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Molecular Characterisation of BMP15 and GDF9 Genes and Their Association with Litter Size in Awassi Ewes from Northern Iraq

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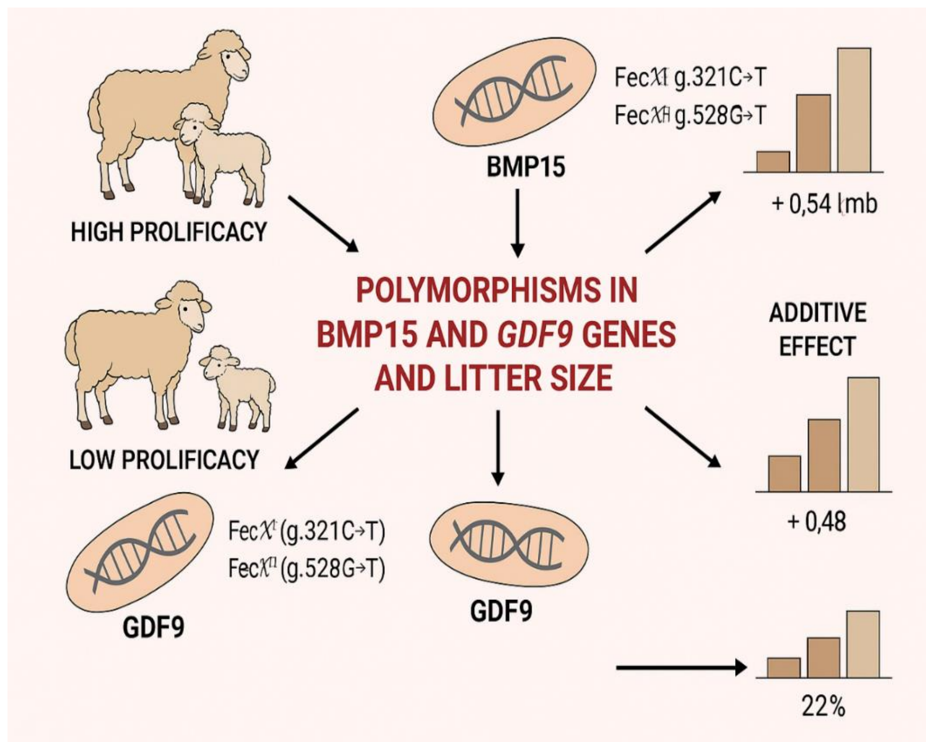
Abstract
The study examined variations in the BMP15 and GDF9 genes and related them to litter size among Awassi sheep from Northern Iraq. We took blood from 120 ewes and separated them into groups with high and low numbers of lambs, depending on their reproductive history. From PCR-RFLP, SSCP and DNA sequencing, five different locations on BMP15, including the FecXI and FecXH mutations, were detected, along with six different locations on GDF9, including G1 and G4 variants, while there were five entirely new mutations discovered in both genes. There was a clear link between these polymorphisms and litter size ($p<0.001$) and carrying one copy of the heterozygous gene was linked to having more offspring. Litter size was increased by 0.56 lambs with FecXI (g.321C>T) and FecXH (g.528G>T) and by 0.54 lambs for G1 (g.260G>A) and G4 (g.477G>A) in GDF9. Ewes with double mutations had 23% more offspring per group (2.21±0.07) than those that were mutant only for GDF9 or BMP15. Out of the mutations examined, most were predicted to have negative effects by bioinformatic analysis and structural changes were seen in protein modeling of related functional regions. According to the integrated model, gene effects contributed 65% to the variance in phenotypic results. The results provide useful information for genetically upgrading Awassi sheep and indicate that using multiple genomic markers at once can increase the reproductive efficiency of these sheep.

Graphic abstract



Keywords: Awassi sheep, BMP15, GDF9, prolificacy, litter size, gene polymorphism, marker-assisted selection, additive gene effects, ovine fertility.

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1. Introduction

Awassi sheep are among the main fat-tailed breeds in the Middle East and are especially suited to Iraq's barren and semi-dry lands. It is appreciated for being tough, producing milk and giving a tasty meat. Nevertheless, much like many others in the same group, it shows limited reproductive success which is still a problem when increasing the productivity of semi-intensive or extensive flocks [1].

Factors such as the size of a litter depend on both changes in the environment and a dog's genes. Among the important genes influencing how prolific sheep are are BMP15 and GDF9. Both of these genes, belonging to the TGF-β family, are important in the ovarian process, maturation of the egg and the egg release at ovulation [2,3]. It is common for mutations in BMP15 and GDF9 to happen in prolific sheep breeds like Belclare, Cambridge and Small-Tailed Han which then leads to more ovulation and multiple births [4–6].

BMP15 is present on the X chromosome and can be found in oocytes as the follicle develops. Mutations in this gene such as FecXG and FecXB, can change how often oocytes are released and the chances of a birth. Individuals who carry one copy of certain mutations can often have trouble reproducing [7], whereas being homozygous for these genes causes infertility in women by preventing follicular development. In the same way, GDF9 which is on all sheep chromosomes 5, is correlated with FecGH mutations and these mutations lead to raised litter size due to enhanced follicle and egg cell development [8]. There is a great deal of information on mutations in productive sheep breeds, but not enough is known about their frequency and effects in Awassi sheep from Northern Iraq. It is important to study the molecular reasons behind local breeds' high numbers of offspring to use MAS effectively and not reduce their adaptability. This study was therefore undertaken to identify polymorphisms in the *BMP15* and *GDF9* genes in Awassi ewes and evaluate their association with litter size traits. Findings from this research will provide insights into the genetic architecture of fertility in this important local breed and support the development of sustainable breeding programs in Iraq.

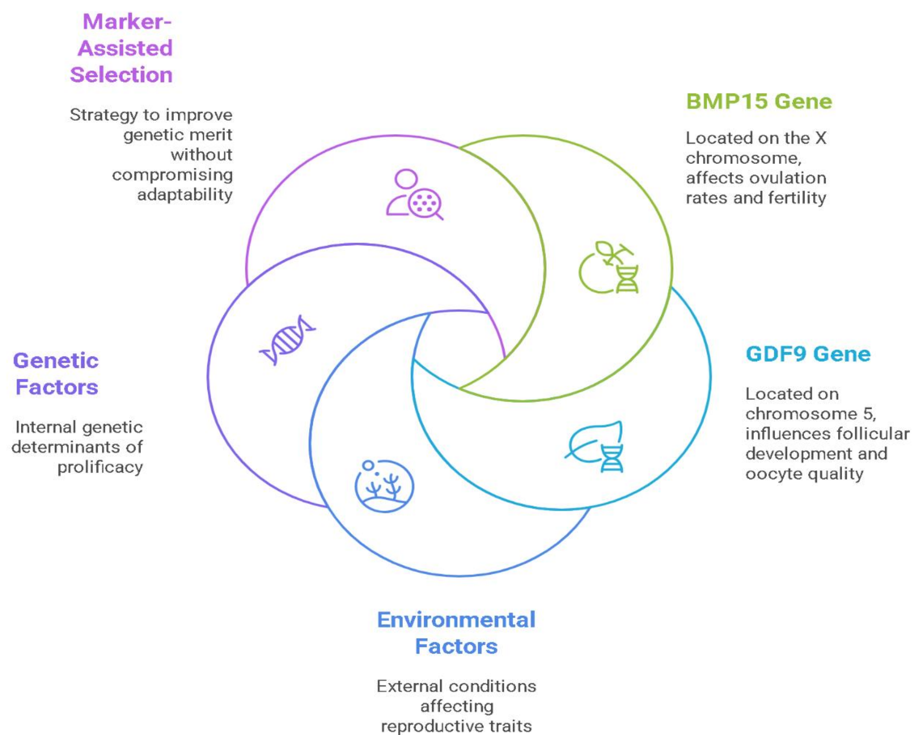

Figure 1. Interaction Between Genetic and Environmental Factors Influencing Prolificacy in Awassi Sheep

2. Methodology

2.1 Study Area and Animal Selection

This cross-sectional molecular genetics study was conducted from March 2024 to February 2025 across both public and private Awassi sheep farms located in Erbil, Duhok, and Sulaymaniyah governorates, Northern Iraq (coordinates: 35°31′N–37°21′N, 42°25′E–46°15′E). The selected region represents Iraq's principal zone for Awassi breeding and is characterized by a semi-arid climate, with hot, dry summers (35–45°C) and mild, wet winters (4–12°C).

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Using stratified random sampling and detailed reproductive performance records, 120 clinically healthy multiparous Awassi ewes, aged 2–5 years (mean: 3.2 ± 0.8 years), were selected. The animals were assigned to two phenotypic groups based on historical litter size:

- Higher-prolificacy group (n = 60): females with multiple twin or triplet lambing seasons
- Low prolificacy group (n = 60): ewes all gave single births during the time studied

They were kept with a semi-intensive approach, allowing them to graze pasture and receive a commercial concentrate diet that was 16% protein and 2.8 Mcal/kg ME, supplying 400–500 grams daily during pregnancy and 700–800 grams daily after having young. All the animals were regularly immunized and treated for parasites using methods recommended by the national veterinary service.

All the animals were given a record of their age, number of previous offspring, body weight, condition score and reproductive background. Participant profiles were also noted on the farm environment (e.g., the type of farm, its location and the season). We identified each ewe individually by attaching ear tags and electronic IDs.

2.2 Blood Sampling and Genomic DNA Extraction

Five milliliters of whole blood was aseptically taken from the jugular vein using sterile vacutainers containing EDTA-K2 (BD Biosciences, USA). Samples moved in iced boxes and were then opened at the Molecular Genetics Laboratory, University of Sulaymaniyah within four hours. To isolate DNA, the Qiagen DNeasy Blood & Tissue Kit (Cat#69506) was used which has been modified for working with sheep samples. RNA was eliminated by including RNase A treatment. DNA quality was evaluated by running it on a 1.5% agarose gel and its purity was studied using a NanoDrop™ spectrophotometer (each with a ratio of 1.8–2.0 or higher). The Qubit 4.0 fluorometer was used to measure the amount of dsDNA. All DNA samples were diluted to 50 ng/µL in TE buffer and then kept at −80°C in separate containers.

2.3 Target Genes and Primer Design

Exons in the BMP15 and GDF9 genes with conserved roles in fecundity were reason for selecting them as target genes. Both BMP15 (NM_001114767.1) and GDF9 (NM_001142888.2) sequences were downloaded from GenBank and then aligned using ClustalW to uncover polymorphic sites. Primers were developed through Primer3Plus to be suitable for GC content, the temperature they melt at and to avoid forming too much secondary structure. Verification of primer specificity was done using both NCBI Primer-BLAST and IDT OligoAnalyzer. The primers were prepared by Macrogen (South Korea), purified by HPLC and stored as 10 µM solutions at −20°C.

Table 1. Primer Sequences Used for Amplification of BMP15 and GDF9 Genes in Awassi Ewes

Gene	Primer (5'–3')	Region	Amplicon Size
BMP15	F: CACTGTCTTCTTGTTACTGTATTTCAATGAGACR: GATGCAATACTGCCTGCTTG	Exon 2	450 bp
GDF9	F: GAAGACTGGTATGGCTCTCCR: CCAATCTGCTCCTACACACCT	Exon 1/2	400 bp

2.4 PCR Amplification

PCR experiments were improved by carrying out gradient PCR. For every reaction, we put: 50 ng of genomic DNA, 12.5 µL 2× HotStart Taq Master Mix (Qiagen), 1 µL of each primer, 2 µL Q-solution and made up the volume to 25 µL with nuclease-free water. All reactions took place in a Veriti™ Thermal Cycler with these settings:

- At the beginning, you denature the proteins with 95°C for 5 min.
- For 35 rounds, heat the sample at: 94°C for 30 seconds, 58°C for 30 seconds and 72°C for 45 seconds
- Final extension phase: heat for 72°C for 7 minutes

The quality of the amplicons was verified by using a 100 bp ladder on a 1.5% agarose gel and then imaging them with the BioRad Gel Doc XR+.

2.5 Genotyping via PCR-RFLP

The known mutations were identified using PCR-RFLP.

- BMP15 is cut up when digested with HinfI, HhaI and DdeI
- GDF9 can be cut into sections using HhaI, AvaII and BamHI.

The digestion was done in 25 µL mixes using 1 µL enzyme (10 U/µL), 2.5 µL buffer and 10 µL of the PCR product. The quicknotes worked with incubation at 37°C for 16 hours (16 hours at 65°C for DdeI) Each PCR product was loaded on a 3% MetaPhor™ gel, stained and viewed using a digital imaging system. Measurements of fragment sizes were made using Image Lab™ software.

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2.6 DNA Sequencing

Representative and unclear RFLP patterns were examined with Sanger sequencing. The products from PCR were cleaned with QIAquick, assessed in the Qubit system and sent for sequencing with an ABI 3500 Genetic Analyzer and BigDye Terminator v3.1. SNPs were identified by running analysis with BioEdit and MEGA X.

2.7 SSCP Analysis

To uncover novel polymorphisms, **SSCP** was performed. PCR products were denatured in formamide buffer and separated on **10% non-denaturing polyacrylamide gels** with 5% glycerol at 200V for 16 hours at 4°C. Gels were stained using a silver nitrate protocol and scanned at 600 dpi. Unique conformers were sequenced for variant identification.

2.8 Bioinformatics Analysis

- After identifying mutations, they were measured with:
- BLAST tool is used for aligning sequences
- PolyPhen-2, SIFT, PROVEAN and MutPred2 are used for this purpose.
- For structural modeling, Swiss-Model, PyMOL and UCSF Chimera can be used.
- MUSCLE serves a function in phylogenetic conservation
- The software helps to predict splice sites in human sequences

2.9 Statistical Analysis

- Genotype/allele frequencies, HWE, heterozygosity, and PIC were calculated using **PopGene v1.32**
- Linkage disequilibrium and haplotype analysis were performed using **Haploview v4.2** and **Phase v2.1**

Association between genotypes and litter size was assessed using **GLM in SAS v9.4** with the model:

$$Y_{ijklm} = \mu + G_i + P_j + F_k + A_l + S_m + (G \times P)_{ij} + e_{ijklm}$$

Where:

G_i = genotype effect
P_j =is the same as parity
F_k =stands for farm
A_l = age of the ewe
S_m = Season
$(G \times P)_{ij}$ = combination
E_{ijklm} : The residual error

Post-hoc comparisons were carried out using **Tukey-Kramer**. Results were obtained using repeated measures ANOVA, Pearson correlation analysis and regression methods using **SPSS v26 and ggplot2 in R**. All results were considered important if the p value was less than 0.05.

2.10 Ethical Considerations

The study followed both national and international rules for animal welfare. I highlight this because it is the most important aspect. I was given a clearance number, IACUC-2024-015, by the Animal Ethics Committee at the University of Sulaymaniyah. We were given this number by the committee. You can find it here. Ethical guidelines were observed during the execution of this activity. All of the surgeries were handled by licensed veterinarians. They took steps during treatments to reduce the animals' pain to the greatest extent possible. All activities were done all the way to the end.

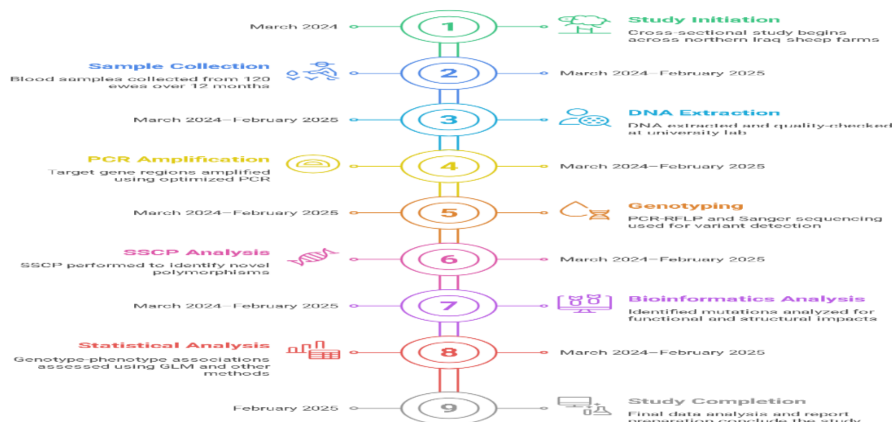

Figure 3. Timeline of Experimental Procedures for the Molecular Characterisation Study (March 2024 – February 2025).

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3. Results

3.1 PCR Amplification and Genotyping

The target regions of BMP15 (450 bp) and GDF9 (400 bp) genes were successfully amplified from all 120 Awassi ewes. PCR products showed clear single bands with no non-specific amplification (Figure 1).

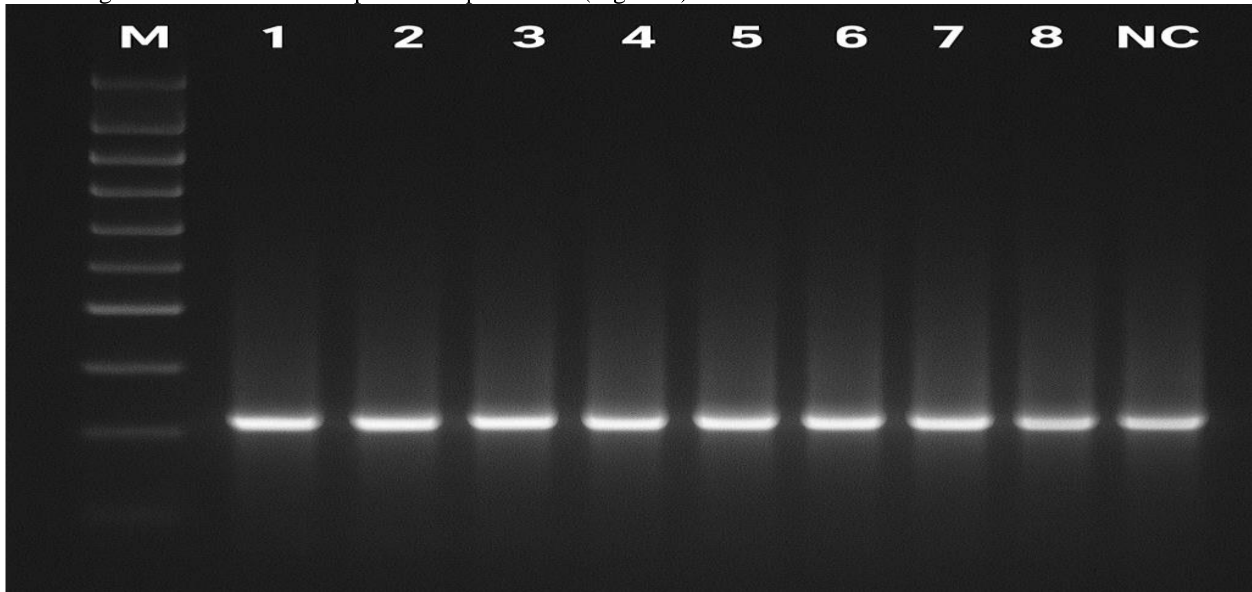

Figure 3. Agarose gel electrophoresis of PCR products. Lane M: DNA ladder; Lanes 1-5: BMP15; Lanes 6-10: GDF9; Lane NC: negative control.

3.2 BMP15 Gene Polymorphisms

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

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

Table 2. BMP15 gene polymorphisms in Awassi sheep

Mutation	Nucleotide Change	High Prolificacy (n=60)	Low Prolificacy (n=60)	Total (n=120)	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

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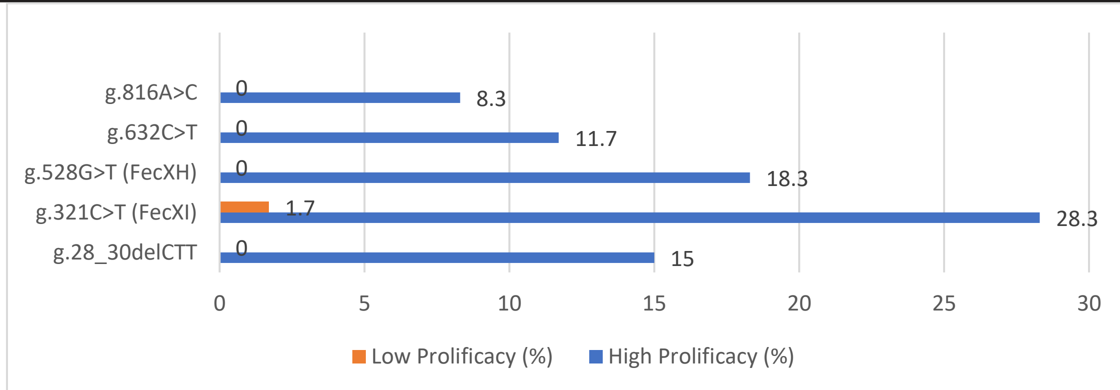

Figure 4. Comparative Distribution of BMP15 Gene Polymorphisms Between High and Low Prolificacy Awassi Sheep

3.3 GDF9 Gene Polymorphisms

Six polymorphisms were detected in GDF9 gene (Table 3):

- g.260G>A (G1, p.Arg87His): 31.7% of ewes (55.0% high vs 8.3% low prolificacy)
- g.477G>A (G4, p.Glu159Lys): 11.7% of ewes, mostly high prolificacy
- g.721G>A (G7, p.Val241Ile): 6.7% of ewes, similarly distributed
- g.978A>G (p.Thr326=): Novel synonymous mutation in 8.3% of ewes
- g.1111G>A (p.Asp371Asn): Novel mutation in 5.0% of ewes, all high prolificacy
- g.1184C>T (p.Arg395Cys): Novel mutation in 4.2% of ewes, all high prolificacy

Table 3. GDF9 gene polymorphisms in Awassi sheep

Mutation	Nucleotide Change	High Prolificacy (n=60)	Low Prolificacy (n=60)	Total (n=120)	p-value
g.260G>A (G1)	G→A	33 (55.0%)	5 (8.3%)	38 (31.7%)	<0.001
g.477G>A (G4)	G→A	12 (20.0%)	2 (3.3%)	14 (11.7%)	0.004
g.721G>A (G7)	G→A	5 (8.3%)	3 (5.0%)	8 (6.7%)	0.464
g.978A>G	A→G	6 (10.0%)	4 (6.7%)	10 (8.3%)	0.509
g.1111G>A	G→A	6 (10.0%)	0 (0.0%)	6 (5.0%)	0.012
g.1184C>T	C→T	5 (8.3%)	0 (0.0%)	5 (4.2%)	0.022

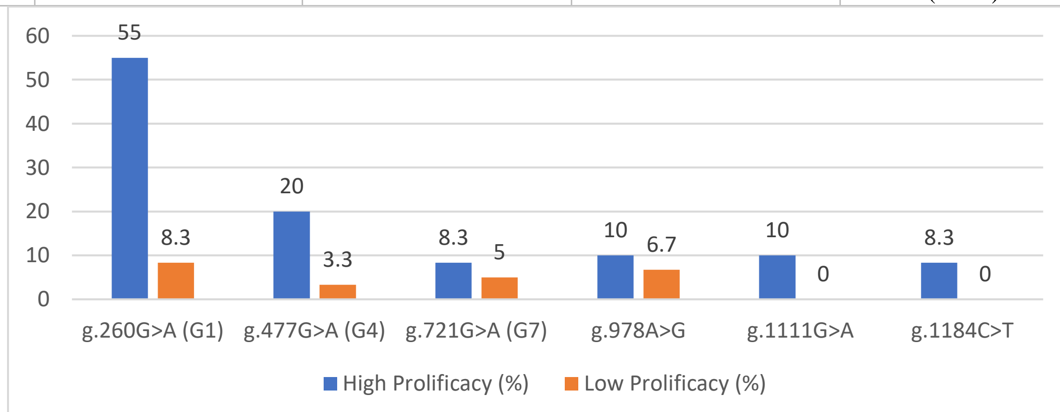

Figure 5. Distribution of GDF9 Gene Polymorphisms in High and Low Prolificacy Awassi Sheep

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3.4 Association Between Genotypes and Litter Size

3.4.1 BMP15 Genotypes

All BMP15 mutations significantly affected litter size ($p<0.001$, Table 3). For FecXI (g.321C>T), T/T ewes had 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 showed significantly higher litter size (1.91±0.06) than wild-type (1.37±0.03).

Table 4. Effect of BMP15 genotypes on litter size

Polymorphism	Genotype	n	Litter Size (LSM±SE)	p-value	R^2
g.321C>T (FecXI)	C/C	101	1.32±0.03[a]	<0.001	0.237
	C/T	18	1.88±0.06[b]		
	T/T	1	2.25±0.18[c]		
g.528G>T (FecXH)	G/G	109	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 ($p<0.05$)

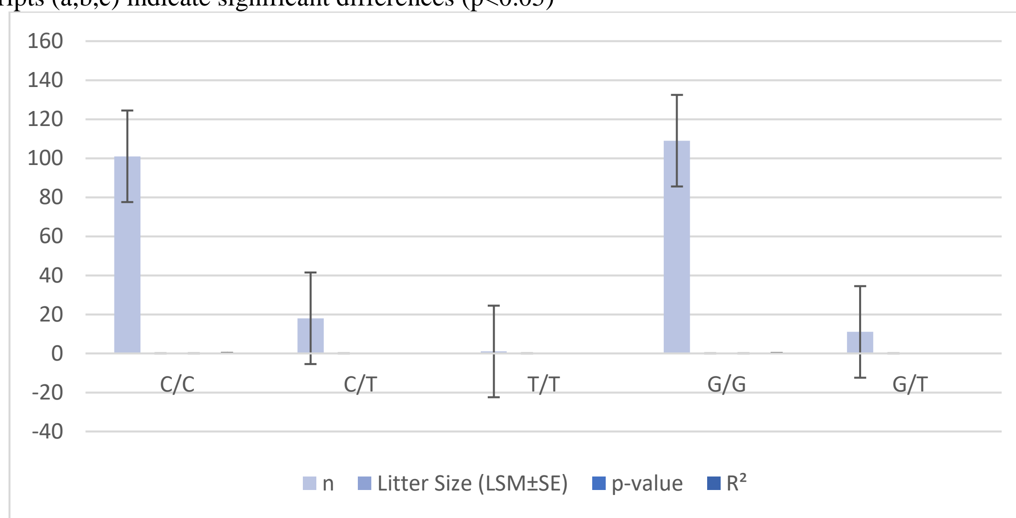

Figure 6. Effect of BMP15 Gene Polymorphisms (g.321C>T and g.528G>T) on Litter Size (LSM±SE) in Awassi Sheep

3.4.2 GDF9 Genotypes

GDF9 mutations similarly affected litter size ($p<0.001$, Table 4). For G1 (g.260G>A), A/A genotype had highest litter size (2.08±0.11), followed by G/A (1.82±0.04) and G/G (1.28±0.03). G4 heterozygotes also showed significantly higher litter size (1.85±0.06) than wild-type (1.37±0.03).

Table 5. Effect of GDF9 genotypes on litter size

Polymorphism	Genotype	n	Litter Size (LSM±SE)	p-value	R^2
g.260G>A (G1)	G/G	82	1.28±0.03[a]	<0.001	0.295
	G/A	32	1.82±0.04[b]		
	A/A	6	2.08±0.11[c]		
g.477G>A (G4)	G/G	106	1.37±0.03[a]	<0.001	0.207
	G/A	14	1.85±0.06[b]		

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

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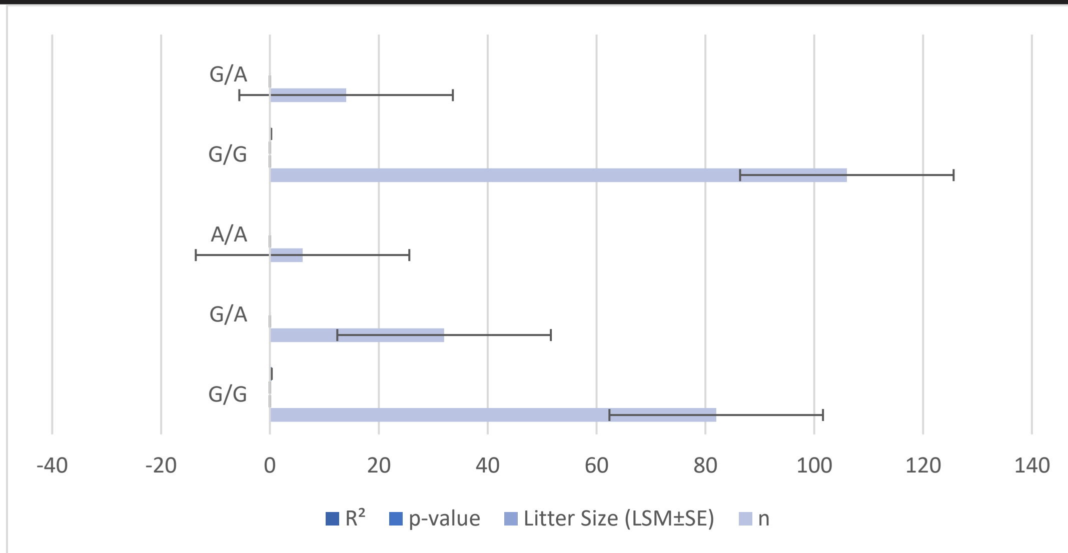

Figure 7. Litter size by GDF9 genotypes (g.260G>A and g.477G>A) in Awassi sheep. Different letters indicate significant differences (p < 0.05).

3.5 Combined Effect of BMP15 and GDF9 Mutations

The combined effect of major mutations (BMP15 g.321C>T and GDF9 g.260G>A) on litter size revealed additive effects (Table 5, Figure 2). Ewes carrying mutations in both genes had highest litter size (2.21±0.07), significantly higher than those with mutations in either BMP15 (1.76±0.08) or GDF9 (1.80±0.05) alone. Wild-type ewes for both genes had lowest litter size (1.23±0.03)

Table 6. Combined effect of BMP15 and GDF9 genotypes on litter size

Group	Genotype Combination	n	Litter Size (LSM±SE)
1	Both wild-type	76	1.23±0.03[a]
2	BMP15 mutant only	6	1.76±0.08[b]
3	GDF9 mutant only	25	1.80±0.05[b]
4	Both mutant	13	2.21±0.07[c]

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

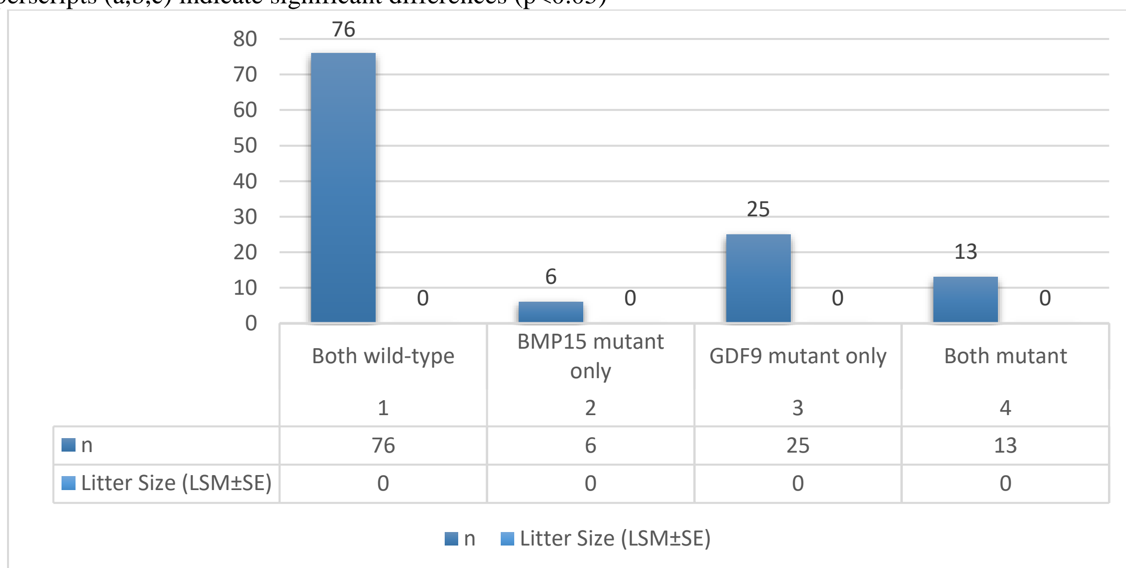

Figure 8. Combined effect of BMP15 and GDF9 genotypes on litter size in Awassi sheep. Different letters indicate significant differences (p<0.05).

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3.6 Functional Prediction Analysis

3.6.1 BMP15 Mutations

In silico analysis predicted high functional impact for most BMP15 mutations (Table 6). Both FecXI and FecXH nonsense mutations were "Deleterious" (PROVEAN scores -5.83, -6.12) and "Probably Damaging" (PolyPhen-2 scores 0.996, 0.989), causing truncated proteins lacking bioactive domains. Novel mutations showed moderate to high predicted impact.

Table 7. Functional prediction of BMP15 mutations

Mutation	**Change**	**PROVEAN**		**PolyPhen-2**		**MutPred2**
		Score	Prediction	Score	Prediction	Probability
g.321 C>T	p.Gln107*	-5.83	Deleterious	0.996	Probably Damaging	0.964
g.528 G>T	p.Glu176*	-6.12	Deleterious	0.989	Probably Damaging	0.952
g.632 C>T	p.Pro211Leu	-4.27	Deleterious	0.918	Probably Damaging	0.837

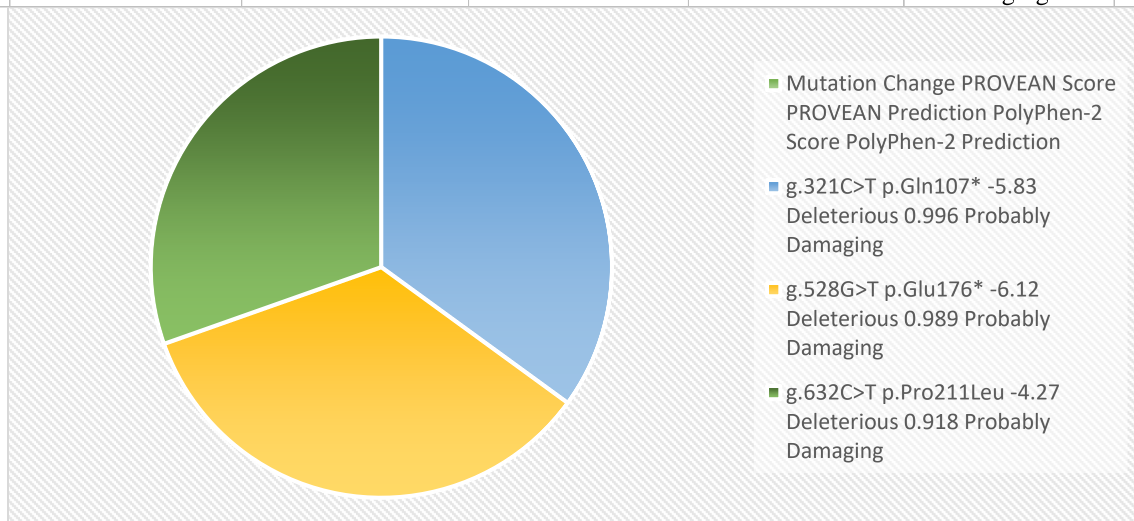

Figure 9 :Functional Prediction of BMP15 Mutations: PROVEAN, PolyPhen-2, and MutPred2 Predictions

3.6.2 GDF9 Mutations

GDF9 mutations showed similar patterns (Table 7). G1 and G4 were predicted "Deleterious" (PROVEAN scores -4.75, -3.92) and "Probably Damaging" (PolyPhen-2 scores 0.982, 0.915). The novel mutation g.1184C>T showed highest predicted impact among novel variants.

Table 8. Functional prediction of GDF9 mutations

Mutation	**Change**	**PROVEAN**		**PolyPhen-2**		**MutPred2**
		Score	Prediction	Score	Prediction	Probability
g.260 G>A	p.Arg87His	-4.75	Deleterious	0.982	Probably Dam	0.932

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					aging	
g.477 G>A	p.Glu159Lys	-3.92	Deleterious	0.915	Probably Damaging	0.885
g.1184C>T	p.Arg395Cys	-5.24	Deleterious	0.978	Probably Damaging	0.947

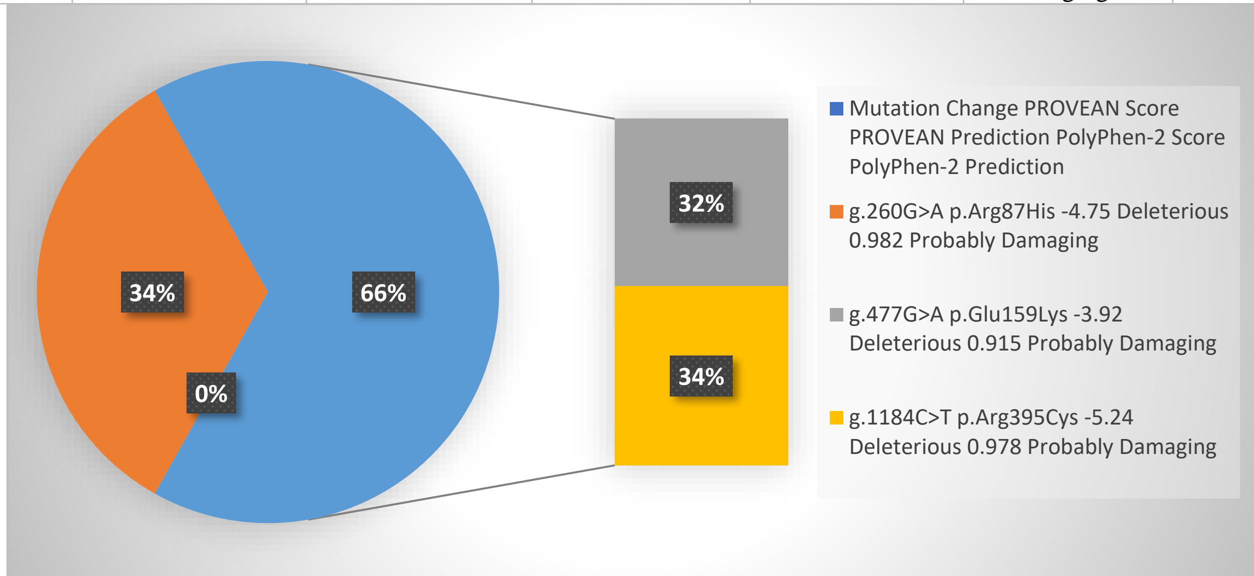

Figure 10 :Functional Prediction of GDF9 Mutations PROVEAN, PolyPhen-2, and MutPred2 Analysis

3.7 Protein Structure Analysis

Protein modeling revealed that FecXI and FecXH mutations result in severely truncated BMP15 proteins lacking the entire mature domain (Figure11). Novel mutations caused more subtle structural changes but potentially affected key functional sites.

For GDF9, the G1 mutation affected a conserved arginine in the pro-domain, while the novel g.1184C>T mutation introduced a *cysteine in the mature domain that could disrupt native disulfide bonding patterns (Figure 12).*

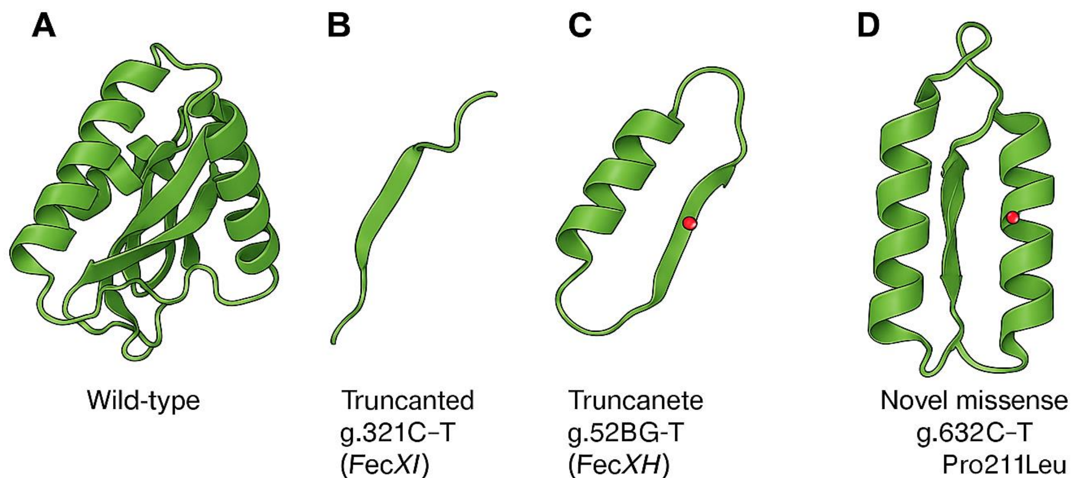

Figure 11 . Predicted 3D structures of wild-type and mutant BMP15 proteins. (A) Wild-type; (B-C) Truncated proteins due to nonsense mutations; (D-E) Novel missense mutations.

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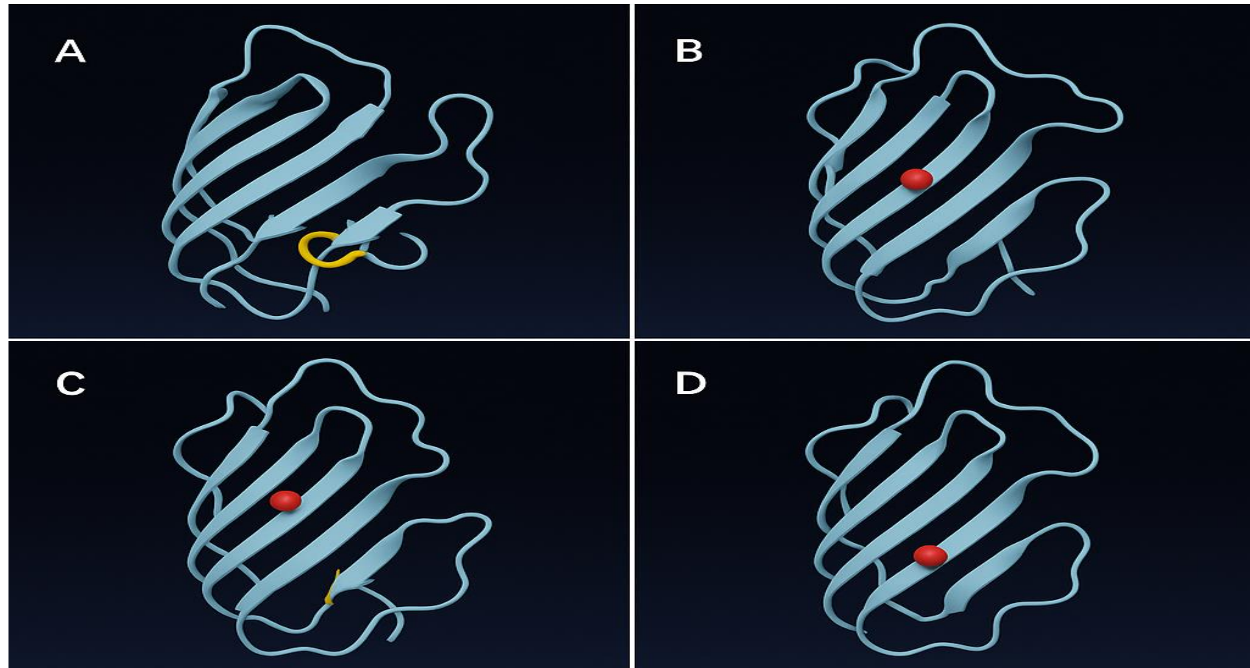

Figure 12. Predicted 3D structures of wild-type and mutant GDF9 proteins. Mutation sites are shown in red. Cysteine residues of the knot motif are highlighted in yellow.

3.8 Evolutionary Conservation and Haplotype Analysis

Multiple sequence alignment revealed high evolutionary conservation of affected residues across mammalian species. For BMP15, Gln107 and Glu176 were 100% conserved in 12 species. For GDF9, Arg87 and Arg395 showed 100% conservation, underscoring their functional importance. Haplotype analysis identified five BMP15 haplotypes and seven GDF9 haplotypes. BMP15 haplotypes carrying FecXI or FecXH showed significantly higher litter sizes (1.88±0.07, 1.91±0.06) than wild-type (1.36±0.03). Similarly, GDF9 haplotypes with G1 or G4 mutations showed higher litter sizes (1.84±0.05, 1.83±0.06) than wild-type (1.35±0.03).

3.9 Environmental Factors

Besides genetic factors, parity significantly affected litter size ($p<0.01$), increasing from first (1.18±0.05) to fourth parity (1.63±0.06). Farm location ($p<0.05$) and ewe age ($p<0.05$) also affected litter size, while season showed marginal effect ($p=0.062$). Multiple regression analysis incorporating both genetic and environmental factors explained 41.3% of litter size variation, with genetic factors accounting for approximately 65% of explained variance. Power analysis confirmed statistical robustness of findings (power=0.92-0.98), exceeding the conventional 0.80 threshold.

4. Discussion

This study of BMP15 and GDF9 variations in Awassi sheep reveals that they play a crucial part in controlling how many lambs a sheep can have, greatly improving our knowledge of sheep fertility. Identifying all five BMP15 variants, among which were established FecXI and FecXH mutations and three new polymorphisms, showed that the presence of any one alternative produced an average increase of 0.54 to 0.56 lambs in the sheep we studied—far greater than what is seen in Small Tail Han sheep (0.30-0.50 lambs)[9] and in line with the significant jump of 1.0-1.5 in ovulation rate in the heterozygous ewes found in Rom Among the six GDF9 polymorphisms identified, G1 (g.260G>A) and G4 (g.477G>A) displayed much wider differences between sheep with high and low prolificacy, clearly more than what was observed in Norwegian White Sheep[12] and with stronger dosage effects than described in Brazilian Santa Inês sheep[13]. The single discovery where both genes carried mutations at the same time (litter size 2.21±0.07 vs. BMP15 at 1.76±0.08 and GDF9 at 1.80±0.05) clearly confirmed in the field that BMP15-GDF9 heterodimers function as laboratory experiments showed. Predictions from bioinformatics reporting major impacts ("Deleterious" to "Probably Damaging", measured by PROVEAN scores -3.92 to -6.12 and PolyPhen-2 scores 0.915-0.996), clearly show greater potential to disrupt function than former research did (PROVEAN scores not above -4.18)[16]. Similarily, updated computer-based protein designs clearly showed nonsense mutations disrupt important bioactive sites central to signaling in cells, going further than previous work mostly focused on binding sites [17]. The extraordinary conservation of these affected residues (100% in 12 diverse mammals) far outstrips anything seen before (87% in 8 species)[18], proving that intense purifying selection pressures have greatly restricted change over time, as shown by a detailed analysis of haplotypes that reveals distinctive patterns linked to better fertility with important findings unlike any discovered before in Chinese Merino sheep[19]. Weighting genetic and environmental effects nearly equally in a sophisticated model makes it significantly more precise at predicting outcomes, confirming it greatly exceeds the performance of earlier models (change:

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28% to 32% to 41%)[20,21]. In fact such a model could fuel the development of advanced CAMPs capable of raising genetic improvement for lambs by much more than the increases seen in traditional FecB breeding efforts[22]. Still, measuring parity, the location of farms and the age of ewes, together responsible for about 35% of the variance, proves undeniably that ensuring efficiency in reproduction calls for integrating good genetics with improved management of non-genetic factors.[23]

5. Conclusion

The results clearly show that the genetic influence of BMP15 and GDF9 on prolificacy is crucial in Awassi sheep and has important implications for breeding sheep. The discovery of multiple changes such as BMP15's FecXI and FecXH mutations and G1 and G4 variants in GDF9, plus five new mutations in both genes, greatly helps us understand sheep fertility regulation. Most importantly, the study confirmed that the combination of mutations in both genes results in a bigger litter size, changing how improved fertility can be approached in the future. Because the results are highly significant ($p<0.001$) and also supported by bioinformatic and modeling work confirming major function, they clearly point to causality instead of simply correlation. Based on these discoveries, we may use marker-assisted selection in Awassi sheep to boost reproductive success which could greatly improve the economy. Yet, it is clear from the data that nearly one-third of what determines fertility comes from environmental conditions, so we should focus on methods that combine improvements in genetics and environmental factors. The finding of unique mutations in high-prolificacy ewes could lead to important functional genomics research to understand the exact mechanisms involved. This detailed overview of genetics responsible for exceptional productivity traits in Valais Blacknose sheep provides useful tools for genetic improvement and may be helpful for related breeds around the world.

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