

Polymorphism of the *GDF9* Gene in Russian Sheep Breeds

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Abstract

Growth differentiation factor 9 (GDF9) plays a key role in the fertility of most mammalian species. Ovine GDF9 gene is localized in the 5th chromosome. The gene's length is 2.5 kb consisting of two exons separated by one intron (1126 bp) and encodes a pro-peptide comprising 453 amino acids. The mature peptide includes 135 amino acids.

The purpose of this paper is to determine the GDF9 polymorphism in sheep of Salskaya and Romanov breeds in Rostov region of Russia. Polymorphism was identified by PCR- RFLP method at points G1 (G260A) and G4 (G721A). AG and GG genotypes were detected at points G1 and AA and AG genotypes were detected at points G4 in Salskaya sheep breed, frequency of A was 0.05 and G allele 0.95 (at point G1) and frequency of A was 0.95 and G allele 0.05 (at point G4). In Romanov breed AG and GG genotypes were detected at points G1 and AA and AG genotypes were detected at points G4, frequency of A was 0.20 and G allele 0.80 (at point G1) and frequency of A was 0.80 and G allele 0.20 (at point G4).

Keywords: GDF9; Gene; Polymorphism; PCR- RFLP; Sheep; Fertility

Introduction

Modern trends in sheep breeding include the use of new methods based on the application of DNA technologies, thus providing the industry being profitable and competitive [1,2]. Marker selection is an important trend in practical genetics (Marker Assisted Selection - MAS), suggesting the use of DNA markers associated with productivity traits [3,4]. The DNA marker-based technologies are widely applied in national breeding programs in several countries with developed sheep breeding [5,6].

In Rostov region there is a gradual increase of sheep livestock after its significant decline, so a more profound approach to restocking based on modern technologies is needed. The traditional assessment should be supplemented by a genetic control system.

Improvement of reproductive traits of sheep is one of the main objectives of breeding work. Direct selection by fertility is characterized by relatively low efficiency, which is connected, on the one hand, with low heritability signs, on the other hand, with a limited manifestation gender. In this regard, the studies aimed at finding DNA markers responsible for the development of these features and their identification are of current interest and demand. Today, the growth differentiation factor 9 (GDF9) is one of the most promising genes for sheep prolificacy [7,8]. Glycoprotein GDF-9, the protein product of the gene is structurally similar to beta (TGF- β), the growth transforming factor. Research on the role of GDF9 in folliculogenesis, revealed that it is the oocyte - specific growth factor and greatly contributes to the growth and differentiation of granulosa cells, as well as to the formation of theca cells and fertility of most mammalian species [9,10].

The ovine GDF9 gene was determined in the 5th chromosome [11,12]. The length of the gene is approximately 2.5 kb consisting of two exons separated by one intron (1126 bp) and coding a propeptide of 453 amino acids, with the mature peptide being composed of 135 amino acids [13].

Eight different mutations (G1-G8) have been identified in the gene GDF9 (Table 1) [12]. Three mutations of eight ones do not result in the modification of amino acid sequence (G2, G3 and G5). Five remaining nucleotide substitutions (G1, G4, G6, G7 and G8) lead to amino acid changes. In this context, the aim is to study the diversity of allelic variants of the GDF9 gene (by points GDF9/G1 and GDF9/G4) in sheep of Salskaya and Romanov breeds.

Variant of GDF9 gene	Base change	Amino acid change
G1	G-A	Arg (R)-His (H)
G2	C-T	UnchangedVal (V)
G3	G-A	UnchangedLeu (L)
G4	G-A	Glu (E)-Lys (K)
G5	A-G	UnchangedGlu (E)
G6	G-A	Val (V)-Ile (I)
G7	G-A	Val (V)-Met (M)
G8	C-T	Ser (S)-Phe (F)

Table 1: Polymorphic sequence variations in GDF9 (Hanrahan et al., 2004).

Materials and Methods

The subjects of study were Salskaya (n=100) and Romanov sheep breed (n=60) of the Rostov region. Isolation of DNA from the samples was DIAtom DNA Prep 100 ("Genlab" LTD, Russia) of *GDF9*. The analysis of allelic variants of the *GDF9* (by points G1 and G4) was

carried out by PCR- RFLP as described by Hanrahan et al. [12]. The specific primers presented in Table 2. PCR conditions: initial denaturation - 2 min at 94°C; denaturation at 94°C-30 sec., annealing at 63°C-40 sec., elongation at 72°C-30 sec. (35 cycles), final elongation at 72°C - 4 minutes.

Points of <i>GDF9</i>	Primers	Fragment length (bp)
G1	5'- GAAGACTGGTATGGGAAATG -3' 5'- CCAATCTGCTCTACACACCT -3'	462
G4	5'- GGAATATTCACATGTCTGTAAATTTTACATGTTGG -3' 5'- GAGGGAATGCCACCTGTGAAAAGCC -3'	161

Table 2: The oligonucleotide primers.

PCR- RFLP analysis of the *GDF9*-G1 gene fragment with the length of 462 bp was performed using restriction enzyme BstHHL (GCG↑C to C↓GCG). Restriction fragments were separated in the 2% agarose gel.

PCR- RFLP analysis of the *GDF9*-G4 gene fragment *GDF9*-G4 with the length of 161 bp was performed using restriction enzyme Bpu14 I (TT↑CGAA to AAGC↓TT). Restriction fragments were separated in the 3% agarose gel. POPGENE software was used to estimate the allele and genotypes frequencies.

Results and Discussion

Salskaya breed (Figure 1a) was developed the Rostov region for almost 20 years (1930-1950). Breeding work of developing a new breed of fine-wool sheep targeted on breeding sheep with strong constitution without exterior defects and shortcomings, well adapted to grazing conditions and capable of walking long distances during migration and of full use of sparse grass in Sal'sk prairie [14].

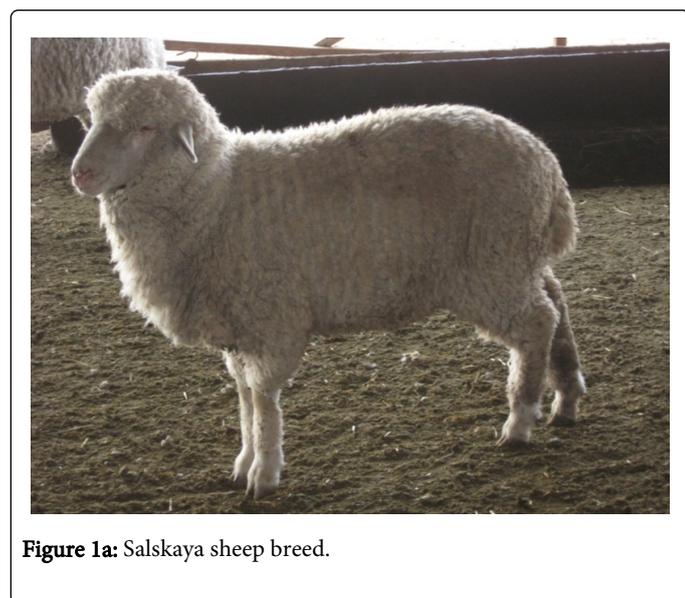


Figure 1a: Salskaya sheep breed.

Romanov breed is an ancient breed and at the same time one of the most promising ones in Russia. The Romanov breed sheep are rather big animals with weight of 80-100 kg (rams) and 60-70 kg (ewes) (Figure 1b). A sheep produces an average of more than two lambs with frequent triplets, and even quadruplets and five lambs.

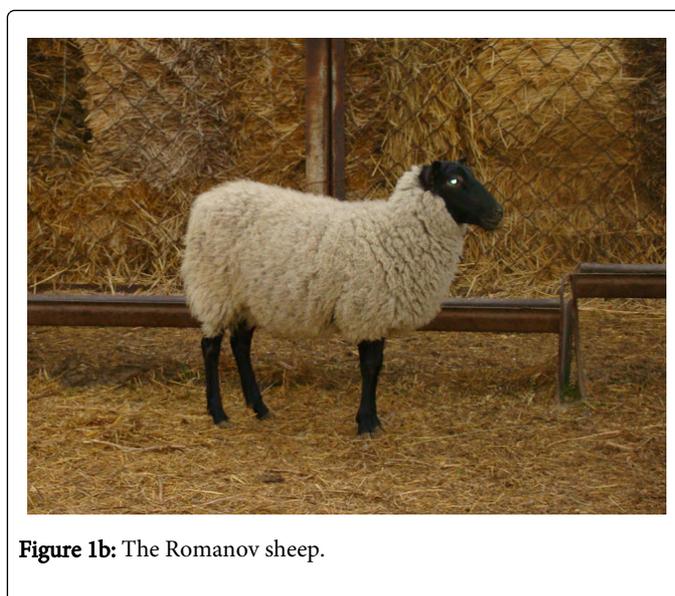


Figure 1b: The Romanov sheep.

The results of studies of Salskaya and Romanov sheep indicated polymorphism of the *GDF9* gene by points G1 (Figure 2) and G4 (Figure 3). The obtained results of allele and genotype frequencies of the *GDF9* gene showed a very low level of polymorphism by points G1 and G4 in the population of Salskaya sheep breeds under study (Table 3). The Salskaya sheep displayed high frequency of G allele (0.95) and GG genotype (90%) at point G1 and A allele (0.95) and AA genotype (90%) at point G4 of *GDF9* gene. Homozygous AA genotype (G1) and GG (G4) in the study population were not observed.

In the population of Romanov sheep under study the results of allele and genotype frequencies of the *GDF9* gene showed a higher level of polymorphism by the points G1 and G4, compared with a population of Salskaya sheep (Table 3). The Romanov sheep also had higher frequencies of G allele (0.80) and GG genotype (60.9%) by point G1 and A allele (0.80) and AA genotype (60.9%) by point G4 of the *GDF9* gene. It should be noted that all individual animals being heterozygous by the point G1, were heterozygous by the point G4, as well.

PCR-RFLP is a reliable and simple method used to study polymorphism gene *GDF9* in various breeds of sheep by some research groups. The data of our study in Salskaya and Romanov

breeds in Rostov region of Russia showed a low frequency of allele A and the absence of genotype AA at point G1

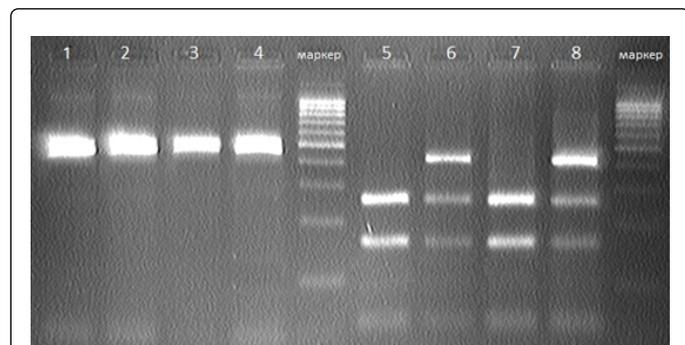


Figure 2: The result of PCR- RFLP of GDF9/G1 in the 2% agarose gel Denotations: 1-4 - PCR product of the gene GDF9/G1 (462 bp.); 5, 7 - GG genotype; 6, 8 - AG genotype; маркер - DNA Marker100 bp.

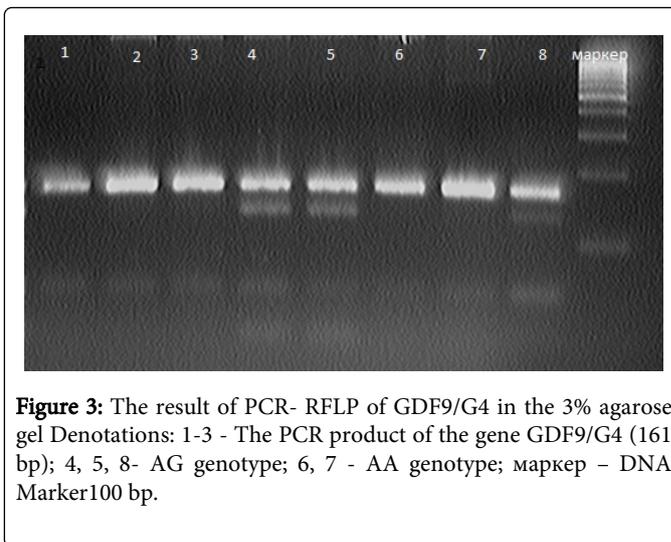


Figure 3: The result of PCR- RFLP of GDF9/G4 in the 3% agarose gel Denotations: 1-3 - The PCR product of the gene GDF9/G4 (161 bp); 4, 5, 8- AG genotype; 6, 7 - AA genotype; маркер - DNA Marker100 bp.

Variant of GDF9 gene	Alleles		Genotypes, %		
	A	G	AA	AG	GG
Salskaya sheep					
G1	0.05	0.95	-	10.0	90.0
G4	0.95	0.05	90.0	10.0	-
Romanov sheep					
G1	0.20	0.80	-	39.1	60.9
G4	0.80	0.20	60.9	39.1	-

Table 3: Frequencies of alleles and genotypes of the GDF9 of Salskaya and Romanov sheep.

These results are in agreement with reports in Sheep Hisari - Tajikistan race [15]. GG, AG and AA genotypes were frequency 93.64, 6.36 and 0% and G and A alleles frequency were 0.97 and 0.03, respectively. Similar results were obtained in Kordi and Arabic sheep [13], where A allele frequencies were 0.09 and 0.08, respectively.

The analysis of polymorphism for GDF9 (G1) in Baluchi sheep indicated all three possible genotypes, however allele G had the highest frequency (0.82), whereas allele A had the lowest frequency (0.18) [16]. The genotype frequencies of GG, AG and AA were 0.72, 0.20 and 8.0%, respectively. Resulted polymorphism in GDF9 in Sangsari sheep showed same results, genotype frequencies for GG, AG and AA were 70.72, 36.88 and 1.40% and allele frequencies for G and A were 0.80 and 0.19, respectively [17].

Conclusion

The diversity of polymorphism of the GDF9 gene (by points GDF9/G1 and GDF9/G4) of Salskaya and Romanov sheep has been studied. The results obtained showed a low level of polymorphism by the points under study, but the presence of heterozygous variants in the investigated population gives grounds to assume that further research in this area will contribute to the identification of informative genes related to productive traits of sheep.

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