2015) and *Bos indicus* cattle in Sudan, i.e. Butana, Kenana and Erashy (Omer *et al.* 2016).

Sequencing analysis of partial exon 10 in bFSHR gene from 41 heads of Pasundan cattle revealed two novel single nucleotide polymorphisms (SNPs) at position g. 2071C/T and g.2119A/C (Fig. 2). Milazzotto *et al.* (2000) reported other two novel SNP at position g.1506T/C and g.1539C/T (GenBank: L22319) in bFSHR gene in Nellore cattle (*Bos indicus*).

Present study was conducted to identify reported polymorphism in the exon 10 of bFSHR gene in Pasundan cattle (g.2037C/G) using PCR-RFLP. This SNP had

moderate PIC value (0.30). Sequencing analysis revealed two novel SNPs at g.2071C/T (PIC=0.24) and g.2119A/C (PIC=0.33). Further work for association between bFSHR/*AluI* polymorphism and reproductive traits of Pasundan cattle is important.

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