Association Between Prolactin Receptor (PRLR) Gene Polymorphisms and Litter Size in Katjang-Boer Hybrid Goats

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Abstract

Goat fertility is essential for farm productivity and economic viability. This study investigates the association between Prolactin Receptor (PRLR) gene polymorphism and litter size in Katjang-Boer hybrid goats. DNA from 43 goats was analyzed using five primers targeting different regions of the PRLR gene. Polymerase chain reaction (PCR) and Single-Strand Conformation Polymorphism (SSCP) were employed to detect genetic variation. The association between litter size, breeding year, and PRLR polymorphism was determined using Kruskal-Wallis and Mann-Whitney U tests. Results indicate a significant association between PRLR gene polymorphisms in intron 1 and intron 2 with litter size ($p < 0.05$). Specifically, the AH genotype in intron 1 was significantly associated with larger litter sizes (1.58 ± 0.19, $p < 0.05$). No significant associations were found for exon 9 and 3'UTR regions. Breeding year also significantly influenced litter size, with the highest numbers observed in the later parity. These findings provide insights for improving farm productivity through genetic selection.

Keywords: PRLR gene, polymorphism, litter size, Katjang-Boer hybrid, SSCP analysis.

Introduction

Domestic goats (*Capra hircus*) have been integral to human agricultural practices for millennia, being among the first animals domesticated by humans (Monteiro et al., 2017). As members of the Bovidae family, goats share ancestry with species such as sheep and cattle, but they possess unique adaptations that enable them to thrive in diverse environments, including arid and mountainous regions (Zheng et al., 2020). This versatility makes goats a vital resource in tropical climates, where other livestock may struggle. Goats are primarily raised for meat, milk, and fiber, with goat milk being a key nutritional source due to its ease of digestion and anti-inflammatory properties (Pragna et al., 2018; AlKaisy et al., 2023). In Malaysia, the goat industry faces several challenges, including declining reproductive performance in breeding herds. Although goats and sheep only account for a small portion of Malaysia's

livestock sector, the rising demand for goat meat and milk highlights the need to improve its productivity (Zaffrie et al., 2022). Increasing litter size, a crucial measure of reproductive efficiency, would directly benefit farm profitability by increasing the number of offspring per birth event. However, current goat breeding programs face limitations due to the lack of genetic markers associated with fertility traits, such as litter size.

Gene mapping efforts in goats remain limited compared to sheep, with less than half of the molecular markers identified for sheep present in goats (Zonaed Siddiki et al., 2020). Molecular markers are critical for identifying genetic variants that influence fertility, and one such candidate gene is the prolactin receptor (PRLR) gene. The PRLR gene mediates the action of prolactin, a hormone essential for reproduction, lactation, and maternal behavior. Previous studies have shown that polymorphisms in the PRLR gene are associated with increased fertility in livestock, including pigs and sheep (Vincent et al., 1998; Ghiasi & Abdollahi-Arpanahi, 2021). Despite the significance of the PRLR gene in other species, its role in goats, particularly in tropical breeds like the Katjang-Boer hybrid, remains poorly understood. The Katjang-Boer hybrid was developed by Malaysia's Agricultural Research Institute (MARDI) to enhance the adaptability and productivity of imported Boer goats (Hifzan et al., 2018). While the hybrid has shown promise in size and resilience, little is known about the genetic factors that influence its reproductive performance, particularly litter size. Few studies have investigated the association between PRLR gene polymorphisms and litter size in goats, particularly in tropical hybrid breeds such as the Katjang-Boer hybrid. This lack of information represents a significant gap in goat breeding programs, where improved reproductive efficiency is critical for economic sustainability. This study aims to fill the gap in understanding the influence of PRLR gene polymorphism on litter size in Katjang-Boer hybrid goats, focusing on the gene's role across different breeding years. By identifying specific genotypes associated with larger litter sizes, this research could provide valuable insights for Marker-Assisted Selection (MAS) programs, ultimately enhancing farm productivity.

Materials and Methods

Animal Ethics

The experimental design and methodology of this study were approved by MARDI Animal Ethics Committee (Approval number: 20231109/R/MAEC00139).

Sample Collection

Animals used for this study were 43 mature female Katjang-Boer hybrid goats housed at MARDI Kluang Station, Johor. The animals were borne from the year 2016 – 2020 and can be divided into F1 and F2 due to different breeding programs. The parity and litter size of each goat were recorded over three years from 2021 to 2023. There is a limitation in the data availability of parity data before the year 2021. Hence, in this study, we describe parity as the

breeding year. Litter size was determined by the number of kids born per kidding and was recorded immediately after birth. Data were collected during each kidding season to ensure accuracy.

Blood Sampling and DNA Extraction

Blood was drawn from the jugular vein using EDTA Vacuum Blood Collection Tubes (Jun Nuo, China) to prevent coagulation. To reduce stress, goats were restrained appropriately, and a cloth was placed over their eyes. Each sample consisted of 10 mL of blood, which was stored at 4°C during transport to the laboratory. Genomic DNA was extracted from blood samples using the Promega Wizard® Genomic DNA Purification Kit (Promega Corporation, Wisconsin, USA) according to the manufacturer's instructions. The extracted DNA was stored at -20°C for long-term preservation.

DNA Quantification

DNA concentration and purity were measured using a Nanodrop™ One Spectrophotometer (Thermo Fisher Scientific, Massachusetts, USA). Absorbance at 260/280 nm and 260/230 nm was used to assess DNA quality. Acceptable purity ratios for the 260/280 nm range from 1.8 to 2.0, while the 260/230 nm ratio should ideally be between 2.0 and 2.2. If these ratios deviate, contaminants such as proteins or phenols may be present. In such cases, the DNA should be re-purified using additional purification steps to remove impurities (García-Alegría et al., 2020).

DNA Verification

The quality of extracted DNA was further verified by agarose gel electrophoresis (Green & Sambrook, 2019). A 0.7% agarose gel was prepared, and 100 bp DNA ladders were used as size markers. The gel was run at 70V for one hour, and bands were visualized using a UV transilluminator to confirm the successful extraction of high-quality DNA.

DNA Amplification

Five sets of primers were used to target different regions of the PRLR gene, including intron 1, intron 2, exon 9, exon 10, and the 3'UTR region (Di et al., 2011; Hou et al., 2013; An et al. 2015; El-Shorbagy, 2022). The primer sequences and annealing temperatures are listed in Table 1. Amplification was carried out using a Bio-Rad C1000 Touch thermal cycler (Bio-Rad Laboratories, California, USA) under the following standard conditions: initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at the primer-specific temperature for 30 seconds, and extension at 72°C for 35 seconds, with a final extension at 72°C for 10 minutes. However, the annealing temperature for each primer was determined. Details of the primers including the respective annealing temperature are listed in Table 1.

Table 1. Primers used for DNA amplification.

Primer	Sequence	Gene region	Product size (bp)	Annealing Temperature (°C)	Reference
Primer 1	F: 5'-CATCTGCTGGAGGTAAGTGC-3' R: 5'-TTCATTGCCCTCTGACGCTT-3'	Intron 1	215	60.0	Di et al. 2011
Primer 2	F:5'-TGTCAGTAAGCGTCAGAGGGC-3' R:5'-GGCTGGTGGAAGGTCACTCTT-3'	Intron 2	176	59.7	Di et al. 2011
Primer 3	F:5'-AAACCCCCTTGTTCTCTGCTA-3' R:5'-CCCAACCCAACTGGAGTCTGC-3'	Exon 10	315	57.0	El-Shorbagy et al. 2022
Primer 4	F:5'-CTTACCACAACATTGCTGAC-3' R:5'-CCTTGGCTGGATTCTATGG-3'	Exon 9	465	59.7	Hou et al. 2013
Primer 5	F:5'-AGTGAGAGTTATGGAAGGATG-3' R:5'-AAGGTTAAGCAACTGGTCTT-3'	3'UTR	443	57.3	An et al. 2015

Primer direction (F = Forward; R = reverse).

Polymerase chain reaction was done as described by Chu et al. (2007) with modifications were carried out using Bio-Rad C1000 Touch thermal cycle (Bio-Rad Laboratories Inc, California, United States) in 0.2 mL microcentrifuge tubes, consisting of 1 µL of DNA sample (100 ng/ µL), 0.5 µL of each primer (20 µmol/µL), 12.5 µL of 2x Bioline MyTaq™ Red Mix (Bioline Reagents Ltd., London, United Kingdom) and 11 µL sterile distilled water. The cycling conditions were initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing for 30s seconds, extension at 72°C for 35 seconds and a final extension of 72°C for 10 minutes. The amplicons were electrophoresed in 2% agarose gels with 1x TAE buffer, containing 6 µL of GelStain (TransGen Biotech Co., Ltd, Beijing, China. The first wells on the top and bottom rows of the gel were loaded with 6.5 µL of 100 bp DNA ladder (Promega Corporation, Wisconsin, United States). The rest of the wells were loaded with 7 µL of PCR product. Once the gel chamber is closed and connected to the power supply, the gel is run at 75V for 40 mins. The gel was then visualised under the GelDoc Go Gel Imaging System (Bio-Rad Laboratories Inc., California, United States).

Single-Strand Conformation Polymorphism (SSCP) Analysis

SSCP analysis was performed using 5% MetaPhor™ agarose gel (Lonza LTD, Basel, Switzerland) to detect genetic variations in the amplified DNA fragments. The samples were denatured at 95°C for 10 minutes, cooled at 4°C for 10 minutes, and then loaded onto the gel. Electrophoresis was conducted at 70V

for 90 minutes, and the bands were visualized using a GelDoc Go Gel Imaging System (Bio-Rad Laboratories, California, USA). Bands corresponding to different conformations of the DNA were identified, indicating potential polymorphisms (Kakavas, 2021).

Statistical Analysis

Data on litter size were analyzed using IBM SPSS version 27. The Kruskal-Wallis test was employed to assess the association between different PRLR gene genotypes and litter size, while the Mann-Whitney U test was used for post-hoc comparisons between genotypes. These non-parametric tests were selected based on the non-normal distribution of the data, as confirmed by the Shapiro-Wilk test (Esmaeili-Fard et al., 2021). Associations between parity and litter size were also tested using the same statistical methods.

Effects of Breeding Year on Litter Size of Katjang-Boer Female Goats

Based on the sequential breeding year, the litter size of the Katjang-Boer goats increased progressively from the earlier parity (0.698 ± 0.12) to the latest parity (1.186 ± 0.15), with a statistically significant difference ($p < 0.005$) observed across the three breeding years (Table 2). These findings are consistent with studies on Black Bengal goats, which also demonstrated an increase in litter size with parity (Haldar et al., 2014). The significant increase in litter size with parity is likely due to the physiological maturity of the reproductive system as does age, resulting in larger uterine capacity and increased fecundity (Briggs et al., 2023). This pattern has also been observed in Nigerian goat breeds, where litter size peaks during the third or fourth parity before declining in subsequent parities (Akpa et al., 2011).

Results and discussion

Table 2. Average litter size according to parity for 2021 to 2023.

Breeding Year	Average Litter Size
2021	0.70 ± 0.12^{b}
2022	0.91 ± 0.11^{b}
2023	1.19 ± 0.96^{a}

Data were illustrated as mean ± standard deviation (Mean ± SD).
^{a,b,c}Means with different superscripts within a column are significantly different at ($p < 0.05$).

Data available for this study is limited to three years. Hence, the number of parities could not be confirmed. However, the parities can be assumed to increase in the subsequent years. Litter size in the year 2023 (1.19 ± 0.96) was significantly higher ($p < 0.05$) than in the year 2021 (0.70 ± 0.12) and 2022 (0.91 ± 0.11) (Table 2). However, there was no significant difference found between the first two breeding years ($p > 0.05$). The results are consistent with a study by El-

Shorbagy et al. (2022) which reports a gradual increase in litter size from the first parity to the highest during the 4th parity. This claim is also supported by a study by An et al. (2015) where the litter size for each genotype increases from the 1st to the 4th parity. Haldar et al. (2014) studied the association between parity and litter size in Black Bengal goats. The result of this study also shows that the litter size is strongly influenced by the parity as larger litter size was observed in goats with higher parity. A study on Nigerian goat breeds such as West African Dwarf, Red Sokoto and Sahel by Briggs et al. (2023) reported an increase in litter size from first to fourth parity.

The litter size then decreases from the fourth to the fifth parity. In another study on Nigerian goats by Akpa et al. (2011), the twinning rate highest during the second parity while triplet rate was highest in the third parity. While the average parity begins to decline after the fifth parity, quadruplets were only observed during the sixth and eighth parity but was not observed in the lower parity. One possible explanation for this is due to the physiological maturity of the doe. As reported in this study where the litter size increases as parity increases, due to the physiological maturity of the doe. This indicates that parity also influenced the reproductivity of goats. In this study, two goats also experienced abortion during the second parity. The abortion could have been caused by various factors such as infections, genetic factors, nutrition, hormonal. However, due to the limited data, it is not possible to point out the exact cause of abortion. Many factors can affect the prolificacy of the does. Litter size could potentially be affected by the physiological maturity of the does as proven in the previous studies. Bhugai et al. (2021) stipulated that early parity leads to lower litter size due to the young age of the does, resulting in the reproductive organs not reaching full maturity. The same study also claims that higher parity leads to larger litter size due to the larger uterine capacity. As the does ages, the reproductive organs will also degenerate, resulting in lower litter size once it has passed the peak value.

Due to financial constraints faced by the farm, the data of parity before the year 2021 could be obtained. As a result, the effects of parity on litter size can only be analyzed from the year 2021. A bias was made by assuming that the first parity was in 2021 to analyze the data and associate the parity with the litter size. As the maternal age of the does cannot be determined in this study, age-related factors were not explicitly considered in this study. One suggestion for future studies is to investigate the association between maternal age and litter size as maternal age could be a separate factor apart from the number of parities. For further studies, it is suggested to consider the maternal age as well when studying the parity.

Polymorphism in PRLR Gene

Polymorphisms in the PRLR gene were detected through Single Strand Conformation Polymorphism (SSCP) analysis, which is a reliable method for identifying mutations or genetic variations in DNA sequences (Sharma &

Rishi, 2021). In this study, polymorphisms were observed in four out of five target regions within the PRLR gene. The variation in genotypes was most pronounced in intron 1 and intron 2, while no polymorphism was detected in exon 10 (Table 3). These findings indicate the presence of genetic diversity within the PRLR gene, which may be associated with the observed variation in litter size among the Katjang-Boer hybrid goats.

Based on Table 3, the SSCP analysis revealed different banding patterns, which are referred to as single band (SB) or double band (DB). SB indicates the presence of a homozygous genotype, while DB signifies a heterozygous genotype. For example, in exon 9, a majority of the goats exhibited the DB pattern (35 out of 43), suggesting the presence of heterozygous genotypes at this locus. Conversely, in exon 10, only a single genotype (AG) was detected, which may reflect a lack of genetic variation in this region for the Katjang-Boer hybrid goats.

In intron 1, two genotypes, AA and AH, were detected, with frequencies of 0.721 ± 0.032 and 0.279 ± 0.022, respectively. Similarly, in intron 2, the CD and DD genotypes were observed with frequencies of 0.419 ± 0.029 and 0.581 ± 0.033. These findings are consistent with previous studies in Jining Grey goats, where a higher frequency of the AA genotype was reported (Di et al., 2011). However, unlike the Jining Grey goats, which exhibited multiple polymorphisms (e.g., AA, AH, AK, HH, HK), the Katjang-Boer hybrids in this study displayed less genetic diversity, potentially due to the smaller sample size or selective breeding practices. The presence of polymorphisms in the PRLR gene was significantly associated with litter size, particularly for intron 1 and intron 2 (Table 3). Goats with the AH genotype in intron 1 had larger litter sizes (1.58 ± 0.19) compared to goats with the AA genotype (1.03 ± 0.10, p = 0.013).

Similarly, in intron 2, the CD genotype was associated with larger litter sizes (1.50 ± 0.15) compared to the DD genotype (0.96 ± 0.11, p = 0.006). These findings suggest that the H allele in intron 1 and the C allele in intron 2 may have positive effects on fecundity in the Katjang-Boer hybrid goats, supporting the use of these polymorphisms as potential markers in breeding programs aimed at improving litter size (Di et al., 2011; Ghiasi & Abdollahi-Arpanahi, 2021).

The relatively small sample size used in this study may limit the generalizability of the findings, particularly in terms of the observed lack of polymorphism in exon 10. Future studies should include a larger sample size and explore additional loci within the PRLR gene to uncover more genetic variations that may be linked to reproductive traits. Additionally, gene sequencing of the single and double bands observed in exon 9 and 3'UTR is recommended to further clarify the underlying genetic structure.

Table 3. Genotype frequency of 5 loci in PRLR gene and the effects of PRLR gene on average litter size in Boer-Katjang hybrid does.

Primer	Target region	Genotype	Sample size	Frequency ± SE	Average litter size ± SE
Primer 1	Intron 1	AA	31	0.721 ± 0.032	1.03 ± 0.10[a]
		AH	12	0.279 ± 0.022	1.58 ± 0.19[b]
Primer 2	Intron 2	CD	18	0.419 ± 0.029	1.50 ± 0.15[b]
		DD	25	0.581 ± 0.033	0.96 ± 0.11[a]
Primer 3	Exon 10	AG	43	1.000 ± 0.000	1.19 ± 0.10
Primer 4	Exon 9	SB	8	0.186 ± 0.040	1.25 ± 0.25[a]
		DB	35	0.814 ± 0.036	1.17 ± 0.10[a]
Primer 5	3'UTR	SB	15	0.349 ± 0.032	1.33 ± 0.19[a]
		DB	28	0.651 ± 0.037	1.11 ± 0.11[a]

Note: Data was illustrated as mean ± standard error (Mean ± SE).
[a,b]Average litter size with different superscripts within a column are significantly different at (p <0.05).

There was no significant difference indicated between the litter size of the two polymorphisms detected in the exon 9 region (Primer 4). However, this could be a result of the small sample size of this study. A study by Hou et al. (2015) reported a significant additive effect of the homozygous GG genotype on Guangzhou and Boer goat breeds which produced an average litter of 1.72 ± 0.02 at the c.1457G>A locus of the exon 9 region as compared to the smaller litter size of the heterozygous GA of Guangzhou and Boer does (1.59 ± 0.05 and 1.54 ± 0.06, respectively). The same study also detected another mutation on the c.1645G>A locus of the exon 9 region where both goat breeds with homozygous GG genotype produced larger litter sizes than does with heterozygous GA genotype. In the 3'UTR region, the polymorphisms (SB and DB) detected by Primer 5 are not statistically different (p > 0.05). While PRLR gene polymorphisms are said to be species-specific and not on the same region, there are still regions of the PRLR gene have yet to be well defined (Banks et al. 2024). In addition, more studies on polymorphism have been done in pigs and sheep as compared to goats which shows that PRLR gene is linked to productivity traits including litter size. This shows that the PRLR gene has the prospect of being used as a selection marker for litter size in livestock. Thus, more studies are required in goats to discover the potential of the PRLR gene and to determine whether the *PRLR* gene is associated with litter size in goats.

Conclusion

The study provides valuable insights into the association between prolactin receptor (PRLR) gene polymorphisms and litter size in Katjang-Boer hybrid

goats. Significant genetic variations were identified in introns 1 and 2, which were positively associated with litter size, demonstrating the potential of these polymorphisms as markers for selective breeding programs. The findings underscore the importance of breeding year in influencing reproductive performance, with litter size peaking around the fourth parity, consistent with the physiological maturity of reproductive organs and uterine capacity. Despite these advances, limitations such as the small sample size and the lack of observed polymorphism in certain gene regions highlight the need for further research. Expanding the study to other loci of the PRLR gene and involving a broader range of goat breeds could offer more comprehensive insights into the genetic determinants of reproductive performance. Such studies could bridge existing gaps in goat gene mapping and enhance marker-assisted selection (MAS) programs, ultimately boosting farm productivity and economic sustainability. This research contributes to the growing body of evidence supporting the utility of PRLR gene polymorphisms in livestock breeding, positioning them as promising genetic markers for improving fertility traits in goats.

Acknowledgement

Appreciation is extended to the officers and staff of the Livestock Science Research Centre, at Malaysian Agriculture Research and Development Institute (MARDI) Serdang Selangor and Kluang Johor as well as the Faculty of Science, UPM for their corporation in providing facilities such as a laboratory and trial plot during the experiment and for the commitment from MARDI and UPM toward the realization of this project. This project was funded by Projek Pemacu Pertanian: K-RL 322-1001- KSR999.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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