Investigation of two recessive disorders in breeder bulls of Abbas Abad animal breeding center

Mohammad Reza Nassiry*, Amir Norouzy¹, Fereidoun Eftekhar Shahroodi¹, Ali Javadmanesh¹ and Mohammad Ali Shad²

¹Animal Science Department, College of Agriculture, Ferdowsi University of Mashhad, P.O. Box: 91775-1163 Mashhad, I.R. Iran ²Center of Animal Breeding in North-Eastern of Iran, Jahad-Keshavarzi Khorasan, Mashhad, I.R. Iran

Abstract
To prevent distribution of recessive alleles in dairy herds all bulls used for AI (Artificial Insemination) have to be tested. In this study 26 blood and 4 semen samples were supplied from Iranian Holstein bulls used for AI. Genomic DNA was extracted from 100 µl of blood and 200 µl of semen. Samples were tested by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. PCR reaction was performed for amplification of polymorphic region of the CD18 gene on chromosome 1 and exon 5 of ASS gene on chromosome 11. We detected one Bovine Leukocyte Adhesion Deficiency (BLAD) carrier and no carrier for bovine Citrullinemia in this study. Hardy-Weinberg test confirmed the equilibrium of BLAD locus in this population.

Keywords: BLAD; CD18; Citrullinemia; Argininosuccinate Synthetase; Holstein; PCR-RFLP.

In animal breeding, genetic disorders are one of the most important points of attention for breeders. At this time, there are identification records for Bulldog (achondroplasia), Mule Foot (syndactylism), Bovine Leukocyte Adhesion Deficiency (BLAD), Complex Vertebral Malformation (CVM), Prolonged Gestation, Hairless, Dwarfism, Imperfect Skin, Deficiency of Uridine Monophosphate Synthase (DUMPS) and Pink Tooth (congenital porphyria) (Citek and Blahova, 2004).

BLAD is a recessive autosomal inherited disease in Holstein-Frisian cattle (Kehrli et al., 1990). Affected calves (BLAD homozygous form) are characterized by recurrent bacterial infections after conventional treatments and early mortality before reaching to sexual maturity (Takahashi et al., 1987; Kehrli et al., 1991; Gilbert et al., 1993). Consequently, genetic diseases like BLAD or Complex Vertebral Malformation (CVM) are economically important diseases; emphasizing the need for genetic screening of the mutant allele in populations.

Two point mutations were identified within the gene encoding bovine CD18 in Holstein-Frisian breed. The mutation at position 775 is silent, as it does not alter the deduced amino acid sequence. The mutation at position 383 causes an aspartic to glycine substitution at amino acid 128 (D128) (Shuster et al., 1992). Mutation in CD18 prevents expression of all β2-integrins LFA-1, MAC-1 and p150, 95 (Leukocyte Adhesion Glycoproteins) on the leukocytes (Kehrli et al., 1990). Leukocyte adhesion to the endothelium, to other leukocytes, and to bacteria is critical in the ability of leukocytes to travel, communicate, inflame, and fight infection.

Citrullinemia is an autosomal recessive error of urea metabolism characterized by high levels of citrulline, and more seriously, of ammonia in plasma, as a result of a deficiency of activity of the urea cycle enzyme, argininosuccinate synthetase (ASS).

The mutation responsible for this disorder has been characterized as a single-base substitution (C→T), converting the CGA codon that codes for arginine-86 to TGA, a translation-termination codon (Dennis et al.,...
This conversion results in a truncated peptide product (85 amino acids long rather than the normal 412 amino acids) that lacks activity. This mutation also eliminates a restriction site for the enzyme AvaII which will cut the normal gene but not cleave the mutant gene. This action forms the basis of a PCR test for detecting genotypes for this disorder (Dennis et al., 1989). Several mutations in ASS gene reported in human (Kobayashi et al., 1991). Bovine citrullinemia gene therapy will be applicable to human trials of the treatment of this disorder and other related urea-cycle disorders (Lee et al., 1999).

The objective of the present study was to identify the bovine citrullinemia and BLAD carrier in AI bulls to prevent their distribution in dairy herds. For this study 26 blood and 4 semen samples were prepared from Holstein AI bulls from Abbas Abad animal breeding center in Khorasan state of Iran. Genomic DNA was extracted from 100 µl of blood and 200 µl of semen by Guanidinium Iso Thiocyanate-Silica gel method (Boom et al., 1990).

PCR reaction was performed for amplification of polymorphic region of the CD18 and ASS genes. The following primers were used for PCR for CD18 and ASS genes respectively (Tammen et al., 1996; Grupe et al., 1996).


1 µl DNA (50 ng) was used for amplification a total volume of 20 µl PCR reaction using the Biometra T- Personal Ver: 1.11 thermocycler. The PCR mix contained: 2.5 µl PCR buffer 10X, 2.5 mM MgCl2, 2 mM dNTPs, 2 µl mix of oligonucleotids (10 pM from each primer), 1u Taq DNA polymerase and 7 µl ddH2O. Electrophoresis was carried out on agarose gel 2% ethidium bromide.

PCR products from polymorphic region of CD18 gene (101 bp) were digested with TaqI and HaeIII enzymes in two separate reactions. Two restriction enzymes were used for increasing the accuracy. Lengths of fragments (bp) after digestion of CD18 PCR product were 52, 32 and 17bp for TL (Free of BLAD), 84, 52, 32 and 17bp for BL (BLAD Carrier) and 84 and 17bp for BLAD (Affected) genotypes. Lengths of fragments (bp) after digestion with HaeIII were 65 and 36bp for TL (Test free of BLAD), 65, 46, 36 and 19bp for BL (BLAD Carrier) and 46, 36 and 19bp for BLAD (Affected) genotypes. PCR products from polymorphic region of ASS gene (199 bp) were digested with AvaII enzyme. Lengths of fragments (bp) after digestion of ASS PCR product were 103 and 82bp for normal homozygous, 185, 103 and 82bp for heterozygote and a 185bp fragment for affected calves. Digestion products were separated by electrophoresis on 8% acrylamid gel and visualized with ethidium bromide or silver staining. An MspI digestion of pUC19 was used as a molecular weight marker.

The frequencies of BL and TL allele frequencies and \( \chi^2 \) were calculated by direct counting. PCR products were obtained from amplification of two genes and both restriction patterns from polymorphic region of the CD18 gene (TaqI and HaeIII) have shown one carrier (BL) bull in this study with a high accuracy (Fig. 1).

**Figure 1.** Acrylamid gel electrophoresis of digestion products with TaqI and HaeIII. Lanes 4, 5, 8 and 9: TL, lanes 3 and 7: BL and lanes 1, 2, 6 and 10: Molecular Marker. (M100: 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100) and (pUC19/MspI: 501, 489, 404, 331, 242, 190, 147, 111, 110, 67, 34, 26).

**Figure 2.** Agarose gel electrophoresis of digestion products from ASS gene revealed same band in all lanes. (103 & 82 bp). M100: 1000, 900, 800, 700, 600, 500, 400, 300, 200, 100.
In restriction patterns from polymorphic region of the ASS gene, all lanes show same band in the agarose gel (Fig. 2), therefore the bulls were homozygous normal. Allele frequencies for BL and TL were 0.98 and 0.02 respectively. Hardy-Weinberg test confirmed the equilibrium of BLAD locus in this population ($\chi^2 = 0.01$).

The massive spread of genetic defects in cattle like BLAD and Citrullinemia in recent years is caused by the extensive use of elite sires and latent heterozygous carriers. In this study we find a BLAD carrier by molecular methods like other similar reports for example, 3.5% BL frequency in Argentinian Holstein cows (Poli et al., 1996), 13.4% in Danish Holstein-Friesian cattle (Jorgensen et al., 1993) and 8.1% in Japanese Holstein animals (Nagahata et al., 1997). Pedigree studies of the carrier bull revealed that the mutation inherited to him from Hawkeye bull (CANM 369995, BL) (Fig. 3). Further studies have shown that Hawkeye has received this mutation from his ancestor Osborndale Ivanhoe Bell 1952 (www.holstein.ca and www.semex.com).

To date, all BLAD cases that have been diagnosed and genetically tested are homozygous for the D128G mutation. Pedigree analysis and DNA testing has shown that the genetic defect likely originated from a single ancestor. This bull, Osborndale Ivanhoe Bell 1952, and sons of Osborndale are also responsible for spreading BLAD in Holstein populations to wide world (Shuster et al., 1992).

In our study Citrullinemia carrier genotype was not detected. Similar results have been reported elsewhere (Robinson et al., 1993 & Grupe et al., 1996).

Acknowledgment

This investigation was supported by Center of Excellence in Animal Science, Ferdowsi University of Mashhad.

References


