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Diversity Identification of Follicle Stimulating Hormone with PstI Restriction Enzyme in Female Mud Buffalo *(Bubalus bubalis)*

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ABSTRACT

Low reproductive efficiency limits the productivity and milk yield in swamp buffaloes. Identifying follicle-stimulating hormone (FSH) is essential, as genetic variation in this gene can indicate reproductive potential. The present study aimed to characterize polymorphisms in the FSH gene, specifically exon 2, in female mud buffalo (*Bubalus bubalis*) using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) with the PstI restriction enzyme. Blood samples (n = 50) were collected through purposive sampling from healthy, mature female buffaloes (3-4 years of age, weighing 300-400 kilograms) across 50 farms in West Sumatra, Indonesia. Genomic DNA was extracted from the blood samples, and exon 2 of the FSH gene was amplified by PCR-RFLP, followed by PstI digestion to detect sequence variations. The PCR-RFLP analysis revealed two distinct genotypes, including a predominant homozygous genotype (+/+) in almost all buffaloes and a heterozygous genotype (+/-) observed in one sample. Additionally, one exhibited no visible DNA band. The allele frequencies for the allele with the PstI site (+) were ~0.99, and the genotype distribution conformed to the Hardy-Weinberg equilibrium. The assessment of FSH gene diversity in Indonesian mud buffaloes confirmed that the exon 2 locus is polymorphic. These preliminary findings offered important insights for targeted breeding programs and promoting the conservation of local buffalo genetic resources.

Keywords: Follicle-stimulating hormone, Genotype, Mud buffalo, PstI enzyme

INTRODUCTION

Buffalo livestock plays a vital role in West Sumatra, especially in agriculture and food production (Nainggolan et al., 2019). Local buffalo are used as working animals in the fields and as the primary source of milk for making curd (Roza et al., 2024). Subsequently, curd is a fermented milk product that holds cultural significance among the Minangkabau of West Sumatra, Indonesia. Curd is a vital component of local cuisine and has significant market potential domestically and internationally. Therefore, ensuring the sustainability of milk production from buffalo is important for maintaining consistent, high-quality curd production (Rehman et al., 2021). However, buffalo milk production in West Sumatra still faces challenges, including livestock fertility. Buffalo is known to have lower fertility rates compared to other livestock, resulting in fewer offspring (Reswati and Putra, 2023). Several studies have reported that buffalo generally exhibit longer calving intervals, lower ovulatory rates, and weaker estrus expression than cattle, which contribute to reduced reproductive efficiency and lower annual calf numbers. According to Fitriani et al. (2023), the gestation period for mud buffalo in Indonesia is 10-11 months, and the age of first calving is between 42 and 48 months. The average time between breeding and calving for buffalo is approximately 3.5 months. These issues are also evident in the artificial insemination fertility rate, which is only 30-40% (Pehan et al., 2025). This limitation directly impacts the amount of milk available for curd production. Therefore, improving fertility and reproductive efficiency in buffalo farming is a crucial strategy to ensure the long-term sustainability of the curd industry in West Sumatra, Indonesia (El Debaky et al., 2019).

One method to improve livestock quality is by enhancing genetic traits through crossbreeding and selective breeding (Widi et al., 2021). Breeders commonly practice crossbreeding to enhance livestock performance, particularly milk production and reproductive efficiency. Crossbreeding is expected to yield livestock well-adapted to tropical climates and with improved milk production potential. Additionally, this genetic combination offers advantages for feed efficiency, animal health, and the potential to produce high-quality meat (Terry et al., 2020).

One approach to increasing livestock fertility is to utilize the genetic diversity in genes involved in the reproductive process, such as the follicle-stimulating hormone (FSH) gene. This gene encodes a hormone that plays a crucial role in

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stimulating the growth of ovarian follicles in females, thereby contributing to the ovulation process (Wang et al., 2021). Follicle-stimulating hormone is a central endocrine regulator of female reproduction, and variations in its gene sequence can alter follicular development, estrous cyclicity, and conception rates. Even minor polymorphisms in FSH-related regions may influence the hormonal balance required for normal reproductive physiology. Genetic variation in the FSH gene can affect fertility and reproductive capacity in livestock, which are directly linked to milk production (Sikdar et al., 2021). These gene variations can be identified using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method (Sofia et al., 2024). The advantages of PCR-RFLP, including its high resolution and ability to detect diverse polymorphisms, make it a consistently relevant technique in genetic research (Utomo, 2018). By analyzing genetic variation in the FSH gene, farmers can select livestock with greater reproductive potential, enhancing breeding efficiency and boosting milk production (Wang et al., 2021).

Additionally, studying FSH gene diversity not only boosts livestock fertility but also helps conserve local buffalo in West Sumatra, Indonesia. Developing breeding strategies based on genetic knowledge is crucial for enhancing buffalo milk production while maintaining the quality and sustainability of local buffalo breeds (Rehman et al., 2021). Typically, using molecular methods such as PstI to identify genetic diversity in the FSH gene has greatly enhanced the efficiency of buffalo breeding programs (Rehman et al., 2021). This genetically-based approach offers considerable potential for supporting the sustainability of local buffalo populations. Although the FSH gene is known to play a crucial role in reproductive regulation, no previous studies have specifically examined polymorphisms in the FSH Exon-2 region using the PstI restriction enzyme in female Buffalo. The present study aimed to identify and characterize the FSH gene in local female buffalo in West Sumatra, Indonesia.

MATERIALS AND METHODS

Ethical approval

The animal procedures conducted in this study adhered to the ethical standards approved by the animal ethics committee of the faculty of veterinary medicine, Udayana University, Indonesia, with certificate number B-120/2024.

Animals

A total of 50 healthy female mud buffalo aged 3-4 years, with an average body weight of 350-400 kg, were used during the present study. Blood samples (3-5 mL) were collected via the jugular vein using EDTA vacutainer tubes (EDTA K2, OneMed, Indonesia). These samples were from reproductively mature animals typically kept in traditional farming systems, where their diet consists of 85% or more natural forage (*Pennisetum purpureum*) and occasionally agricultural waste such as straw.

DNA isolation

DNA isolation was performed using the modified method described by Afriani et al. (2022). The equipment used includes Eppendorf tubes, micropipettes, microtips, Eppendorf centrifuges, sterile tissues, and vortexes. The materials used consisted of 50 blood samples from female mud buffalo, cell lysis (CL) buffer, binding lysis (BL) buffer, wash A (WA) buffer, wash B (WB) buffer, column elution (CE) buffer, 1x buffer tris boric acid EDTA (TBE, 1 M Tris, 0.9 M Boric Acid, 0.01 M EDTA pH 8.0), spin column, RNase A, proteinase, isopropanol, DNA rehydration solution, and ethanol 70%. DNA extraction was performed using the G-Spin^{TM} total DNA extraction mini kit (iNtRON Biotechnology, Korea) according to the manufacturer's protocol for genomic DNA extraction. The purity and concentration of extracted DNA were evaluated using a Nanodrop spectrophotometer (Thermo Scientific, USA).

Follicle-stimulating hormone gene amplification

The isolated DNA was amplified using primers designed with the Primer3 platform, including forward primer F: 5'-AAT TGT TTG TTG CCC ATG GCC C-3' and reverse primer R: 5'- TGG CTA AAG GAC TCA TGG CT-3'. These primers produced a 301 bp fragment of exon 2 of the FSH gene. Furthermore, PCR amplification was performed using a thermal cycler (Bio-Rad, USA), using Eppendorf tubes, micropipettes, and microtips. The amplification process used the GoTaq Green Master Mix 2x protocol (Promega, USA), which consisted of 2 µL of DNA template, 8.5 µL of nuclease-free water, 1 µL of forward primer, 1 µL of reverse primer, and 12.5 µL of Master Mix, totaling 25 µL.

The materials were sequentially loaded into a 200 µL Eppendorf tube, which was then placed in the PCR machine (Bio-Rad, USA) for amplification. The PCR program conditions were initial denaturation at 95°C for five minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 30 seconds, then a final extension at 72°C for 10 minutes. Furthermore, PCR products were subjected to electrophoresis on a 1.5% agarose gel at 100 volts for 70 minutes. The gel was stained with GelRed, and a 100 bp DNA ladder was used as a molecular marker. The results were observed utilizing a UV transilluminator (Bio-Rad, USA) and subsequently documented. Gene amplification was confirmed when bands of the target size, specifically 301 bp, were visible on the agarose gel. The electrophoresis results were subsequently recorded with a camera.

Gene genotyping

Genotyping of the FSH gene using the PstI restriction enzyme was performed according to established PCR-RFLP procedures as described by Utomo (2018). The equipment used included Eppendorf tubes, micropipettes, microchips, and water bath incubators. The materials consisted of nuclease-free water, PstI restriction enzyme, and purified PCR products. A total of 5 µL of the PCR product was placed into a 200 µL Eppendorf tube, followed by the addition of 1 µL of PstI enzyme (10 U), 2 µL of buffer O, and 12 µL of nuclease-free water. The tube was subsequently placed in the PCR machine at 37°C for two hours to allow enzymatic digestion. Following incubation, the restriction fragments were analyzed by electrophoresis on a 3% agarose gel stained with GelRed, using a 50 bp DNA ladder as a molecular size marker. The gel was run on an electrophoresis machine at 100 volts for 70 minutes, and the banding patterns produced by PstI digestion were visualized under a UV transilluminator.

Genetic parameter calculation

Genotype and allele frequencies were calculated based on established formulas, and Hardy-Weinberg equilibrium (HWE) was assessed using the chi-square test (Chen et al., 2016; Abramovs et al., 2020). Genotype frequencies (*Xi*) were computed using the following formula.

$$Xi = \frac{\sum ni}{N} \quad \text{(Formula 1)}$$

Where ni is the number of individuals with genotype i, and N is the total number of samples. Allele frequency (Xi) was calculated using Formula 2.

$$Xi = \frac{2nii + \sum j{\neq}i\; nij}{2N} \quad \text{(Formula 2)}$$

Where nii represents homozygous genotype, and nij denotes heterozygous genotypes.

HWE was assessed using the chi-square (X^2) goodness-of-fit test based on the equation described by Abramovs et al. (2020) using Formula 3.

$$X^2 = \sum \frac{(O - E)}{E} \quad \text{(Formula 3)}$$

where O and E denote observed and expected genotype counts, respectively. The population was considered in HWE when $X_h^2 < X_t^2$ at significance levels of α = 0.05 and α = 0.01.

Statistical analysis

All statistical calculations, including allele and genotype frequency determination and HWE testing, were performed using Microsoft Excel 2019. Additional confirmation was conducted utilizing PopGen32 (version 1.32). A significance threshold of α = 0.05 was applied. The HWE was assessed through the chi-square (χ^2) goodness-of-fit test. The observed number of genotypes was compared with the expected frequencies under HWE assumptions, and the χ^2 value was calculated. The results were interpreted by comparing the computed χ^2 (X^2h) with the tabulated χ^2 (X^2t) at α = 0.05. The population was considered to be in equilibrium when $X^2h < X^2t$.

RESULTS AND DISCUSSION

DNA isolation

DNA was successfully isolated from all 50 female mud buffalo blood samples, yielding concentrations ranging from approximately 4 to 68 ng/µL. Samples with elevated DNA concentrations generally yielded more prominent bands on agarose gels, whereas samples with lower concentrations yielded less intense bands. This is consistent with the notion that DNA quantity influences band visibility in electrophoresis (Afriani et al., 2022). Previous studies have reported that parameters such as incubation temperature and duration during cell lysis notably affect the quality and quantity of extracted DNA (Marquina et al., 2024). Furthermore, optimizing incubation conditions and buffer composition improves DNA yield and purity in extraction protocols (Kamilari et al., 2025).

The DNA purity (A260/280 ratio) showed considerable variation, ranging from -3.313 to 2.95. An A260/280 ratio outside the expected range of 1.8-2.0 may indicate the presence of contaminants or inaccuracies in measurement (Davidescu et al., 2021). Several measured DNA samples had ratios either below 1.8 or above 2.0, suggesting likely protein/phenol carryover or RNA contamination, respectively. Deviations from the ideal A260/280 ratio suggest the presence of particular contaminants. Ratios below 1.8 typically indicate protein or phenol contamination, while ratios exceeding 2.0 generally indicate the presence of RNA (Utomo and Safitri, 2021). Although impurities were present, the DNA quality was mostly suitable for PCR amplification. Minor technical issues during the isolation process, such as incomplete supernatant separation or suboptimal precipitation, may have reduced yields and purity in some samples (Knudsen et al., 2016). Similar issues have been documented by Kamilari et al. (2025), who emphasized that careful handling at each stage, such as appropriate phase separation and comprehensive washing, is essential for optimal DNA recovery. Overall, the quantity and quality of extracted DNA were adequate for subsequent genetic analyses.

Follicle-stimulating hormone gene amplification

After confirming DNA quality, FSH gene fragments were amplified by PCR. The PCR results using primers targeting exon 2 of the FSH gene yielded a vivid, single band of the expected length (301 bp) from all samples (Figure 1), indicating 100% amplification success. Each sample exhibited a distinct 301 bp band without any nonspecific amplification or primer-dimer artifacts, indicating that the PCR conditions were ideal for targeting the FSH gene exon 2 (Afriani et al., 2022). Having a single band of the correct size in each lane meets the criterion for successful amplification, as supported by Yurnalis et al. (2017). The current results are consistent with those of Afriani et al. (2022), who observed that obtaining a single, strong band of the expected size indicates specific amplification with minimal byproducts. The high-quality PCR product obtained in the present study provides a suitable template for subsequent restriction RFLP analysis (Yurnalis et al., 2017).

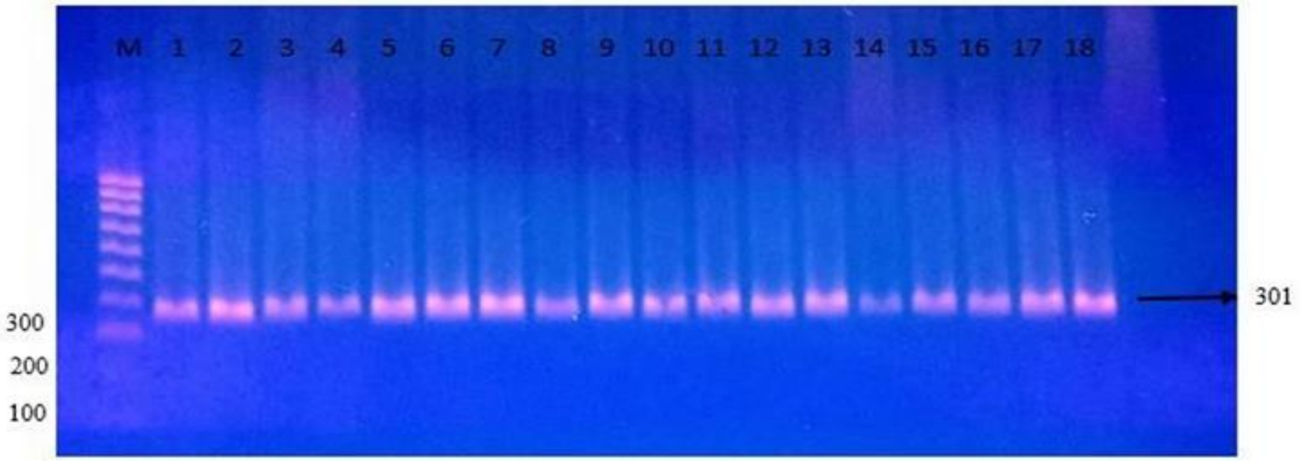


Figure 1. The follicle-stimulating hormone gene PCR products from the female mud buffalo. M: 100 bp DNA ladder, Samples 1-18: Lanes 1-18 show the PCR amplification product. Each sample exhibited a distinct 301 bp.

Follicle-stimulating hormone gene genotyping by PstI restriction

The RFLP banding pattern exhibited that the PstI enzyme recognized the sequence CTGCAG and cut the FSH exon 2 fragment (Figure 2). Among 50 buffalo samples, two different genotype patterns were identified, based on whether the PstI site was present or absent.

The homozygous truncated genotype (+/+) produced two fragments, including 78 bp and 223 bp, a pattern observed in 48 samples. The heterozygous genotype (+/-) exhibited three bands at approximately 301 bp, 78 bp, and 223 bp in one sample. One sample did not exhibit a distinct band following digestion, likely attributable to inadequate PCR product loading or a technical error during gel electrophoresis. No homozygous (−/−) non-cleaved genotypes, which would manifest as a single 301 bp band following digestion, were observed during the current study. Figure 3 illustrates the position of the PstI recognition site within the FSH gene as a reference.

The presence of two RFLP patterns confirmed the presence of a single-nucleotide polymorphism (SNP) at the PstI site in the FSH exon 2 region in this buffalo. Individuals with the (+/+) genotype have the PstI recognition sequence on both alleles, leading to complete digestion and the formation of two smaller fragments. In contrast, (+/−) animals carry the sequence on only one allele. The polymorphism at this restriction site indicated genetic diversity in the FSH locus. This variation could be linked to differences in the DNA sequence, such as mutations at the restriction site, which may prevent the enzyme from cleaving the allele. These findings are consistent with those of Utomo (2018), who reported that the FSH gene in an Indonesian cattle population exhibited polymorphic banding patterns using a similar PCR-RFLP method. General principles of RPLP analysis indicated that additions, substitutions, deletions, or point mutations can create or eliminate restriction sites, leading to a variable banding pattern (Afriani et al., 2022). Variations in these patterns, whether within a population or across different populations, can also result from changes in the sequence of the mtDNA D-loop base pairs (Mishra et al., 2015). Notably, the polymorphism identified in the present study was the first report of an FSH exon 2 SNP in local buffalo.

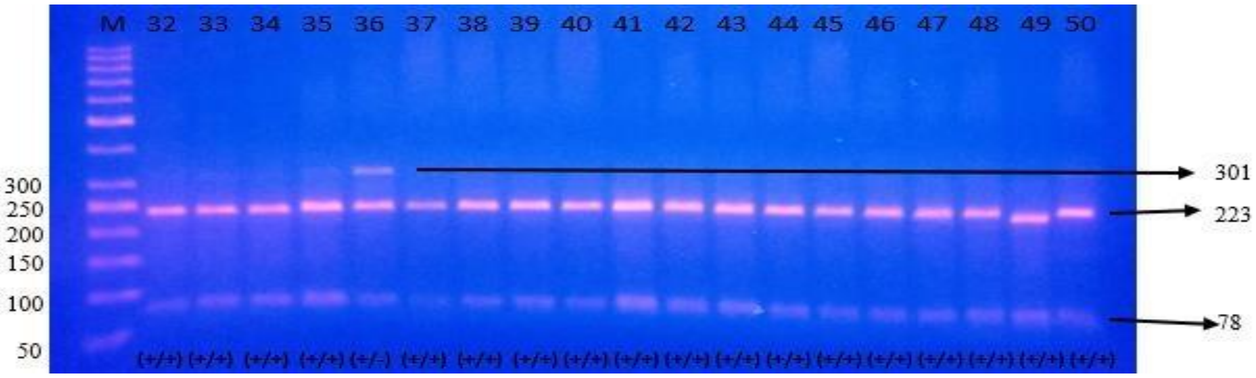
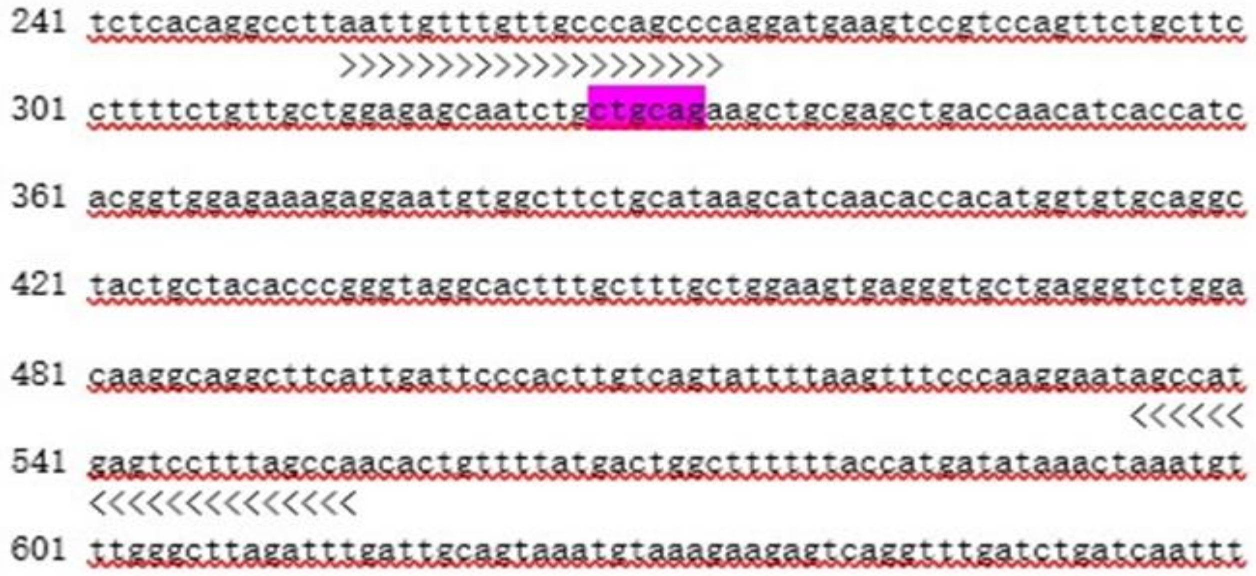


Figure 2. The follicle-stimulating hormone gene PCR products after PstI digestion in female mud buffalo. M: 50 bp DNA ladder, lanes 32-50 display restriction fragments for the samples 32-50. All lanes except lane 36 show the truncated homozygous genotype (+/+), whereas lane 36 shows the heterozygous genotype (+/-).



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241 tctcacaggccttaattgtttgttgcccagcccaggatgaagtccgtccagttctgcttc
                      >>>>>>>>>>>>>>>>>>>>>
301 cttttctgttgctggagagcaatctgctgcagaagctgcgagctgaccaacatcaccatc

361 acggtggagaaagaggaatgtggcttctgcataagcatcaacaccacatggtgtgcaggc

421 tactgctacacccgggtaggcactttgctttgctggaagtgagggtgctgagggtctgga

481 caaggcaggcttcattgattcccacttgtcagtattttaagtttcccaaggaatagccat
                                                          <<<<<<
541 gagtcctttagccaacactgttttatgactggctttttaccatgatataaactaaatgt
    <<<<<<<<<<<<<<
601 ttgggcttagatttgattgcagtaaatgtaaagaagagtcaggtttgatctgatcaattt
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Figure 3. Sequence of the follicle-stimulating hormone gene exon 2 showing the PstI restriction site (cutting point), highlighted in magenta in the gene.

Genotype and allele frequencies

The genotype and allele frequencies for the FSH gene in the current buffalo population are presented in Table 1. Two genotypes, including (+/+) and (+/-), were identified, with the homozygous (+/+) genotype showing high dominance. Approximately 98% (48/49) of the successfully genotyped samples were +/+, while 2% (1/49) were +/-. The non-truncating homozygous genotype (−/−) was not found in samples. These frequencies indicated an allele frequency of about 0.99 for the (+) allele, which contained the PstI site, and 0.01 for the (-) allele, which lacked the PstI site. In other words, the female mud buffalo population almost possesses the PstI truncating allele, with just a single copy of the non-truncating allele remaining detected. Although the (−) allele is rare, the locus meets the criteria for polymorphism, as defined by Chen et al. (2016), which states that a locus is considered polymorphic when the minor allele frequency is ≥ 0.01. Based on the current results, the (+) allele frequency was slightly below 0.01 threshold, and the (-) allele was detectable. Therefore, the FSH exon 2 locus was classified as genetically polymorphic in the mud female buffalo population. The current findings are consistent with Gaina and Amalo (2022), who used the criterion of an allele frequency below 99% to define polymorphism. Additionally, the present results are aligned with the findings of Sofia et al. (2024), who reported polymorphisms in the FSH beta subunit gene in buffalo using PCR-RFLP with PstI. Polymorphisms in the FSH gene have been reported in different breeds, including Friesian, Brahman, Limousin, and Simmental cattle (Yurnalis et al., 2017; Primasari et al., 2021; Gaina and Amalo, 2022). In a similar study in coastal cattle, Afriani et al. (2022) observed polymorphism in the FSH gene using a different restriction enzyme (TasI), highlighting that several regions of the FSH gene contain genetic diversity. The widespread occurrence of FSH gene polymorphism across ruminant species highlighted the evolutionary conservation of genetic variability in reproductive genes. A study conducted by Zhang et al. (2011) indicated that SNPs in exon 3 of the FSH beta gene in goats were notably associated with litter size and follicle development. Meanwhile, Ishak (2012) identified polymorphic sites in the FSH beta subunit of Bali cattle associated with differences in reproductive performance. Additionally, Sosa et al. (2017) identified genetic variation in the FSH beta gene in water buffalo and proposed that these polymorphisms could influence hormonal regulation and ovarian function activity. These results indicated that FSH beta polymorphisms in swamp buffalo have a similar functional impact, reinforcing the gene's viability as a molecular marker to boost reproductive efficiency. Several factors influence differences in allele and gene frequencies, such as gene mutations, selection, gene flow between two populations with different gene frequencies, outcrossing, inbreeding, and genetic drift. Weak selection pressure on this gene likely allows the minor (-) allele to persist in buffalo. Conversely, strong artificial selection can decrease genetic diversity. However, buffalo in West Sumatra have not been subject to specific selection on FSH gene variants, which probably explains the observed polymorphism (Setyorini et al., 2023; Khan et al., 2024).

The FSH plays a crucial role in female reproductive physiology; therefore, even silent or neutral SNPs can serve as genetic markers when associated with key traits (Wang et al., 2021; Sikdar et al., 2021). The presence of minor alleles, at approximately 1%, indicated genetic diversity that breeders could utilize or observe.

Table 1. Allele and genotype frequencies of the follicle-stimulating hormone gene in female mud buffalo in Indonesia

Types of genotypes	**Total individuals**	**Genotype frequency**	**Total alleles**		**Allele frequency**	
			+	-	+	-
(+/+)	48	0.92	48	0	0.97	0.02
(+/-)	1	0.02	1	1		
(-/-)	0	0	0	0		
Total	49	1	49	1		

(+/+): Truncated homozygous genotype individuals, (+/-): Heterozygous genotype individuals, (-/-): Non-truncated homozygous genotype individuals

Table 2. Hardy-Weinberg equilibrium of the follicle-stimulating hormone gene (PstI polymorphism) in female mud buffalo in Indonesia

Types of Genotypes	**Frequency and types of genotypes**			**Total**	X^2h	X^2t
	(+/+)	**(+/-)**	**(-/-)**			
Observation (O)	48	1	0	49	1.53	65.17
Expectation (E)	46.1	1.90	0	48		
$(O-E)^2/E$	0.096	0.43	0	0.526		

χ^2: Chi-square, X^2h: Computed χ^2, X^2t: Tabulated χ^2

Hardy-Weinberg equilibrium analysis

To understand the genetic dynamics of the FSH locus in the sampled population, data from the present study were analyzed to assess whether genotype frequencies align with HWE expectations. Using the allele frequencies presented in Table 1, the expected genotype proportions were computed and compared with the observed distributions using a chi-square test, as shown in Table 2. Chi-square analysis revealed no significant deviation from HWE, as the calculated chi-square was lower than the critical chi-square value at $\alpha = 0.05$. In practice, the observed genotype frequencies, approximately 98% for (+/+) and 2% for (+/−), did not differ significantly from those predicted by HWE, given the allele frequencies of approximately 99% (+) and 1% (−). Therefore, the genotype distribution of the FSH gene in exon 2 of the female mud buffalo population was consistent with HWE expectations (Chen et al., 2016; Abramovs et al., 2020). The current findings align with those of Fouda et al. (2021), who stated that a population is considered to be in HWE level when the calculated chi-square value is less than the critical value for the specified degrees of freedom. This equilibrium might be attributable to random mating and the lack of selection across successive generations, thereby sustaining stable gene frequencies. In these closed buffalo herds, the two gene versions (+) and (−) are equally common because mating is random and neither version currently confers a breeding advantage. Similarly, Prihandini et al. (2021) observed that traditional buffalo populations often maintain HWE at neutral loci, reflecting random mating and stable allele transmission between generations.

Maintaining HWE and genetic diversity at the FSH gene can be advantageous. This result suggested that the *in-situ* conservation of these buffalo has preserved multiple alleles, potentially crucial for future adaptation. The persistence of the FSH polymorphism without selective pressure indicates that it could be a neutral variant. Studies in other species highlighted the significance of FSH gene variation. For instance, polymorphisms in the FSH beta-subunit gene are associated with reproductive traits in male goats (Khan et al., 2024). Meanwhile, variants in the FSH receptor gene have been associated with infertility, including repeated breeding, in buffalo (Fouda et al., 2021). These studies indicated that genetic variation in the FSH signaling pathway can significantly affect reproductive performance.

CONCLUSION

In female mud buffalo, two genotypes were identified in exon 2 of the FSH gene. The truncated homozygous (+/+) genotype was predominant, with only a single individual carrying the heterozygous (+/-) genotype. The allele distributions confirmed that the current buffalo population was genetically polymorphic at the FSH locus and in HWE analysis. The present study provided new insights into the genetic diversity of Indonesian mud buffalo and highlighted the potential utility of molecular markers, such as polymorphisms in the FSH gene, for guiding breeding strategies. Future studies should investigate how this FSH gene variation influences reproductive performance and its potential application in genetic improvement programs, such as marker-assisted selection to enhance fertility. By incorporating this genetic information into reproductive management, breeders can boost fertility and productivity while keeping the genetic diversity for the long-term health and vitality of the species. Furthermore, it is recommended to undertake more extensive genetic studies of buffalo reproductive genes and to implement breeding management strategies to facilitate the conservation and sustainable productivity of the local mud buffalo population.

DECLARATIONS

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Authors’ contributions

All authors contributed to the development of the experiments. Tinda Afriani and Jaswandi conceptualized the study. Khasrad and Faina Farhani were responsible for collecting samples and conducting laboratory analyses. Tiara Putri Artha and I Made Merdana performed the data analysis. Tinda Afriani, Jaswandi, and Khasrad composed the manuscript. All authors participated in revising, reviewing, and confirming the final edition of the manuscript.

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Competing interests

The authors declared that they have no conflict of interest.

Availability of data and materials

The data presented in this study will be available upon reasonable request from the corresponding author.

Ethical considerations

The authors declared that this manuscript has been thoroughly reviewed and meets all aspects of publication ethics, including the absence of plagiarism, data manipulation or falsification, and multiple publication or submission. The authors declared that no AI tools were used in preparing and writing this manuscript.

REFERENCES

Abramovs N, Brass A, and Tassabehji M (2020). Hardy-weinberg equilibrium in the large scale genomic sequencing era. Frontiers in Genetics, 11: 210. DOI: https://www.doi.org/10.3389/fgene.2020.00210

Afriani T, Purwati E, Yurnalis J, Mundana M, Rastosari A, and Farhana A (2022). Identification of single nucleotide polymorphism (snp) in exon 9 and intron 9 of follicle stimulating hormone receptor (fshr) gene in pesisir cattle. Journal of Animals and Health Production, 10(4): 479-484. DOI: https://www.doi.org/10.17582/journal.jahp/2022/10.4.479.484

Chen YS, Su YC, and Pan W (2016). Effect of spatial constraints on hardy-weinberg equilibrium. Scientific Reports, 6(1): 19297. DOI: https://www.doi.org/10.1038/srep19297

Davidescu MA, Ciorpac M, and Creanga S (2021). Highly effective method of DNA extraction from blood: A first step for analysis of genetic diversity of indigenous cattle breeds. Scientific Papers, 64(2): 40-44. Available at: https://ibn.idsi.md/sites/default/files/j_nr_file/vol2021_2_0.pdf#page=36

El Debaky HA, Kutchy NA, Ul-Husna A, Indriastuti R, Akhter S, Purwantara B, and Memili E (2019). Potential of water buffalo in world agriculture: Challenges and opportunities. Applied Animal Science, 35(2): 255-268. DOI: https://www.doi.org/10.15232/aas.2018-01810

Gaina CD and Amalo FA (2022). Genetic polymorphism of myostatin gene in sumba ongole (*Bos indicus*) cattle and its association with growth traits. Journal of Advanced Veterinary and Animal Research, 9(4): 565-572. DOI: https://www.doi.org/10.5455/javar.2022.i625

Fitriani Z, Riyanto J, Dewanti R, Cahyadi M, Wati AK, Pawestri W, and Yanti Y (2023). Reproductive performance of buffalo (*Bubalus bubalis*) in small scale farm in Boyolali Regency Central Java. Jurnal Ilmu-Ilmu Peternakan, 33(3): 327-335. DOI: https://www.doi.org/10.21776/ub.jiip.2023.033.03.03

Fouda M, Hemeda S, El-Bayomi K, El-Araby I, Hendam B, and Ateya A (2021). Genetic polymorphisms in fshr/alui and esrα/bg1i loci and their association with repeat breeder incidence in buffalo. Journal of the Hellenic Veterinary Medical Society, 72(2): 2869-2878. DOI: https://www.doi.org/10.12681/jhvms.27525

Ishak ABL, Sumantri C, Noor RR, and Arifiantini I (2011). Identification of polymorphism of FSH beta-subunit gene as sperm quality marker in Bali cattle using PCR-RFLP. Journal of the Indonesian Tropical Animal Agriculture, 36(4): 221-227. DOI: https://www.doi.org/10.14710/jitaa.36.4.221-227

Kamilari M, Papaioannou C, Augustinos A, Spinos E, Giantsis IA, Ramfos A, and Batargias C (2025). From Shell to Sequence: Optimizing DNA Extraction and PCR for Pen Shell Identification. Water, 17(8): 1162. DOI: https://www.doi.org/10.3390/w17081162

Khan A, Thakur MS, Joshi S, Shukla SN, Khare A, Khare V, Vandre RK, and Sharma M (2024). Genetic polymorphism of follicle stimulating hormone beta (fshβ) gene and its association with body weight, scrotal measurements and seminal quality traits in indigenous goats: Genetic polymorphism of fshβ gene in goats. Indian Journal of Experimental Biology, 62(5): 309-316. DOI: https://www.doi.org/10.56042/ijeb.v62i05.4242

Knudsen BE, Bergmark L, Munk P, Lukjancenko O, Priemé A, Aarestrup FM, and Pamp SJ (2016). Impact of sample type and DNA isolation procedure on genomic inference of microbiome composition. mSystems, 1(5): e00095-16. DOI: https://www.doi.org/10.1128/mSystems.00095-16

Marquina D, Roslin T, Łukasik P, and Ronquist F (2022). Evaluation of non-destructive DNA extraction protocols for insect metabarcoding: gentler and shorter is better. Metabarcoding and Metagenomics, 6: e78871. DOI: https://www.doi.org/10.3897/mbmg.6.78871

Mishra B, Dubey P, Prakash B, Kathiravan P, Goyal S, Sadana D, Das G, Goswami R, Bhasin V, and Joshi B (2015). Genetic analysis of river, swamp and hybrid buffaloes of north-east india throw new light on phylogeography of water buffalo (*Bubalus bubalis*). Journal of Animal Breeding and Genetics, 132(6): 454-466. DOI: https://www.doi.org/10.1111/jbg.12141

Nainggolan HL, Aritonang J, Sihombing M, Supriana T, and Tafsin MR (2019). Structural modelling of rice fields-buffalo livestock based integrated agricultural systems in the context of regional development in Humbang Hasundutan, Indonesia. Future of Food: Journal on Food, Agriculture and Society, 7(2): Nr-106. DOI: https://www.doi.org/10.17170/kobra-20190709595

Pehan EA, Miah M, Rahman MH, Shejuty SF, Haque MN, Huda MN, Habib MR, and Ali MY (2025). A holistic review of buffalo productivity, reproductive efficiency, genetic improvement, and disease management in Bangladesh. Veterinary and Animal Science, 29: 100496. DOI: https://www.doi.org/10.1016/j.vas.2025.100496

Prihandini PW, Primasari A, Luthfi M, Pamungkas D, Sari APZNL, Dina TB, and Maharani D (2021). Identification of restriction enzyme in the FSHR gene of Indonesian local cattle. IOP Conference Series: Earth and Environmental Science, 888: 012024. DOI: https://www.doi.org/10.1088/1755-1315/888/1/012024

Primasari A, Efendy J, and Prihandini P (2021). Polymorphism of follicle-stimulating hormone beta sub-unit (fsh-p) gene as a molecular marker for reproductive status in Peranakan Ongole x Bali crossbred (Poba) cattle. IOP Conference Series: Earth and Environmental Science, 902: 012052. DOI: https://www.doi.org/10.1088/1755-1315/902/1/012052

Rehman SU, Hassan F-u, Luo X, Li Z, and Liu Q (2021). Whole-genome sequencing and characterization of buffalo genetic resources: Recent advances and future challenges. Animals, 11(3): 904. DOI: https://www.doi.org/10.3390/ani11030904

Reswati and Putra AA (2023). The profile of buffalo farming in Matur District, Agam Regency, West Sumatra. Journal of Agricultural Sciences and Veterinary, 11(1): 97-106. doi: https://www.doi.org/10.31949/agrivet.v11i1.5997

Roza E, Yellita Y, Susanty H, and Rizqan R (2024). Improving milk production and quality of Murrah buffalo through local forage-based feeding. Journal of Animal Health and Production, 12(3): 337-342. DOI: http://www.doi.org/10.17582/journal.jahp/2024/12.3.337.342

Setyorini YW, Kurnianto E, Sutopo S, and Sutiyono S (2023). Identification polymorphism of lhr and fshr genes in Indonesian Holstein dairy cattle associated with productive and reproductive traits. Biodiversitas Journal of Biological Diversity, 24(5): 2898-2905. DOI: https://www.doi.org/10.13057/biodiv/d240544

Sikdar S, Das T, Sajib EH, Rahman KMU, Siddiki AZ, and Uddin MB (2021). Multi-omics and molecular biology perspective in buffalo genome. Journal of Buffalo Science, 10: 21-31. DOI: https://www.doi.org/10.6000/1927-520X.2021.10.04

Sofia A, Sari E, Abdullah M, and Wahyuni S (2024). Identification of growth hormone releasing hormone in Gayo buffalo (*Bubalus bubalis*) using PCR-RFLP. Technological innovations in tropical livestock development for environmental sustainability and food security. CRC Press, pp. 83-88. DOI: https://www.doi.org/10.1201/9781003468943

Sosa AS, Mahmoud KGM, Kandiel MM, Eldebaky HA, Nawito MF, and Abou EME (2017). Genetic characterization of FSH beta-subunit gene and its association with buffalo fertility. Asian Pacific Journal of Reproduction, 6(5): 193-196. DOI: https://www.doi.org/10.4103/2305-0500.215928

Terry SA, Basarab JA, Guan LL, and McAllister TA (2020). Strategies to improve the efficiency of beef cattle production. Canadian Journal of Animal Science 101(1): 1-19. DOI: http://www.doi.org/10.1139/cjas-2020-0022

Utomo B (2018). Identification polymorphism of fsh ß-subunit gene and fshr gene in Madura cattle results of artificial insemination with Limousin cattle with PCR-RLP technique. International Journal of Advanced Scientific Research and Management, 3(10): 38-43. Available at: https://api.semanticscholar.org/CorpusID:199564043

Utomo B and Safitri E (2021). DNA sequence analysis of follicle-stimulating hormone (fsh) gene and follicle-stimulating hormone receptor (fshr) gene in madrasin cattle with ovarian hypofunction. Journal of Hunan University Natural Sciences, 48(4): 18-22. Available at: https://jonuns.com/index.php/journal/article/view/547/544

Wang HQ, Zhang WD, Yuan B, and Zhang JB (2021). Advances in the regulation of mammalian follicle-stimulating hormone secretion. Animals, 11(4): 1134. DOI: https://www.doi.org/10.3390/ani11041134

Widi TSM, Udo H, Oldenbroek K, Budisatria IGS, Baliarti E, and der Zijpp Av (2021). Designing genetic impact assessment for crossbreeding with exotic beef breeds in mixed farming systems. Outlook on Agriculture 50(1): 34-45. DOI: https://www.doi.org/10.1177/0030727020915206

Yurnalis Y, Arnim A, Sarbaini S, and Jamsari J (2017). New diversity at the end of the growth hormone gene of local cattle in West Sumatra. Indonesian Journal of Animal Science, 19(3): 107-113. DOI: https://www.doi.org/10.25077/jpi.19.3.103-109.2017