POLYMORPHISMS IN EXON 2 OF BMP15 GENE IN AFSHARI SHEEP BREED THROUGH PCR-SSCP

Amir Ajaki[1], Shahin Eghbalsaied[1] and Hamid-Reza Amini[2]
[1]*Dept. Anim. Sci., Isfahan branch, Islamic Azad University, Isfahan, Iran.*
[2]*Young Researchers Club, Khorasgan (Isfahan) Branch, Islamic Azad University, Isfahan, Iran.*
** Corresponding Author E-mail address: Shahin080@gmail.com*

INTRODUCTION

Several genes have recently been shown to affect female fecundity in domesticated sheep. Among the genes merely expressed in the oocyte, bone morphogenetic protein 15, BMP-15, is a member of the TGFβ superfamily that play a pivotal role in female fertility and associated with increased ovulation rate in sheep. Ovulation rates in BMP15 mutants are high in the heterozygotes while the homozygous mutants show a primary ovarian failure resulting in complete sterility. On the other side, for detection of effective mutation in BMP15 gene, it is impossible to detect these mutations using PCR-RFLP techniques, due to lack of incision site with corresponding restriction enzyme for these loci (B1, B2 and B4 mutations). Therefore, the present study was conducted to determine polymorphism in exon 2 of BMP15 gene in Afshari sheep by PCR-SSCP (single strand conformation polymorphism).

Materials and Methods

Venous jugular blood samples (10 mL per ewe) were collected from 85 Afshari sheep with known single or twin lambing. Genomic DNA was extracted from whole blood by the phenol-chloroform method and quantitative and quality of DNA was determined with spectrophotometer and 1% agarose gel, respectively. Then DNAs were dissolved in TE buffer and kept at −20°C. An 840 bp fragment that includes B2, B3 and B4 mutation in exon 2 of BMP15 gene was amplified by PCR for SSCP analysis. Aliquots of 5 µl PCR products were mixed with 10 µl denaturing solution (98% formamide, 0.025% xylenecyanole and 0.025% bromophenol blue, 10 mM EDTA), incubated at 98°C for 10 min and then chilled on ice. Denatured DNA was loaded on 12% poly acrylamide gel and constant voltage 250V for 5.5 h at 4°C. For detection of band patterns, gels stained with silver nitrate in three stages with fixer, stainer and developer solutions.

Results and Discussions

SSCP results revealed three banding patterns (AA, BB and AB) in this population, and their frequency were 0.764, 0.223, and 0.012, respectively. The results showed that the studied part of exon 2 in BMP15 is polymorphic in Iranian Afshari sheep and PCR-SSCP method could be applied for analysis of this region.

Keywords: Litter size, BMP15 gene, Afshari sheep, PCR-SSCP, Polymorphism.