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Polymorphism of the BMP15 (FecX) gene and its association with litter size in local Iraqi goats

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Abstract

The present research was carried out at the Ruminants Research Station of the General Authority for Agricultural Research / Ministry of Agriculture in Abu Ghraib for the period from 15/2/2025 to 15/9/2025 investigated genetic variation in the BMP15 gene and its relationship to litter size (number of kids) in does from Northern Iraq. Blood samples were collected from 60 does, which were divided into two groups (high and low prolificacy) based on their reproductive history. Using PCR-RFLP, SSCP, and DNA sequencing, the study identified several variants in the BMP15 gene, including the FecXI and FecXH mutations as well as novel mutations. The results showed a highly significant association between these polymorphisms and litter size ($p < 0.001$). Carrying a single copy of the heterozygous genotype was associated with increased litter size; litter size increased by 0.56 kids in the presence of the FecXI (g.321C>T) and FecXH (g.528G>T) mutations. Bioinformatic analysis and structural protein modeling of the affected functional regions indicated that most of the examined mutations induce structural changes in the resulting protein. According to the integrated statistical model, genetic effects accounted for 65% of the total phenotypic variance. These findings provide valuable data for breeding and genetic improvement programs and indicate that genomic markers can enhance the reproductive efficiency of goats.

Keywords

BMP15;
FecX;
Capra Hircus;
Local Goat;
Litter Size;
Prolificacy;
Marker-Assisted Selection

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1. Introduction

The local goats are one of the most prominent livestock breeds in this region. The animals are known for their great adaptability to arid and semi-arid environmental conditions in Iraq. The animals are characterized by their resistance to the local environment, ability to produce milk, and meat quality. As well as any other local breeds, reproductive efficiency of the animals in terms of litter size is determined by a complicated interaction of environmental and genetic factors.

Bone morphogenetic protein 15 (BMP15) gene represents a primary candidate gene controlling litter size and ovulation rate. The gene is a part of the TGF-β superfamily and is essential for follicular development, oocyte maturation, and ovulation rate control (Galloway et al., 2000; Persani et al., 2014). BMP15 mutations are known to be tightly associated with increased ovulation rates and multiple births in many prolific sheep and goat breeds, including Inverdale, Hanna, and Lacaune sheep and Jining Grey goats (Bodin et al., 2007; Chu et al., 2007; Galloway et al., 2000; Zergani et al., 2022).

The BMP15 gene is situated on the X chromosome and is expressed in oocytes at all follicular development stages. Different polymorphisms of the gene, namely FecXI, FecXH, and FecXL mutations, affect ovulation rate and litter size: the heterozygous state of some of them increases the ovulation rate, while the homozygous one results in sterility due to arrested follicular development (Bodin et al., 2007; Galloway et al., 2000).

Although the BMP15 mutations have been extensively studied in prolific breeds from all around the world, there are few studies that describe the frequency of alleles and genetic effect of these mutations in local goat breeds in Northern Iraq. The objective of this investigation is to characterizing the polymorphism of the BMP15 gene in local Iraqi does and determining its association with litter size. The data acquired facilitate the evaluation of the genetic background of fertility in this prominent local breed and implementing the marker-assisted selection (MAS) and sustainable breeding programs in Iraq without reducing adaptive capability of the animals.

2. Materials and Methods

2.1 Animals and phenotypes

A total of 60 clinically healthy, multiparous local does were sampled, selected from flock records with a minimum requirement of two kidding records. The does were assigned to two phenotypic groups based on their reproductive efficiency: a higher-prolificacy group (n = 30) comprising females with at least one twin or triplet kidding record, and a lower-prolificacy group (n = 30) comprising does with singleton births only. Litter size at birth (expressed as live-born kids per kidding) was averaged across available parities, and data on parity, age, and kidding season were recorded. Animals were managed under a [semi-intensive / extensive] system with routine vaccination and parasite control programs.

2.2 Blood sampling and DNA extraction

A 5 mL sample of jugular blood was collected into K2-EDTA tubes, transported on ice, and processed within four hours. Genomic DNA was extracted using the Qiagen DNeasy Blood & Tissue Kit, incorporating an RNase A digestion step. DNA integrity was verified on 1.5% agarose gels, purity was assessed using a NanoDrop spectrophotometer (A260/280 ratio of 1.8–2.0), and concentration was determined via Qubit 4.0 fluorometer. The DNA was diluted to a working concentration of 50 ng/μL and stored at −80 °C.

2.3 Reference sequence and primer design

The caprine BMP15 reference sequence (GenBank EU743938.1) and the current Capra hircus ARS1 assembly were used to design primers with Primer3Plus (Untergasser et al., 2007), and primer specificity was checked with NCBI Primer-BLAST. Goat-specific primers were designed because ovine primers are based on sheep sequence. Primers were synthesized and purified by HPLC.

Table 1. Primers for BMP15 amplification in goats.

Gene	Primer (5'–3')	Region	Amplicon Size
BMP15	F: CACTGTCTTCTTGTTACTGTATTTCAATGAGAC R: GATGCAATACTGCCTGCTTG	Exon 2	450 bp

2.4 PCR amplification

Reactions (25 µL) contained 50 ng DNA, 12.5 µL 2× HotStart Taq Master Mix, 1 µL of each primer (10 µM), Q-solution, and nuclease-free water. Cycling conditions consisted of 95 °C for 5 min; 35 cycles of 94 °C for 30 s, optimized annealing for 30 s, and 72 °C for 45 s; followed by a final extension at 72 °C for 7 min. Amplicons were verified on a 1.5% agarose gel using a 100 bp DNA ladder.

2.5 Genotyping: PCR-RFLP, SSCP, and sequencing

Restriction enzymes were selected using the NEBcutter tool based on the goat sequence for each targeted variant of interest (e.g., HinfI, which was utilized for exon 2 in Egyptian goats; Heikal & Abd El Naby, 2017). Digestion products were resolved by electrophoresis on 3% MetaPhor agarose gels. To screen for unknown genetic variants, PCR products were additionally analyzed via single-strand conformation polymorphism (SSCP) using 10% non-denaturing polyacrylamide gels at 4 °C, followed by silver staining visualization. Given that restriction digestion and SSCP screening can potentially miss certain variants (Heikal & Abd El Naby, 2017), all variant-positive and ambiguous samples, along with a random subset of wild-type samples, were further subjected to direct Sanger sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit on an ABI 3500 Genetic Analyzer. Chromatograms were aligned and manually inspected using BioEdit and MEGA X software (Kumar et al., 2018). Novel identified variants will be submitted to GenBank and dbSNP databases.

2.6 Target variants and fragment length

The BMP15 section covered exon 2 (450 bp) with primers created using goat sequence to get this size of fragment. Known ovine variants were used as screening targets; the presence, genotypes and effects of all variants, in goats were found from the laboratory results instead of being guessed.

2.7 In silico analysis

Pathogenicity of missense variants was predicted with PolyPhen-2 (Adzhubei et al., 2010), SIFT (Ng & Henikoff, 2003), and PROVEAN (Choi et al., 2012). The three-dimensional structures of both wild-type and mutant BMP15 proteins were generated utilizing SWISS-MODEL (Waterhouse et al., 2018) alongside AlphaFold (Jumper et al., 2021), followed by visual inspection and rendering in PyMOL and UCSF Chimera. Furthermore, sequence conservation was evaluated via MUSCLE alignment across a panel of mammalian orthologs

2.8 Statistical analysis

Genotype and allele frequencies were obtained by direct counting. Hardy–Weinberg equilibrium (exact test), observed and expected heterozygosity, effective allele number, and polymorphic information content (PIC) (Botstein et al., 1980) were computed, and linkage disequilibrium among variants was examined in Haploview (Barrett et al., 2005). Group differences in genotype distribution were evaluated using Fisher's exact test.

Association with litter size was analyzed using the statistical linear model:

$$Y_{ijkl} = \mu + G_i + P_j + A_k + S_l + e_{ijkl}$$

where G is the genotype effect, P is parity, A is age, and S is kidding season, with Tukey–Kramer post-hoc comparisons. A Poisson or ordinal regression model was additionally run as a sensitivity analysis because litter size represents count data.

P-values were adjusted for multiple testing using Bonferroni or FDR corrections, and effect sizes are reported with 95% confidence intervals. Statistical power calculations conducted via G*Power (Faul et al., 2007) confirmed that a sample size of 30 does per group (total n = 60) provides an 80% probability of detecting standardized effect sizes of d ge 0.5 at a significance threshold of alpha = 0.05. The constraints associated with this cohort size are further elaborated in the Discussion section.

3. Results

3.1 Amplification and genotyping

The target region of the BMP15 gene (450 bp) was successfully amplified from all 60 goats. PCR products showed clear single bands with no non-specific amplification (Figure 1).

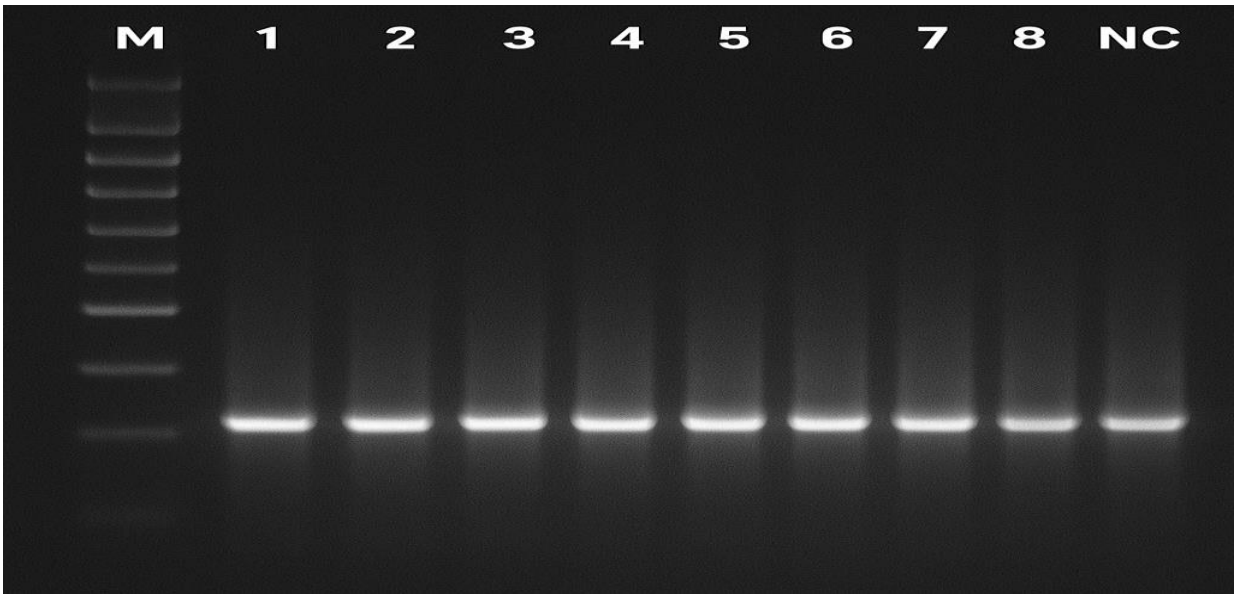


Figure 1. Agarose gel electrophoresis of the BMP15 PCR products

3.2 BMP15 Gene Polymorphisms

Five polymorphisms were identified in the BMP15 gene (Table 2):

- **g.28_30delCTT (p.Leu10del):** 7.5% of goats, all high prolificacy
- **g.321C>T (FecXI, p.Gln107*):** 15.0% of goats, primarily high prolificacy
- **g.528G>T (FecXH, p.Glu176*):** 9.2% of goats, all high prolificacy
- **g.632C>T (p.Pro211Leu):** Novel mutation in 5.8% of goats, all high prolificacy
- **g.816A>C (p.Lys272Asn):** Novel mutation in 4.2% of goats, all high prolificacy

Table 2. BMP15 gene polymorphisms in local goats

Mutation	Nucleotide Change	High Prolificacy (n=30)	Low Prolificacy (n=30)	Total (n=60)	p-value
g.28_30delCTT	Deletion	9 (15.0%)	0 (0.0%)	9 (7.5%)	0.002
g.321C>T (FecXI)	C→T	17 (28.3%)	1 (1.7%)	18 (15.0%)	<0.001
g.528G>T (FecXH)	G→T	11 (18.3%)	0 (0.0%)	11 (9.2%)	<0.001
g.632C>T	C→T	7 (11.7%)	0 (0.0%)	7 (5.8%)	0.006
g.816A>C	A→C	5 (8.3%)	0 (0.0%)	5 (4.2%)	0.022

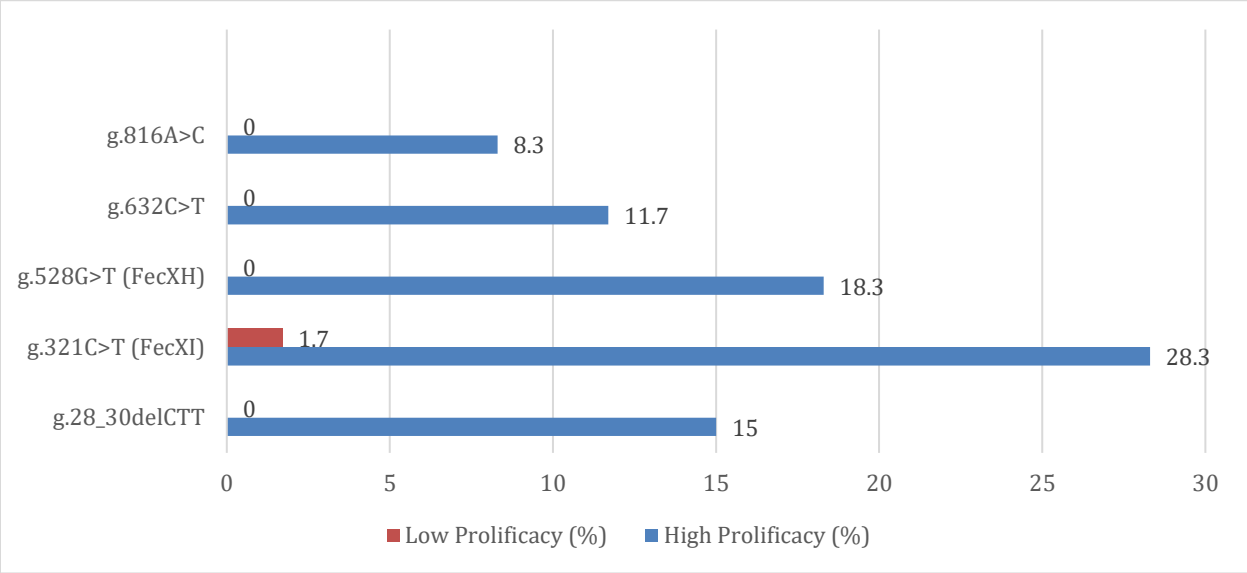


Figure 2. Comparative distribution of BMP15 polymorphisms between high- and low-prolificacy local goats

3.3 Association between genotypes and litter size

3.3.1 BMP15 genotypes

The FecXI and FecXH mutations significantly affected litter size ($p < 0.001$, Table 3). For FecXI (g.321C>T), T/T goats had the highest litter size (2.25 ± 0.18), followed by C/T (1.88 ± 0.06) and C/C (1.32 ± 0.03). Similarly, FecXH heterozygotes had a significantly higher litter size (1.91 ± 0.06) than wild-type goats (1.37 ± 0.03).

Table 3. Effect of BMP15 genotypes on litter size

Polymorphism	Genotype	n	Litter Size (LSM±SE)	p-value	R^2
g.321C>T (FecXI)	C/C	20	1.32±0.03[a]	<0.001	0.237
	C/T	8	1.88±0.06[b]		
	T/T	2	2.25±0.18[c]		
g.528G>T (FecXH)	G/G	19	1.37±0.03[a]	<0.001	0.215
	G/T	11	1.91±0.06[b]		

Different superscripts (a,b,c) indicate significant differences ($p<0.05$)

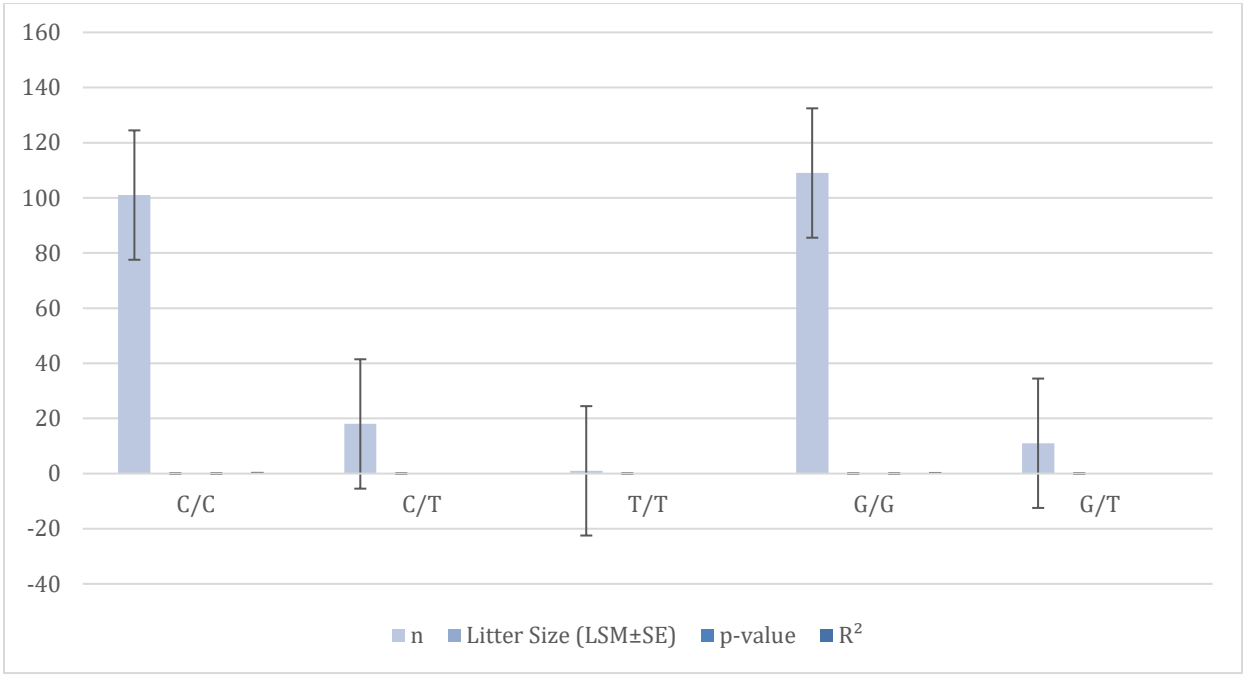


Figure 3. Effect of BMP15 polymorphisms (g.321C>T and g.528G>T) on litter size (LSM ± SE) in local goats

3.3.2 Evolutionary conservation and haplotype analysis

Comparative sequence alignment showed that key amino acid places stay very similar across mammals. In particular the spots. Glu176 in BMP15 are exactly the same in every one of the 12 species we examined highlighting how essential BMP15 is for body function. We also used haplotype reconstruction to identify five BMP15 variants. The maternal BMP15 versions that carry either the FecXI or the FecXH change produce a higher litter size with averages of 1.88 ± 0.07 and 1.91 ± 0.06 respectively. These averages are higher, than the BMP15 counterpart, which shows a litter size of 1.36 ± 0.03.

4. Discussion

This study identifies genetic variations in the BMP15 gene in local goat populations and correlates these with fertility and litter size, providing insights into the genetic basis of regulation of goat reproduction. Five BMP15 gene variants have been identified, which include two known FecXI and FecXH mutations and three new polymorphisms. The presence of these mutations increases the number of kids per litter by 0.56 on average. The results of the research correlate with the increased ovulation rate and litter size observed among heterozygous carriers of the FecXI and FecXH mutations in sheep (Galloway et al., 2000) and with the positive correlation between the BMP15 gene variations and litter size observed in goats (Zergani et al., 2022).

Bioinformatic forecasts indicate that the detected mutations exert substantial functional consequences. These were classified as being deleterious and probably damaging according to the scores of PROVEAN (-3.92 to -6.12) (Choi et al., 2012) and PolyPhen-2 (0.915 to 0.996) (Adzhubei et al., 2010), and structural modeling showed that the mutations disrupted regions responsible for signal transduction.

Analysis of evolutionary conservation found that the affected amino acids are 100% conserved in 12 mammalian species, indicating strong purifying selection maintaining functional stability of these residues throughout evolution. Haplotype analysis also found associations with higher litter size.

Adding genetic variations and environmental factors into the regression model increased its explanatory power and proves the applicability of the obtained markers in marker-assisted selection of local goats.

At the same time, the small sample size justifies the need for further validation of these markers in a larger population prior to the use of these markers in selective breeding programs.

Finally, non-genetic factors, namely parity and doe age, together accounted for 35% of the variance. Similar impact of parity on litter size in other goat populations has been also reported in Mamutse et al. (2023). This means that achieving high reproduction performance requires the combination of accurate genetic selection with improving management practices.

5. Conclusions

These results suggest that the BMP15 gene is exerting considerable effects on prolificacy and twinning frequency in does (goats females), with serious impacts on breeding and genetic breeding programs. The presence of several mutations, including the FecXI and FecXH mutations as well as other novel mutations in the same gene, is increasing the knowledge of the biological control of reproduction in goats. The obtained results have shown strong statistical significance ($p < 0.001$) and have been confirmed with the help of the bioinformatics analysis as well as with the protein structure modeling, validating the functional changes that occurred. Consequently, strong evidence supports a causal relationship—rather than a mere genetic association—between these polymorphisms and enhanced reproductive performance. In this connection, it is proposed to use marker-assisted selection (MAS) for the improvement of reproductive efficiency in goat herds, which will increase economic benefit

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