

Impact of Environmental Geographic toward Point Mutations in Exon 1 of Growth Differentiation Factor (GDF9) Gene in Kosta and Lakor Goat Breeds

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Abstract: This study aims to analyze the impact of environmental biogeography on point mutations in Kosta and Lakor goat breeds as local Indonesian germplasm using the GDF9 Exon 1 gene sequence. A total 43 follicle samples of Kosta and Lakor goats which collected in Serang regency and Lakor island were analyzed. To isolate the total genome of the samples, the tissue isolation method was used. The polymerase chain reaction (PCR) method was used for amplification of the exon-1 region of the GDF9 gene with two pairs of primers. Analysis of genetic variation and single nucleotide polymorphisms (SNP) using MEGA software version X. Multiple alignment results showed that there are 2 nucleotides that have mutations, but not encode different amino acids. This results indicates that the mutation has association with litter size of two breeds. This finding shows that Lakor goats have polymorphism i.e SNP g.54C>T which is significantly associated with litter size. The analysis of GDF9 gene showed that all samples from the Two local Indonesian goat breeds (Kosta and Lakor) are monomorphic. But, although both are monomorphic, geographical isolation still contributes to genetic drift within and between populations.

Keywords: Biogeography; GDF9 gene; Lakor goat; Kosta goat; point mutations

Introduction

The conventional breeding program is typically centered to enhance the production performance of farm animals to fulfill the developing demand of population. but, choice simplest for production tendencies will cause declination of many fitness trends due to terrible genetic correlation (Mishra, 2014). Therefore, a simultaneous improvement of manufacturing in addition to reproduction tendencies is an utmost necessary in breeding application. development of reproductive traits of any domestic species which include small ruminants is the key interest of animal breeders' community as mild boom in clutter size can cause outsized earnings. The various goat breeds (including

the Marica, Bengal, Etawah, Kosta, Kacang, Gembrong, Muara, Samosir, Kosta, and Lakor goats) have adapted to the climate and geography of Indonesia (Batubara et al., 2013). Goats are also often used in traditional and religious rituals apart from being livestock producing meat, milk and fur (Budisatria et al., 2008). They are kept as a necessary supplement for fiber, meat, and milk. Among all ruminant species, goats are thought to be the most productive (Yadav and Yadav 2008).

Profitability in goat farming is heavily influenced by the litter size. The number of eggs that are ovulated by the dams determines the litter size. So far, genetic development packages in Indonesian goats were carried out by way of the usage of crossbreeding. Kosta goat is a cross between local Indonesian goats, commonly

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known as Kacang goat/Javanese goat, with khashmir goats that are spread throughout India. Currently, Kosta goat can be found in Tangerang Regency, Tangerang City, South Tangerang City, Serang Regency, Serang City and Pandeglang Regency. Lakor goat is one of the domestic goat breeds from Southwest Maluku Regency with adaptability and tolerates hot habitats. The Lakor goat breed has been designated as a local goat family by the Decree of the Minister of Agriculture No. 2912/Kpts/OT.140/6/2011 with a habitat on Lakor Island. Research Salamena et al. (2014), asserted that the Lakor Goat is the result of a cross between two goat breeds, i.e the Etawah goat and the Kacang goat (an endemic goat of Indonesia). The domesticated goat (*C. hircus*) is a completely adaptable animal and geographically broadly distributed across numerous continents (Aziz, 2010). Conventional breeding methods using data recording are an easy process but need to be supported by molecular data, confirming the productivity identity of each breed for improved genetic performance. In this regard, it is very important to find and explore the genes of the right candidates and who play a role and are responsible for prolific abilities in goats.

The Growth differentiation factor 9 (GDF9) gene belongs to the transforming growth factor region β the superfamily and plays an important role in the mechanism of follicular development in the ovaries and ovulation rate (Elvin et al., 1999; McNatty et al., 2005). This region is found on autosomal chromosome number 5 in sheep and goats (Sadighi et al. 2002). Regarding the function of the GDF9 gene in the process of ovulation and reproduction, this gene can be used as a candidate gene to study reproductive activity related to litter size ability in relation to mutation and proliferation rates in goats, and sheep (McNatty et al. 2003). Study conducted by Hanrahan et al. (2004) found GDF9 gene polymorphisms in two sheep breeds i.e Cambridge and F700-Belclare and concluded that these polymorphisms are responsible for prolonging ovulation cycles in heterozygous parents and infertility in homozygous parents. The results of further analysis of the impact of

GDF9 gene polymorphism on several sheep breeds have a correlation with phenotypic appearance including litter size ability (Feng et al., 2012; An et al., 2012; Silva et al., 2010). This study aims to identify point mutations in two goat breeds from Indonesia, namely Kosta and Lakor as native livestock from Banten and Southwest Maluku regency, based on exon region 1 analysis of the GDF9 gene in relation to litter size ability in both breeds.

Method

Ethical approval

This take a look at does not require ethical approval due to the fact the use of non-invasive samples (hair follicles).

Sample collection

In overall, Forty Three animals belonging to two Indonesian goat breeds (Kosta and Lakor) were analyzed in this study. Hair follicles from goat tails were gathered and saved in envelopes to preserve dry. DNA turned into extracted the use of a DNA isolation package (gSYNC™ DNA Extraction package, Geneaid) and saved at -20°C until in addition examination.

PCR amplification

Region of exon 1 of the GDF9 gene, with a size of 462 bp amplified using a pair; forward: (5'-GGCTCTTCCTGATTTTAGG-3') and reverse: (5'-TAGCCTACATCAACCCAT GA-3') designed using an online primer3 program using a genbank database sequence with accession number (KT290893.1) (Figure 1). The following PCR programs for target region amplification are set as follows: Pre-Denaturation at 94°C for 5 minutes, 94°C denaturation for 1 minute, Annealing 61°C for 1 minute, 72°C extension for 30 seconds and final extension at 72°C for 4 minutes. This in vitro amplification process lasts for 35 cycles. PCR products visualized on standard 1.5% agarose gel stained with bioatlas fluorescence and observed on UV transluminator ($\lambda=260$ nm).

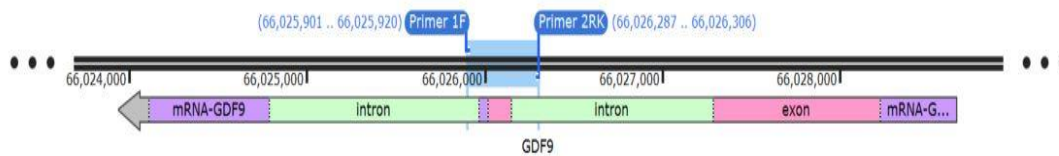


Figure 1. Location of the exon-1 region of the GDF gene

Sequencing and Single Nucleotide Polymorphism (SNP) analysis

Purified PCR products are then sequenced directly by 1st Base Sequencing INT (Malaysia). SNP analysis was performed using exon sequence database 1 of the GDF9 gene from comparative sequences from the National Centre for Biotechnology Information (NCBI) by first aligned using Clustal W in MEGA version X.0 software. Comparison sequence accession number data i.e *C. hircus* accession number i.e (MF416088.1), (LC721860.1), and *C. hircus* breed Jhakhrana growth (HM462256.1).

Result and Discussion

The GDF9 gene was amplified as a single fragment for all goat samples Kosta and Lakor were studied after electrophoresis on 1,5% agarose gel with 1 kb DNA Ladder (1st base) (Figure 2). The amplified GDF9 gene fragment consists of GDF9 exon-1 with a nucleotide length of 652 bp. This indicates that the amplification of exon-1 of the GDF9 gene is carried out at optimal temperature.

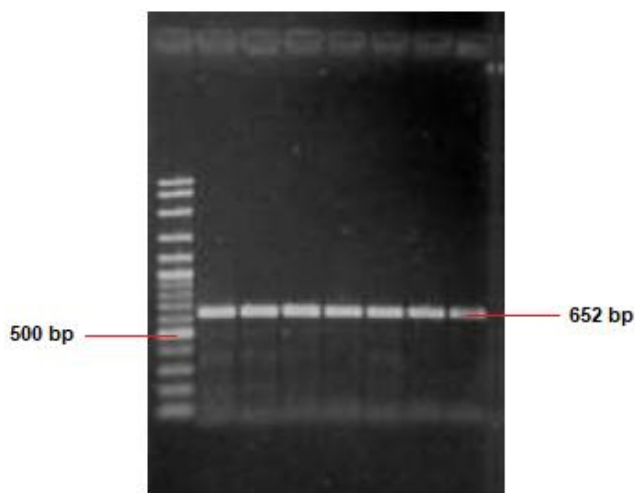


Figure 2. Amplification results of exon 1 of the GDF9 gene

Multiple alignment results of the exon-1 from GDF9 gene, showed that there are 2 nucleotides that have mutations (Figure 3), but mutations of these 2 nucleotides do not encode different amino acids (Figure 4). This indicates that the mutation has association with litter size of Lakor and Kosta goat populations. The results of translating mutated nucleotides into amino acids showed that they produce synonymous amino acids. It is suspected that a mutation. What occurs in

exon 1 of the GDF9 gene in the Lakor goat population is a muted mutation and a neutral mutation so that it does not change the function of the amino acid and protein of the GDF9 gene. Various inheritance patterns have been successfully analyzed as having links with the GDF9 gene. Some studies report that GDF9 gene polymorphisms are associated with infertility and increased ovulation rates in sheep. The study by Hanrahan et al. (2004), reported that several mutations were found in the GDF9 and BMP15 genes in sheep of the Cambridge and F700-Belclare breeds. The results of the analysis concluded that the ovulation rate in both offspring was caused by mutation factors in the GDF9 and BMP15 genes.

Alignment results do not show insertions and deletions (indels) so that they have the same size. The success of amplifying the GDF9 region is largely determined by conditions attachment of primer to the sample genomic DNA, PCR reagent material and the type of PCR machine used (Sambrook and Russel, 2001). The results of this study confirm that the level of variation in a gene, it is different at each taxon level, i.e in the Order, Family, Genus, or mammal species. Study by Kunda et al 2017, stated that the position of the nucleotides is mutated in the first or third base of the triple codon, generally does not change the amino acid encoded. This study shows that the mutation occurs in the 54 nucleotide site (C-T) between Lakor goats (Cytosine nucleotides) with Kosta goats and references from genbank (Thymine nucleotides). The results of the nucleotide translation showed that the amino acids are translated is Cysteine.

Hanrahan et al. (2004), reported that mutations occur in functional genes always associated with certain characters. In this case mutations (C-T) occur between populations Lakor and Kosta goats are very likely associated with processing capability ovulation and sterility in female goats between the two populations. It is suspected that the base mutation (C-T) what happened had a huge impact on the Lakor goat population in terms of ability prophylactic. This needs to be further tested to ensure mutations and their correlation to traits profile between the two populations. Rumanta et al. (2020) stated that the cross pressure is deep (*Inbreeding depression*) is very large in the Lakor goat population. The result that happened as a result of inbreeding depression is decreased performance and fitness in this population.

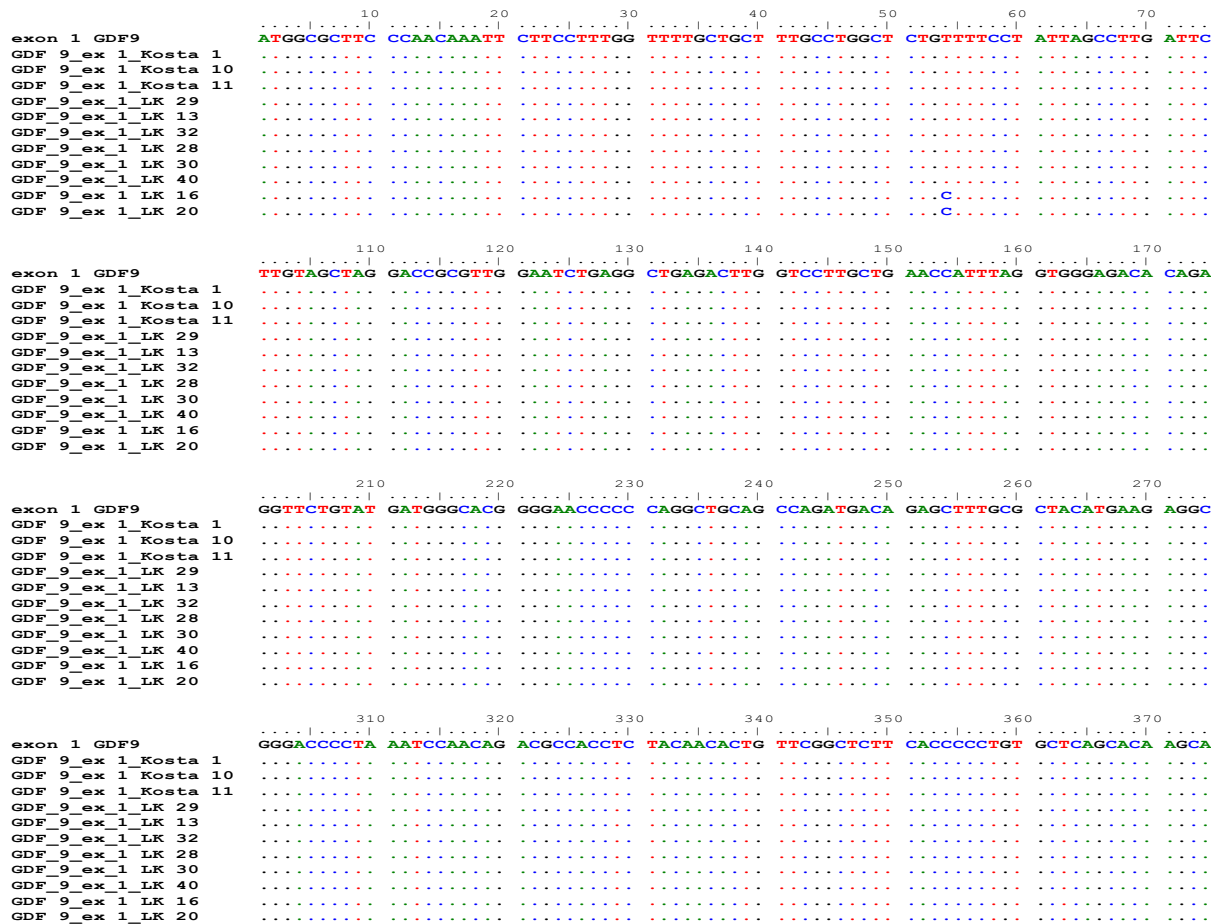


Figure 3. Alignment of the Exon-1 of GDF9 gene sequences



Figure 4. The amino acid profile of the nucleotide translation of the GDF9 gene

The ability to reproduce is regarded as the most crucial economic characteristic in sheep and goats. Heritability of the litter size is low and its improvement through conventional choice methods is a tremendously slow process. Marker-assisted selection, collectively with traditional choice techniques, ought to benefit a higher genetic development in litter size. In goat, candidate gene markers for litter size have been identified such as GDF9 (Davis 2004; Ran et al., 2009; Ma et al., 2010; Dutta et al., 2013; Gazooei et al., 2013). The GDF9 gene can be regarded as a possible candidate gene associated with prolificacy in both sheep and goat.

Kunda et al. (2015) stated that differences in the rate of substitution are caused by differences in the population used, resulting in different nucleotide variations. Also Primack et al. (1998) stated that a species includes one or more geographically separated populations and one population consisting of one or millions of individuals. Thus it can be concluded that substitution is one of the factors (material raw) which can affect population genetic diversity and drive evolution (Jones, 1997). According to Jones (1997), and Brum et al. (1994), each individual in a population usually genetically different from one another. Genetic variation between individuals in a population or between populations, occurs because every individual in the population has the composition of the nucleotides that make up genes that are dynamic and have different chromosome units codes for a specific type of protein.

This process occurs to support metabolic activity and survival an individual in a particular habitat. The appearance of variations in an individual in many populations dominated by the mutation process in the DNA molecule that will form the components the chromosomes of each individual member of the population. Various alleles of a gene will affect development and physiology of an individual differently. The expression that occurs in GDF-9 affects the development of specific cells ovaries in normal follicular stages, granulosa cell development and development oocytes. The effect of GDF-9 expression was also seen after ovulation and 1.5 days after the occurrence of fertilization, then regulates the early development of the embryo (Davis, 2004).

Research by Hanrahan et al. (2004), reported that mutations in GDF-9 occurred at base 1184 in ewes, namely a change from the base C to T resulting in acid changes amino acids from serine to phenylalanine and to produce the FecGH allele. The buck have a mutation of the GDF-9 gene and have a homozygous FecGH allele associated with sterility in buck. Mutation of the GDF-9 gene with the FecGH / FecG + allele in a heterozygous state resulted in an increase in the ovulation rate (Hanrahan et al 2014). Mutations that are occurs in the

GDF-9 gene associated with a mutation that occurs in the BMP-15 gene the X chromosome, whether homozygous or heterozygous, has a significant impact similar to the reproductive conditions in sheep (Galloway et al., 2002). It is suspected that a mutation.

Conclusion

This study proved that polymorphisms in the GDF9 gene were detected in Lakor goats. SNP g.54C>T is significantly related to litter size. Thus, these SNPs may prove valuable as genetic markers for future research on Lakor goat populations. GDF9 gene analysis showed that all samples from two local Indonesian goat breeds (Kosta and Lakor) were monomorphic. However, although both are monomorphic, geographic isolation contributes to genetic drift within and between populations.

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Author Contributions

MR and RMK as the originator of the idea and designed of this study, MR, RMK, and IMM designed collected samples in field. SDV and RMK conducted study in the laboratory. MR, IMM and and RMK wrote the manuscript's first draft after analyze the data. The final manuscript was read by all authors, who also gave their approval.

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Conflicts of Interest

The authors declare that they do not have any competing interests.

References

- An, X.P., Hou, J.X., Zhao, H.B., Li, G., Bai, L., Peng, J.Y., M, Yan Q., Song, Y.X., Wang, J.G., & Cao, B.Y. (2013). Polymorphism identification in goat GNRH1 and GDF9 genes and their association analysis with litter size. *Animal Genetics*, 44(2), 234-238. <https://doi.org/10.1111/j.1365-2052.2012.02394.x>
- Aziz, M. A. (2010). Present status of the world goat populations and their productivity. *World*, 861, 42-52. Retrieved from https://lohmman-information.de/content/l_i_45_artikel17.pdf
- Batubara, A., Noor, R.R., Farajallah, A., & Tiesnamurti, B. (2013). Genetic Diversity of Y-chromosomal DNA in Six Local Indonesian Goat Breeds.

- Proceedings IAARD Press, Indonesian*, 316-325.
- Budisatria, I.G.S., Udo, H.M.J., van der Zijpp, A.J., Baliarti, E., & Murti, T.W. (2008). Religious festivities and marketing of small ruminants in central java-Indonesia. *Asian Journal of Agriculture and Development*, 5(2), 57-73. Retrieved from <https://core.ac.uk/download/pdf/6231843.pdf>
- Brum, G.L, Meckane., & Karp, G. (Eds) (1994). *Biology Exploring Life*. New York: John Wiley and Son.
- Davis, G.H. (2004). Fecundity genes in sheep. *Animal Reproduction Science*, 82, 247-253. <https://doi.org/10.1016/j.anireprosci.2004.04.001>
- Dutta, R., Das, B., Laskar, S., Kalita, D.J, Borah, P., Zaman, G & Saikia, D.P. (2013). Polymorphism, sequencing and phylogenetic characterization of growth differentiation factor 9 (GDF9) gene in Assam Hill goat. *African Journal of Biotechnology*, 12(50), 6894. Retrieved from https://academicjournals.org/article/article1386776256_Dutta%20et%20al.pdf
- Elvin, J.A., Clark, A.T., Wang, P., Wolfman, N.M., & Matzuk, M.M. (1999). Paracrine action of growth differentiation factor-9 in mammalian ovary. *Molecular endocrinology*, 13(6), 1035-1048. <https://doi.org/10.1210/mend.13.6.0310>
- Feng, T., Geng, X., Lang, X.Z., Chu, M.X., Cao, G.L., Di, R., Fang, L., Chen, H.Q., Liu, X.L., & Li, N. (2012). Polymorphisms of caprine GDF9 gene and their association with litter size in jining grey goats. *Molecular biology reports*, 39, 4029-4038. <https://doi.org/10.1007/s11033-011-1184-5>
- Gazooei, Y.M., Niazi, A & Zamiri, M.J. (2013). Single nucleotide polymorphism analysis of the bone morphogenetic protein receptor IB and growth and differentiation factor 9 genes in Rayini goats (*Capra hircus*). *Journal of Livestock Science and Technologies*, 1(2), 45-50. Retrieved from https://lst.uk.ac.ir/article_549_e2487315e069d735011fb6ae1eee2d99.pdf
- Galloway, S. M., McNatty, K. P., Cambridge, L. M., Laitinen, M. P., Juengel, J. L., Jokiranta, T. S., ... & Ritvos, O. (2002). Mutation in an oocyte-derived growth factor gene (BMP15) cause increased ovulation rate and infertility in a dosage-sensitive manner. *Nature genetics*, 25(3), 279-283. <https://doi.org/10.1038/77033>
- Hanrahan, J. P., Gregan, S. M., Mulsant, P., Mullen, M., Davis, G. H., Powell, R., & Galloway, S. M. (2004). Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). *Biology of reproduction*, 70(4), 900-909. <https://doi.org/10.1095/biolreprod.103.023093>
- Jones, A.M. (Eds.) (1997). *Environmental Biology*. Routledge. London and New York.
- Kunda, R.M., Handayani, N.S.N., Wijayanto, H., & Widayanti, R. (2017). DNA barcoding of cuscuses (Marsupialia: Phalangeridae) from Maluku and Papua. *Asian J. Anim. Vet. Adv.*, 12(5), 227-238. <https://doi.org/10.3923/ajava.2017.227.238>
- Kunda, R.M., Handayani, N.S.N., Wijayanto, H., & Widayanti, R. (2015). Study of genetic marker of cuscuses (Marsupialia: Phalangeridae) from Maluku and Papua based on cytochrome B gene sequences. *Pakistan Journal of Biological Sciences: PjBS*, 19(3), 122-135. <https://doi.org/10.3923/pjbs.2016.122.135>
- Mishra, C. (2014). Genetic basis of Prolificacy in sheep. *Int J Livest Res*, 4(1), 46-57. Retrieved from <https://www.bibliomed.org/?mno=48125>
- McNatty, K.P., Juengel, J.L., Reader, K.L., Lun, S., Myllymaa, S., Lawrence, S.B., Western, A., Meerasahib, M.F., Mottershead, D.G., Groome, N.P., Ritvos, O., & Laitinen, M.P. (2005). Oocyte signalling molecules and their effects on reproduction in ruminants. *Reproduction, Fertility and Development*, 17(9), 66-66. <https://doi.org/10.1071/SRB05Abs020>
- McNatty, K.P., Juengel, J.L., Wilson, T., Galloway, S.M., Davis, G.H., Hudson, N.L., Moeller, C.L., Cranfield, M., Reader, K.L., Laitinen, M.P., Groome, N.P., Sawyer, H.R & Ritvos, O. (2003). Oocyte-derived growth factors and ovulation rate in sheep. *Reproduction*, 61, 339-351. Retrieved from <https://pubmed.ncbi.nlm.nih.gov/14635946/>
- Ma, X., Liu, X., Zhang, C & Li, J. (2010). Candidate genes polymorphism and its association to prolificacy in Chinese goats. *Journal of Agricultural Science*, 2(1), 88-92. <https://doi.org/10.5539/jas.v2n1p88>
- Primack, R.B., Supriatna, J., Indrawan, M., & Kramadibrata, P. (Eds.) (1998). *Conservation Biology (In Indonesian)*. Yayasan Obor Indonesia, Jakarta, Indonesia.
- Ran, X., Lin, J., Du, Z., Qing, C & Wang, J. (2009). Diversity of BMP15 and GDF9 genes in White goat of Guizhou Province and evolution of the encoded proteins. *Zoological Research*, 30, 593-602. Retrieved from <https://hdl.handle.net/1807/64400>
- Rumanta, M., Kunda, R.M., Volkandari, SD., Indriawati., & Kakisina, P. (2020). Genetic characterization and phylogenetic study of Lakor goat from Southwest Maluku Regency based on mitochondrial COI gene. *Veterinary World*, 13(6), 1209. <https://doi.org/10.14202/2Fvetworld.2020.1209-1220>
- Salamena, J.F., Malle, D., Latupeirissa, C.C.E., & Siwa, I.P. (2014). Potential development of local animal

- genetic resources in Maluku. *Occas. Pap*, 54, 17-25. Retrieved from [http://cpi.kagoshima-u.ac.jp/publications/occasionalpapers/occasional/vol-54/occasional_papers_54\(pp17-25\).pdf](http://cpi.kagoshima-u.ac.jp/publications/occasionalpapers/occasional/vol-54/occasional_papers_54(pp17-25).pdf)
- Sadighi, M., Bodensteiner, K.J., Beattie, A.E., & Galloway, S.M. (2002). Genetic mapping of ovine growth differentiation factor 9 (GDF9) to sheep chromosome 5. *Animal genetics*, 33(3), 244-245.
- Silva, B.D.M., Castro, E.A., Souza, C.J.H., Paiva, S.R., Sartori, R., Franco, M.M., Azevedo, H.C., Silva, T.A.S.N., Vieira, A.M.C., Neves, J.P & Melo, E.O. (2010). A new polymorphism in the growth and differentiation factor 9 (GDF9) gene is associated with increased ovulation rate and prolificacy in homozygous sheep. *Anim. Genet*, 42, 89-92. <https://doi.org/10.1111/j.1365-2052.2010.02078.x>
- Yadav, A & Yadav, B.R. (2008). DNA Fingerprint: Genetic relationship in Six Indian goat breeds. *Indian J. Biotechnol.* 7, 487-490. Retrieved from <https://nopr.niscpr.res.in/handle/123456789/2350>