

# Identification of *FecX<sup>G</sup>* and *FecB* Mutations and its Association with Litter Size in Kacang and Boerka Goat

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**Abstract** | The Kacang goat is one of Indonesia's prolific native goat breeds, while the Boerka goat is a crossbreed between Kacang and Boer goats. It is widely known that the ovulation rate and litter size in sheep breeds are significantly impacted by the BMP15 (*FecX<sup>G</sup>*) and BMPR-1B (*FecB*) gene alterations. Based on this knowledge, the study's objective was to determine the presence of BMP15 and BMPR-1B gene polymorphisms and how they affected the prolific traits of Kacang and Boerka goats. This study indicates that the *FecX<sup>G</sup>* and *FecB* mutations were associated with litter size in Kacang and Boerka goat breeds. The *FecX<sup>G</sup>* has two genotypes in both Kacang and Boerka goats, namely homozygote wild type ++ and heterozygote mutant carrier G+ with a litter size of 1.56 and 1.65 (Kacang); 1.61 and 1.75 (Boerka), respectively. Meanwhile, *FecB* mutations produce three genotypes: homozygote wild type ++, heterozygote mutant carrier B+ and homozygote mutant BB with a litter size of 1.00, 1.37, and 1.65 (Kacang); and 1.00, 1.18, and 1.74 (Boerka), respectively.

**Keywords** | Goat, *FecX<sup>G</sup>*, *FecB*, Polymorphic, Litter size.

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## INTRODUCTION

In Indonesia, the increase in the goat population is generally related to empowering the economy of rural households, supporting food security, and improving the nutritional quality of families. Goats have great potential to increase rural and urban prosperity due to their ability to adapt well to tropical climates, have better productivity, and can utilize low-quality fiber (Kumar et al., 2016; Ahl-awat et al., 2015) so that it can be effortlessly maintained in a dry land farm.

The Kacang goat is one of the native Indonesian goats that have high fertility, with a litter size of 1.56–1.98 kids per birth, 52.2% for twins, and 2.6% for triplets (Pamungkas

et al., 2009). While the Boerka goat is a hybrid goat resulting from a cross between Kacang and Boer goat. As a hybrid breed, Boerka goats have the potential to be developed as meat-producing goats because of their better growth and meat quality. Boerka goats have a birth weight of 2.6–2.8 kg with a weaning weight of 10–12 kg, while the birth weight of Kacang goats was 1.6–1.8 kg with a weaning weight of 6–8 kg. Boerka goats also have prolific characteristics, with a litter size of 1.45–1.54 kids per birth (Mahmilia and Elieser, 2008).

Prolific traits are reproductive traits that have high economic value in goats, where high litter size can accelerate goat population growth. The tendency to produce twins or triplets in goats and sheep is the same, and this trait

is passed on to their offspring. The genetic mechanism of increasing litter size due to the FecB and FecX mutations has been widely reported. However, prolificacy regulation at the gene level is different in sheep and goats. Mutations in the BMP15 gene make granulosa cells more sensitive to FSH, accelerating the formation of follicular and early ovulation of tiny follicles, thereby increasing ovulation in ewes (Moore and Shimasaki, 2005). Granulosa cells and oocytes express the BMP receptor (BMPR) and then bind to BMP15. The ovulation rate is enhanced by one copy due to an additive effect of the BMPR-1B gene mutation (Pramod et al., 2013; Guo et al., 2018).

As in sheep, the genetics of prolificacy has also been studied extensively in goats. Several studies have shown that the BMP15 gene is also the primary gene that regulates prolificacy in goats, such as in Funiu white (Wang et al., 2011), Jining Gray (Chu et al., 2007), Beetal and Teddy (Islam et al., 2019), and Markhoz goats (Ghoreishi et al., 2019). Polymorphism in the BMPR-1B gene was also reported to be associated with prolific traits in Black Bengal (Polley et al., 2009), Beetal, and Teddy goats (Islam et al., 2019). On the contrary, Hua et al. (2008) showed none of the FecB and FecX polymorphism in prolific goat breeds, including Haimen, Huanghuai, Boer, Nubi and Matou.

Based on the abovementioned, the mechanism and regulation pathways of fecundity genes in prolific goats are not clear, so additional study is still required in various breeds and a large sample size of a goat. In goats, litter size is a reproductive trait with low heritability and is regulated by more than one gene. Therefore, it is crucial to provide scientific information for designing a set of DNA markers that can be useful for marker-assisted selection (MAS) in Kacang and Boerka goats.

## MATERIAL AND METHOD

### EXPERIMENTAL ANIMALS AND DNA ISOLATION

The experiments were carried out with a total sample was 211 female goats, consisting of 111 Boerka and 100 Kacang goats. All samples were collected at PT. Sadhana Arif Nusa, East Lombok, West Nusa Tenggara, Indonesia. For association analysis, the number of kids for every birth and the average litter size for each ewe of the first three parities were recorded. Blood samples were collected from each animal using a K2EDTA Venoject tube and preserved at -25°C. The Genomic DNA extraction was performed according to the Genomic DNA Mini Kit Geneid procedure.

### PCR AMPLIFICATION AND GENOTYPING

Primer 3.0 software was used to design two pairs of PCR primers based on the *Ovis aries* genbank database (GenBank access code: NC\_022322) and the published nucleotide

sequence of the caprine gene. The forward and reverse primers for exon 2 of BMP15 and exon 6 of BMPR1B genes are presented in Table 1.

DNA amplification was carried out in the Nexus Master-cycler PCR machine with a reaction volume of a 15 µl consisted 1 µl DNA, 0.5 µl Primers, 6.25 µl MyTaq HS Red Mix, 2x and 7.25 µl Water (dH<sub>2</sub>O). Amplification conditions were as follows: pre-denaturation conditions for 5 minutes at 95 °C, followed by 35 cycles of denaturation step at 95 °C for 10 s, annealing at 60 °C for 20 s, and elongation at 72 °C for 30 s; with the final elongation stage at 72 °C for 5 minutes in one cycle. About 5 µl of each PCR product were digested separately with HinfI 10 U for BMP15 and AvaII 10 U for BMPR-1B at 37 °C overnight. The digested product was separated by 2.5% agarose gel electrophoresis and visualized in an AlphaImager (Alpha Innotech Corporation, USA).

### DATA ANALYSIS

Popgene version 1.31 software was used to investigate the genotype and allele frequencies, heterozygosity, polymorphism information content, and Hardy-Weinberg equilibrium. SNP position determination was corrected to Ensembl's DNA sequence using the MEGA-X program (ENSCHIG00000024611). The association of genotypes with the litter size of Kacang and Boerka goats was examined using the General Linear Model procedure with SAS 9.1.3 software (SAS Institute, Cary, NC, USA). The linear model applied was:

$$Y_{iklm} = \mu + C_i + B_k + (BC)_{ik} + S_l + E_{iklm}$$

Where,  $Y_{iklm}$  is the trait observation of the  $iklm^{th}$  animals;  $\mu$  is the overall mean;  $C_i$  is the genotype effect of the  $i^{th}$ ;  $B_k$  is the breed effect of the  $k^{th}$ ;  $(BC)_{ik}$  is the interaction between the  $i^{th}$  and the  $k^{th}$ ;  $S_l$  is the sire effect of the  $l^{th}$ , and  $E_{iklm}$  is the random error.

## RESULT AND DISCUSSION

### POLYMORPHISM OF BMP15 GENE IN KACANG AND BOERKA GOAT

Amplifying the BMP15 gene in Kacang and Boerka goats produced DNA fragments (PCR products) with a length of 141 bp (Figure 1). The BMP15 gene mutation was identified by the RFLP method using the HinfI restriction enzyme with the G|ANTC cleavage site (Figure 2). The wild type of the exon 2 BMP15 gene sequence has a cleavage site for the HinfI enzyme that produces alleles with sizes of 111 bp and 30 bp (+ allele), respectively. The C/T transition mutation caused a change in the cleavage site of the HinfI enzyme at position 718 (c.718C>T) of the BMP-15 gene, which resulted in a mutant allele (G allele) with a size of 141 bp.

**Table 1:** Gene sequences and primers

| Gene   | Primer Sequence 5'-3'                                            | Amplicons | Restriction enzymes | Annealing temp. (°C) |
|--------|------------------------------------------------------------------|-----------|---------------------|----------------------|
| BMP15  | F: CACTGTCTTCTTGTACTGTATTTCAATGAC<br>R: GATGCAATACTGCCTGCTTG     | 141 bp    | HinfI               | 60                   |
| BMPR1B | F: GTCGCTATGGGGAAGTTTGGATG<br>R: CAAGATGTTTTCATGCCTCATCAACACGGTC | 140 bp    | AvaII               | 59                   |

F: forward, R: reverse

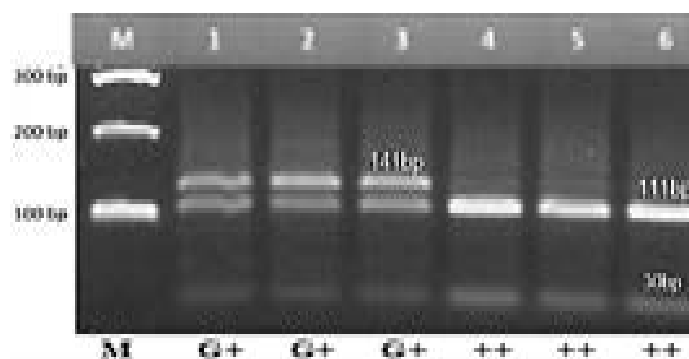
**Table 2:** Genotype and Allele Frequency of BMP15 gene on Kacang and Boerka Goats

| SNP Position | Goat Population | N   | Genotype Frequency |       |       | Allele Frequency |       |
|--------------|-----------------|-----|--------------------|-------|-------|------------------|-------|
|              |                 |     | GG                 | G+    | ++    | G                | +     |
| c.718C>T     | Kacang          | 100 | 0.000              | 0.330 | 0.670 | 0.165            | 0.835 |
|              | Boerka          | 111 | 0.000              | 0.396 | 0.604 | 0.198            | 0.802 |

N: Number of goats; HWE: Hardy-Weinberg equilibrium



**Figure 1:** Amplicons of BMP15 gene (lines 1-7) and marker 100 bp (M).



**Figure 2:** PCR-RFLP product of BMP15|HinfI gene (lines 1- 6) and marker 100 bp (M)

Identification of allele and genotype of the BMP15 gene using the method of RFLP-HinfI resulted in wild type + and mutant type G allele with homozygote wild type ++ and heterozygote mutant carrier G+ genotypes, while the homozygote mutant GG genotype was not identified on Kacang and Boerka goats (Figure 2). The allelic and genotypic frequency distributions indicate a very contrasting difference ( $P < 0.01$ ). In the two goat breeds, the genotype ++ was significantly higher than the G+, and the + allele was significantly higher than the G allele (Table 2). The presence of polymorphic sites for exon 2 of the BMP15 gene has been published in various breeds of goats, such as

Jining Grey (Chu et al., 2007); Guizhou White (Ran et al., 2009); Taihang black and Funiu white (Wang et al., 2011); Beetal and Teddy goats (Islam et al., 2019).

In this study, the homozygous mutant GG was not found in the Kacang and Boerka goat populations. Similarly, homozygous mutant GG also was not found in several goat breeds, such as Teddy goats (Islam et al., 2019), Markhoz goats (Ghoreishi et al., 2019), Taihang black (Wang et al., 2011), and Jining gray (Chu et al., 2007); and some breeds of sheep, including the Small Tailed Han (Chu et al., 2007), Belclare (Mullen et al., 2013), Barki and Rahmani (El-Seedy et al., 2017), six Egyptian sheep breeds (Saleh et al. 2020), and Lombok Fat Tail (Maskur et al., 2016). The Possible reasons need to be explained that no GG ewes were observed in the present study. A tendency towards accumulating and losing certain genotypes could be due to intensive selection. GG ewes may exist in the Kacang and Boerka breeds, but this research used experimental ewes with a litter record, so all infertile ewes were excluded. Another possible reason that GG ewes may not exist in the Kacang and Boerka breeds is that there are no reports of infertility among Kacang and Boerka goats, as in Belclare sheep.

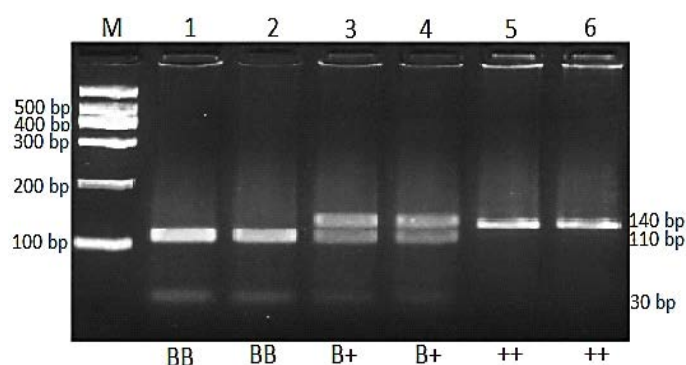
## POLYMORPHISM OF BMPR-1B GENE IN KACANG AND BOERKA GOAT

Amplifying the BMPR-1B gene in Kacang and Boerka goats produced DNA sequences with a length of 140 bp (Figure 3). The wild type sequence of exon 6 of the BM-PR-1B gene has no cleavage site for the AvaII restriction enzyme, so the wild type allele is 140 bp (+). Mutations at this locus are transition mutations that convert adenine into guanine at the base position 746 coding regions of the BMPR-1B gene (c.746A>G). These mutations cause the formation of a cleavage site for the AvaII restriction enzyme (G|GACC), so the digestion produces mutant alleles

measuring 110 and 30 bp (B) in length.



**Figure 3:** Amplicons of BMPR1B gene (lines 1-7) and marker 100 bp (M).



**Figure 4:** PCR-RFLP product of BMPR1B|AvaII gene (lines 1- 6) and marker 100 bp (M)

Identification of allele and genotype using the method of RFLP-AvaII resulted in two alleles, namely mutant B (110 bp and 30 bp) and wild + allele (140 bp), with three genotypes ++ (homozygote wild type), B+ (heterozygote mutant carrier), and BB (homozygote mutant) (Figure 4). The allelic and genotypic frequency distributions indicate a highly contrasting difference ( $P < 0.01$ ). The frequency of the wild + allele was significantly higher than the mutant B allele, and the genotypic ++ was significantly higher than B+ and BB in the two goats breed (Table 3). However, the previous research in several sheep breeds indicates the abundance of mutant alleles compared to wild alleles, such as in prolific Garole (Polley et al., 2010); Indonesian fat-tailed (Maskur et al., 2016); Small-Tail Han (Chu et al., 2007; Wen et al., 2021); and Bayanbulak sheep (Zuo et al., 2013).

Similar to this study, the FecB mutation has been previously found in several goat breeds, including Black Bengal (Polley et al., 2009), Markhoz (Shokrollahi and Moram-mazi, 2018), Beetal and Teddy goats (Islam et al., 2019). In contrast, it has not been found in some other goat breeds, including the Rayini goats of Iran (Gazooei et al., 2013), the prolific Raighar (Palai et al., 2013), the Indian Berari

and Surti goat (Sharma et al., 2016; Dangar et al., 2019), and some Egypt sheep breeds (Abulyazid et al., 2011; El-Seedy et al., 2017; Saleh et al., 2020). The possible reasons for the differences between the results obtained from different breeds may be due to: (1) the accumulation of certain genotypes and alleles as a result of intensive selection, (2) the possibility of inbreeding, and (3) the possibility of previous genes introgression as a breeding strategy.

### GENETIC DIVERSITY WITHIN THE GOAT BREEDS

The genetic diversity of a population is represented by the number of alleles, expected heterozygosity (He), observed heterozygosity (Ho), and Polymorphic Information Content (PIC) values as the fundamental data for individual and population discrimination (Seo et al., 2016). Data in Table 4 indicate a genotypic imbalance in the two goat populations where the observed heterozygosity was significantly different from the Hardy-Weinberg expectation agreement. In this study, the low level of Ho and He values revealed low genetic diversity in the two goat populations. The occurrence of heterozygosity deficits in the population is caused by selection pressure at certain loci and inbreeding.

The PIC value of the FecX<sup>G</sup> (c.718C>T) in both Kacang and Boerka goat breeds was 0.211 and 0.221, while FecB (c.746A>G) was 0.170 and 0.131, respectively. Bolstein et al. (1980), the PIC level classified as highly ( $PIC > 0.50$ ), reasonably ( $0.50 > PIC > 0.25$ ), and slightly ( $PIC < 0.25$ ) informative. Thus, in recent research, the PIC value in both BMP15 and BMPR1B was classified as slightly informative ( $PIC < 0.25$ ).

### ASSOCIATION OF THE FECX<sup>G</sup> AND FECB MUTATIONS WITH LITTER SIZE IN THE GOAT BREEDS

The mutations of FecX<sup>G</sup> (c.718C>T) and FecB (c.746A>G) were investigated as potential gene candidates for prolificacy in Indonesian Kacang and Boerka goats. The mutations and their influence on litter size and ovulation rates were initially found in sheep and subsequently identified in other species, such as goats. On the other hand, no FecB and FecX<sup>G</sup> mutations and/or their association with litter size were detected in earlier studies in various breeds of sheep and goats.

The influence of The FecX<sup>G</sup> (c.718C>T) mutation on goat litter size is apparent from the data of this study (Table 5). We found two genotypes in both goat breeds, namely homozygote wild type ++ and heterozygote carrier G+, in which the G+ had a higher number of kids than the ++ genotype. These findings are similar to the previous study done on Jining Grey goats, in which the heterozygote carrier AB had 1.13 more offspring than the homozygote AA ( $p < 0.01$ ) (Chu et al., 2007). Conversely, some other re

**Table 3:** Genotype and Allele Frequency of BMPR-1B gene on Kacang and Boerka Goats

| SNP Position | Goat Population | N   | Genotype Frequency |       |       | Allele Frequency |       |
|--------------|-----------------|-----|--------------------|-------|-------|------------------|-------|
|              |                 |     | BB                 | B+    | ++    | B                | +     |
| c.746A>G     | Kacang          | 100 | 0.040              | 0.130 | 0.830 | 0.105            | 0.895 |
|              | Boerka          | 111 | 0.027              | 0.099 | 0.874 | 0.077            | 0.923 |

N: Number of goats; HWE: Hardy-Weinberg equilibrium

**Table 4:** Genetic diversity measures in Kacang and Boerka goat populations across BMP15 (c.718C>T) and BMPR1B (c.746A>G) gene loci.

| Goat population | N   | SNP Position | Ho    | He    | se    | PIC   | X <sup>2</sup> (HWE) |
|-----------------|-----|--------------|-------|-------|-------|-------|----------------------|
| Kacang          | 100 | c.718C>T     | 0.278 | 0.239 | 0.024 | 0.211 | 3.905                |
|                 |     | c.746A>G     | 0.130 | 0.188 | 0.038 | 0.170 | 9.507                |
|                 |     | means        | 0.204 | 0.214 | 0.031 | 0.191 | <b>6.706</b>         |
| Boerka          | 111 | c.718C>T     | 0.298 | 0.253 | 0.024 | 0.221 | 6.782                |
|                 |     | c.746A>G     | 0.099 | 0.141 | 0.035 | 0.131 | 9.942                |
|                 |     | means        | 0.199 | 0.197 | 0.030 | 0.176 | <b>8.362</b>         |

N: sample size; Ho: observe heterozygosity; He: expected heterozygosity; PIC: polymorphic information content; and HWE: Hardy-Weinberg Equilibrium.

**Table 5:** Association of BMP15 (c.718C>T) and BMPR-1B (c.746A>G) gene polymorphism with litter size in the goat breeds

| Goat population | Locus    | Genotype | Number of individuals | Litter Size (LS)        |
|-----------------|----------|----------|-----------------------|-------------------------|
| Kacang          | c.718C>T | G+       | 33                    | 1.65±0.339 <sup>a</sup> |
|                 |          | ++       | 67                    | 1.56±0.291 <sup>b</sup> |
|                 | c.746A>G | BB       | 4                     | 1.00±0.000 <sup>a</sup> |
|                 |          | B+       | 13                    | 1.37±0.220 <sup>b</sup> |
|                 |          | ++       | 83                    | 1.65±0.280 <sup>c</sup> |
| Boerka          | c.718C>T | G+       | 44                    | 1.75±0.396 <sup>a</sup> |
|                 |          | ++       | 67                    | 1.61±0.406 <sup>b</sup> |
|                 | c.746A>G | BB       | 3                     | 1.00±0.000 <sup>a</sup> |
|                 |          | B+       | 11                    | 1.18±0.252 <sup>b</sup> |
|                 |          | ++       | 97                    | 1.74±0.369 <sup>c</sup> |

searchers observed that the homozygote mutant goats had the most offspring, followed by the heterozygote carrier and homozygote wild-types, such as Beetal and Teddy goats (Islam et al., 2019), Anglo-Nubian (Abdel-Rahman et al., 2013), Funiu white and Taihang black (Wang et al., 2011).

In some sheep breeds, such as the Small Tailed Han (Chu et al., 2007; Zhang et al., 2011), the Chios (Liandris et al., 2012), the Rahmani, and the Rahmani x Barki Cross (Saleh et al., 2020), previous studies also demonstrated that the heterozygote carrier genotype for BMP-15 had a larger litter size than the homozygote wild-type. In contrast, several other studies found that the homozygote wild type had a higher litter size than the heterozygote carrier. Meanwhile, homozygote carrier sheep for this mutation were infertile. Mc Natty et al. (2005) state that FecX<sup>G</sup> mutation causes premature stop codon at amino acid residue 239 (CAG --> TAG, Q239Ter), resulting in immature pro-

tein, which may result in loss of BMP15 function. The relationship between BMP15 mutations and ovulation rates is based on the concept that the rate of ovulation will increase if the expression of the BMP15 gene is lower, but if the level of BMP15 is too low, it will cause total dysfunction (Fabre et al., 2006).

The important role of BMP15 in modulating litter size and the rate of ovulation in sheep and goats was supported by several previous studies. The BMP15 gene expression was significantly (P<0.05) higher in the prolific goat than in non-prolific goats' ovaries (Yang et al., 2012; Pramod et al., 2013; Pan et al., 2015). However, Crawford et al. (2011) found that the BMP15 gene expression in the oocytes of prolific was lower than in wild Booroola Romney sheep. This finding suggests that BMP15 plays a distinct role in goats and sheep, and that it may regulate female fertility and ovulation rate in a species-specific manner (Al-Musa-wi et al., 2013).

In the current research, we observed a negative relationship between *FecB* mutation (A746G or Q249R) and Litter Size (LS) in prolific Kacang and Boerka goats (Table 5). The Litter Size of the wild-type ++ goats was greater than carrier B+ and mutant BB goats. Polley et al. (2009) found a positive effect of *FecB* mutation on Black Bengal goats in which the genotypes of homozygote wild type, heterozygote carrier and homozygote mutant had litter sizes of 2.7, 3.04, and 3.11, respectively. However, some previous studies showed the *FecB* mutation had low polymorphism and no association with litter size in Anhui White, Wendeng Dairy, Jining Grey, Boer, Liaoning Cashmere, Barbari, Malabari, Osmanabadi, and Ganjam goats (Ahlawat et al., 2014; Chu et al., 2010). The different genetic impacts of these mutations are possibly caused by the intense selection pressure on the population resulting in the accumulation of certain genotypes. This in turn would have an impact on the effective population size in the goat genotype group and would alter the results of the significant association analysis. Another possible reason is that the *BMPR-1B* gene expression may vary between breeds and environmental conditions or due to the interactions between breeds and the environment (Fogerty, 2008).

In sheep breeds, some researchers have confirmed that the *FecB* mutation has an effect on ovulation rate and/or litter sizes, such as Dorset, Mongolian, Small-Tail Han, Hu and Bayanbulak sheep (China: Jia et al., 2019; Wen et al., 2021; Wang et al., 2018; Zuo et al., 2013); Garole, Nilagiri, Nellore and Deccani sheep (India: Polley et al., 2010; Praveena et al., 2017; Chaudhari et al., 2019); Mehraban and Kalehkoochi (Iran: Abdoli et al., 2013; Talebi et al., 2018; Mahdavi et al., 2014). The important role of *FecB* mutations in the regulation of sheep reproduction has been confirmed in several previous studies. The *FecB* mutation reduces the *BMPR-1B* inhibitory effect on granulosa cell steroidogenesis so that they are more sensitive to FSH; thus, the cell division becomes more active, leading to the maturation and ovulation of multiple ovarian follicles (Fabre et al., 2006). The basic concept of the prolific nature is that an increased ovulation rate leads to a more significant number of ovulated oocytes and if fertilization occurs and the parent is able to maintain a viable embryo, it will be followed by the birth of more than one kid (Wilson et al., 2001).

## CONCLUSION

According to these results, we can state that *BMP15* and *BMPR1B* genes were polymorphic and significantly affected the litter size in Kacang and Boerka goats. The results of this study will hopefully provide a basis for the development of marker-assisted selection (MAS) for goat breeding programs in the future. However, our study has limitations on the effective population size of each geno-

type group in the test goats, which will impact the association analysis results. Therefore, further investigations on large and balanced populations are required to confirm the association of *BMP15* and *BMPR1B* genes with increased prolificacy in Kacang and Boerka goats.

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## NOVELTY STATEMENT

The authors declare that the article is original and sourced from unpublished research data. The Faculty of Animal Science University of Mataram's ethics committee granted all the research materials and procedures with registration number: 10/UN18.F2/EC/2021.

## AUTHOR'S CONTRIBUTIONS

**Maskur** was responsible for designing, analyzing, interpreting data, and preparing the manuscript; **Muhammad Muhsinin** was responsible for phenotypic and genotypic analysis, and preparation of the manuscript; and **Sulaiman Ngongu Depamede** was responsible for the experiment, laboratory analysis, and article preparation.

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