

## MOLECULAR CHARACTERIZATION OF CD18 GENE AND IDENTIFICATION OF BLAD CARRIERS BULLS IN IRAN

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### INTRODUCTION

Bovine leukocyte adhesion deficiency (BLAD) is an autosomal recessive genetic disorder in Holstein cattle (Waldrop *et al.*, 1987). It is caused by a point mutation which substitutes guanine to adenine in CD18 gene, resulting substitution of aminoacid aspartic acid to a glycine in D128G position (Shuster *et al.*, 1992). This change causes a lack of performance of Mac1 (CD11b/CD18) a surface glycoprotein of neutrophils, which interferes in immune response against bacterial infections. This results in lack of adhesion and migration capability of neutrophils to inflammatory tissues. Therefore homozygote cases are prone to recurrent infections such as pneumonia, diarrhea, gingivitis, delay in wound healing, enteritis, neutrophilia and death in early months of life (Hagemoser *et al.*, 1983 ; Nagahata *et al.*, 1997 ; Kehrl *et al.*, 1990).

### MATERIAL AND METHODS

**Samples.** All imported and domestic semen samples plus carrier offsprings of 1 562 herd distributed in 27 provinces of Iran were studied. Blood samples from active bulls and sperm samples used for breeding program during 1965 - 2001 from animal breeding center of Iran were collected .

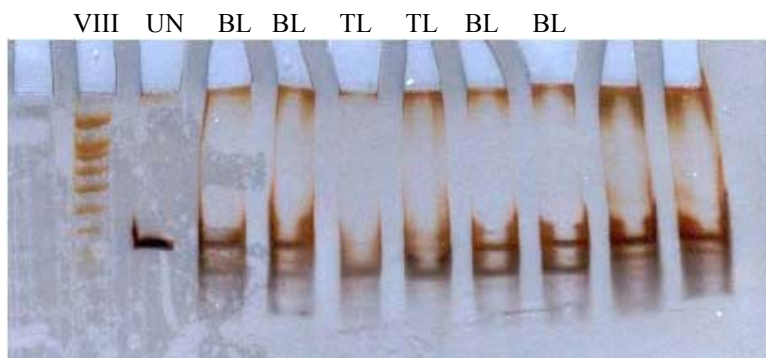
**Primer design and PCR.** DNA samples were extracted from blood and semen by phenol/chloroform method. Then, a primer set, 20 mer was designed for amplification of exon number 5 of CD18 gene using computer software and modified for amplification of 158 bp fragment.

**Enzymatic digestion.** for enzymatic digestion, 10 unit *TaqI* enzyme was added to 30 µl PCR product and incubated for 1hour at 65°. After enzymatic digestion with *TaqI* restriction enzyme, the digested PCR product were electrophoresed on a 10 % acrylamid gel and silver stained. DNA ladder (No. VIII) used in the study was purchased from Roche company.

### RESULTS

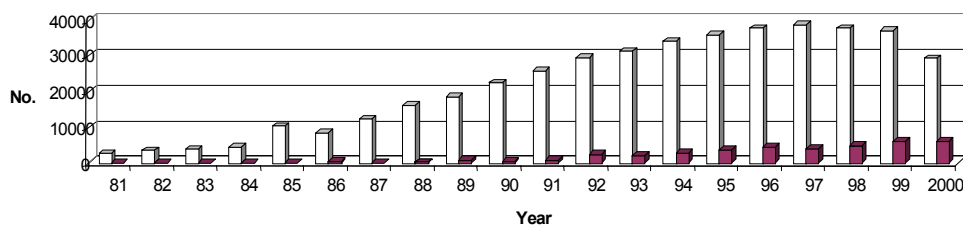
Mutation of D128G position in BLAD carriers resulted in changing adenine to guanine and alteration of *TaqI* recognition site (TCGA) to *HaeIII* recognition site (GGCC).

The results of this study confirmed the existance of BLAD carrier in Iranian holstein cattle. After detection of carrier sperms, the prevalence of BLAD frequency was estimated approximately 2.3 %, and ranged from 1.83 % for imported bulls to 3.06 % for locally produced sperms.



**Figure 1. Pattern of PCR product from normal and Blad carrier after digestion with *TaqI***

VIII : DNA ladder no.VIII Roche company ; UN : undigested PCR product ; BL : Blad carrier digest ; TL : normal hemozygote digest



**Figure 2. Number of offsprings of Blad carriers bulls originated from Animal Breeding Center**

## DISCUSSION

According to the information from 1965-2001, the carrier progeny produced on the animal breeding center were identified. Distribution of carriers in different provinces varies and correlated to distribution of carrier sperms. Therefore, higher the carrier sperm distribution the higher the carrier progenies prevalence. The profile of carrier inducing sperm showed the imported carrier sperms was almost the only cause of disease, between 1991 – 1994, however some carrier cases in local sperm was also observed during 1993 – 2001.

## ACKNOWLEDGEMENTS

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