Genetic study on BMP-15 as candidate gene of prolificacy in Ile de France sheep breed

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Abstract

The aim of this experiment was to detect the possible polymorphism in BMP-15 gene by PCR-RFLP. Sheep BMP-15 gene is located on X chromosome and it is directly related to prolificacy, ovulation rate and twining in sheep. The present study was carried out with 50 ewes from Ile de France sheep breed, part of the Institute of Animal Science – Kostinbrod. Blood samples were collected from jugular vein. Genomic DNA was extracted from the whole blood by manual purification kit. Specific set of primers was used for the PCR amplification. The PCR products were digested by restriction enzyme DdeI and the obtained fragments were visualized on 3% agarose gel stained with GelRed. In all tested animals it was detected only wild allele A and only wild homozygous genotype *AA* (153 bp), respectively. Therefore, in this experiment, the studied region of the BMP-15 gene can be defined as monomorphic, which means that it could not be used as a candidate gene of prolificacy in this herd.

Key words: genetic diversity, PCR-RFLP, BMP-15 gene, Ile de France sheep breed, prolificacy

Introduction

Twining trait is one of the important factors affecting prolificacy in sheep. Improvement of reproduction traits by traditional selection is slow and uncertain process because of the low heritability rate. The application of marker assisted selection (MAS) based on study of various candidate genes associated with different productive traits in livestock can significantly improve the quality of selection and therefore the quantity and quality of the obtained animal production. Modern techniques like DNA analysis could provide irreplaceable resource of information that can accelerate improvement of reproduction performances. For sheep as a species with limited twining, the marker-assisted selection (MAS) could play an essential role due to the fact that the growth characteristics are associated mainly with twining (Pramod et al., 2013; Lassoued et al., 2017; Dinçel et al., 2018).

BMP-15 is a protein that blocks FSH receptor expression in the ovaries. Therefor heterozygous individuals have multiple ovulations and increased ovulation rate. In sheep BMP-15 gene is located on X chromosome and eight different mutations have been identified in this locus (Inverdale-FecXI Hanna-FecXH, Belclare-FecXB, Galway-FecXG, Lacaune-FecXL, Rasa Aragone-sa-FecXR, Grivette-FecXGr, Olkuska-FecXO) (Davis, 2005; Bodin et al., 2007; Monteagudo et al., 2019; Demars et al., 2013).

The aim of this experiment was to detect the possible polymorphism in BMP-15 gene by PCR-RFLP method in 50 ewes from Ile de France sheep breed.

Materials and methods

The experiment was carried out in the Laboratory of Genetics part of the Institute of Animal Science – Kostinbrod, Bulgaria.

Animals

In this experiment were tested 50 ewes from Ile de France sheep breed. Approximately 6 ml blood were collected from each individual in vacuum tubes containing EDTA by method of Miller et al. (1988). When receiving blood samples, all animal welfare requirements were met. The samples were stored at -20 °C till experimental part. During blood collection all requirements for the welfare of animals were observed.

DNA extraction

DNA was extracted from whole blood by manual commercial kit Blood-Animal-Plant DNA Preparation Kit (Jena Bioscience) according to the manufacturer's instruction. After manual DNA extraction were received 50 samples with concentration about 10–15 ng/µl. The quality of the obtained DNA was tested using gel monitoring on 1% agarose (Bioline) gel prepared with 1xTAE buffer (Bioline).

PCR amplification

PCR amplification was carried out in total volumes of 25 µl, containing 100 ng DNA template, 50 pM of each primer and 12 µl of 2×(1.5 mM $MgCl_2$) MyTaq TM HS Red Mix 2x (Bioline). The polymerase chain reactions were performed using thermal cycler TC-TE (BOECO, Germany) under the condition described in table 2. The primer sequences were shown in table 1.

Restriction analysis

The genotypes of tested animals were established using restriction fragment length polymorphism (RFLP) analysis. The digestion reactions were carried out in 10 µl final volume, containing 6 µl PCR product and 10 U/µl specific restriction enzyme. The digestion process for BMP-15 was performed by speed enzyme DdeI (Fermentas), for 12 h at 37 °C in thermo-block. The fragment sizes were determined using GeneRuler™ Ladder, 50 bp (Fermentas) supplied with 1 ml 6x DNA Loading dye (Thermo) on 3% agarose gel, stained with GelRed and then visualized under UV light on trans-illuminator Hi-UVTM Duo Capture (HIMEDIA).

Results and Discussion

After DNA extraction were received 50 samples with concentration approximately 10–15 ng/µl. In all tested animals were obtained PCR products with expected length of 153 bp (fig. 1). The restriction fragment length polymorphism analysis was performed by specific endonuclease DdeI that cleaved the BMP-15 gene in polymorphic region in specific site:

5'	C ↓	T	N	A	G	3'
3'	G	A	N	T ↑	C	5'

Table 1. Locus, region, primer sequencies, length of PCR products of BMP-15 gene

Locus	Region	Primer sequencies	Length of PCR product	References
BMP-15	$FecX^B$	F: 5'GCCTTCCTGTGTCCCTTATAAGTATGTTCCCCTTA-3' R: 5'- TTCTTGGGAAACCTGAGCTAGC -3'	153 bp	Barakat et al. (2017)

Table 2. Specific PCR conditions for amplification of BMP-15 gene in Il de France sheep breed

Locus	Primary denaturation	Cycles	Denaturation	Annealing	Elongation	Final elongation	Store	References
BMP-15	94 °C / 5 min	35	94 °C / 45 sec	58 °C / 40 sec	72 °C / 1 min	72 °C / 10 min	4 °C	Barakat et al. (2017)

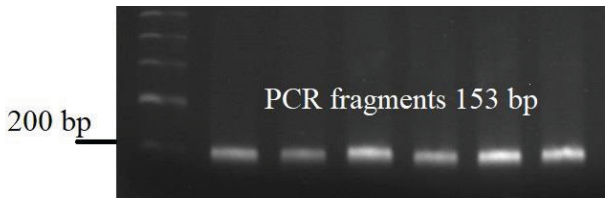

Fig. 1. Amplified PCR products of sheep BMP-15 gene in animals of Ile de France breed

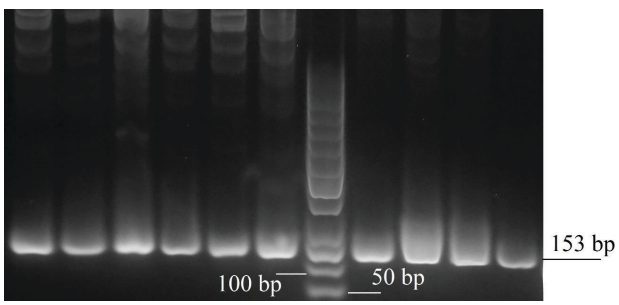

Fig. 2. Results of the PCR-RFLP analysis of the BMP-15 gene, visualized on 3% agarose gel, after genotyping of the samples with restriction endonuclease DdeI

Investigation by PCR-RFLP method revealed the presence of allele A only and one genotype AA, respectively. The size of fragments was identical to PCR products 153 bp. The results of genotyping of tested animals from Ile de France sheep breed were shown on fig. 2.

BMP-15 or FecX is one of the most studied genes belonging to the group of fecundity genes. However, this is the first study in Bulgarian sheep breeds in this region of the gene. Worldwide researchers have been located mutations in different regions of the BMP-15. Moradband et al. (2011) reported that 6 point mutations were identified in sheep in the BMP15 gene locus (FecXI, FecXH, FecXL, FecXG, FecXB, and a 17 bp deletion from the coding region of the FecXR gene in the Rasa Aragonesa breed). Each of them has a significant effect on fertility, as it changed gene expression.

Deletion in the coding region of the BMP-15 gene was announced by Martinez-Royo et al. (2008). The authors analyzed a population of Rasa Aragonesa sheep and found a drop of 17 bp in the initial sequence of exon 2. This changed codon 154, shifts the reading frame, and introduced a new stop codon at position 208.

Three consecutive studies by Davis et al. (1991; 1993) and Hanrahan et al. (2004) were found that sterile sheep had two mutant alleles from the BMP-15 gene (homozygous animals), while the heterozygous genotype (animals with one mutant allele) had a beneficial effect on ovulation and increased frequency of twining rate. The authors observed a positive effect on ovulation and fertility due to mutation in BMP-15 locus when the animals had heterozygous genotypes, with the effect being additive. This hypothesis was also supported in later studies by Gemmell and Slate (2006) and Martinez-Royo et al. (2008). Published results proved that the animals with the highest fertility had heterozygous genotypes for the BMP-15 gene.

Similar results as in present study were announced by Dinçel et al. (2018) in 77 animals from Chios sheep. All individuals were digested by DdeI restriction enzyme and showed wild-type genotype and did not carry FecXB mutation. The authors suggested that the high prolificacy in Chios sheep may be based on another region of this gene or it could be due the effect of other genes.

Conclusions

In present experiment were tested 50 ewes from Ile de France sheep breed by means of PCR-RFLP technique. Only allele A and only genotype AA were identified. As a result of this study it can be concluded that tested region of the BMP-15 gene can be defined as monomorphic, which means that it could not be used as a candidate gene of prolificacy in this herd. Nevertheless we suggest that future studies would be useful in order to be investigated other regions of BMP-15 gene.

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