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IDENTIFICATION OF FACTOR XI DEFICIENCY IN HOLSTEIN FRIESIAN CROSSBRED COW WITH REPEAT BREEDING

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Abstract: In the present study the HF crossbred cattle was screened for reproductive disorders especially for Factor XI deficiency by using Polymerase Chain Reaction (PCR). Total 70 blood samples were collected from Bhgyalaxmi Cattle Farm, Manchar Dist. Pune, Maharashtra with history of reproductive disorders like repeat breeding and Anestrous. The previous reports has suggested the possibility of presence of mutant gene affecting reproductive performance in HF crossbred cattle in India which makes it necessary to screen the animals to avoid the risk of spreading FXI in the breeding cattle population.

Keywords: FXID, PCR, FXI.

INTRODUCTION

Factor XI deficiency (FXID) is the major genetic defect affecting the reproductive efficiency of dairy cattle. It is monogenic recessive autosomal defect causing embryonic deaths, abortions and still births, leading to negative influence on reproductive efficiency. The causal mutation responsible for FXID is an autosomal recessive disorder, with partial deficiency of FXI protein which is involved in blood clotting. Factor XI is an inherited deficiency which results in a bleeding disorder and also important protein involved in the blood coagulation. Some other important autosomal recessive genetic disorders affecting reproductive performance in cattle are Deficiency of Uridine Monophosphate Synthase, Bovine Citrullinemia, Complex Vertebral Malformation and Bovine Leucocyte Adhesion Deficiency. The carrier cattle usually shows the symptoms of mild haemophilia while affected calves can survive for years with no clinical signs and symptoms but shows higher mortality, morbidity with bleeding form of umbilicus, hemoptysis, epestaxis, hypogenesis syndrome and weak neonatal calf. In pregnant cows, abortion and failure to conceive have been observed. Mummified calf foetuses are commonly reported.

The molecular basis of factor XI deficiency is a 76 bp insertion of an imperfect poly-adenine tract occurring in exon 12, followed by a repeat segment of 14 bp, which corresponds to the

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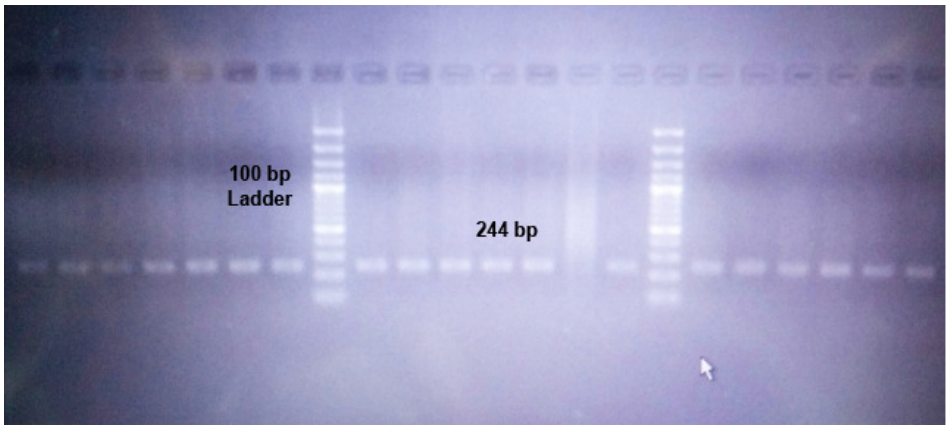
normal coding sequence immediately preceding the insertion (Marron *et al*., 2004). The carriers of this genetic disorder are likely to be present within the population leading to great economic losses. The increased use of artificial insemination has made it necessary to screen the breeding population for the presence of these genetic disorders. Considering these losses in performance of dairy animals, the present study was undertaken to screen HF crossbred cattle for FXI deficiency loci.

MATERIALS AND METHODS

For the present study total 70 blood samples were collected on Holstein Friesian (HF) crossbred cattle with the history of repeat breeding and anestrous maintained at Bhagyalaxmi dairy farm, district Pune of Maharashtra State. Aspectically collected blood Samples were subjected to isolation of DNA using traditional Phenol Chloroform method. The isolated DNA was checked for its purity by electrophoresis using 0.8 per cent agarose gel for 30 minutes at 90V and assessed under UV Spectrometer. The quantification was checked with OD_{260}/OD_{280} ranging between 1.7 and 2.0 was subjected for further analysis. The final volume of 25 µl is prepared for the FXID PCR protocol containing 1X PCR buffer 2.5µl, dNTP 2.0 µl, $MgCl_2$ 1.5µl, Forward primer 0.5 µl (5' CCCACTGGCTAGGAATCGTT3'), Reverse primer 0.5 µl (5' CAAGGCAATGTCATATCCAC 3') Taq DNA Polymerase 0.2 µl, Template DNA 1.0 µl (~100 ng), Nuclease free water 16.8µl.The PCR reaction included the following steps: Initial Denaturation at 94°c for 3 minutes, Denaturation 94°c for 1.5 min, Annealing temperature 55°c for 1 min repeat cycle for 30 times and final extension for 10 min at 72°c. The PCR products were resolved by electrophoresis using 1.7 per cent agarose gel in 1X TAE buffer. The amplified PCR products were confirmed under UV Photo spectrometer.

RESULTS AND DISCUSSION

The samples without impurity and having optical density in between 1.7 to 2.0 were selected for PCR reactions. The PCR conditions were optimized with respect to parameters such as template concentration, primer concentration, $MgCl_2$ concentration and annealing temperature. A suitable annealing temperature was tested from a range 50-60°C for amplification of fragments of FXI Gene. Consistent results obtained at an annealing temperature of 55°C for FXI gene. Patel *et al.* (2007) and Meydan *et al*. (2009) also reported similar annealing temperature for FXI PCR.



(Fig 1) PCR amplification of FXI locus

DNA fragment of 244 bp of FXI gene amplified with the primers described by Marron *et al*. (2004) (Fig.1). The PCR products of all animals analyzed on 1.7 per cent agarose gel revealed single 244 bp FXI fragment. The carriers of the FXI deficiency exhibits two DNA fragments of size 320 bp and 244 bp, homozygous recessive animal exhibits only one fragment of size 320 bp, while normal animal exhibits only one fragment of size 244 bp. The present findings suggested that none of the animal was carrier for FXI deficiency. The present findings are similar to that of Mukhopadhyaya *et al*., (2006) who screened 307 HF cattle and 259 water buffaloes in India. Eydivandi *et al*., (2011) studied 330 Khuzestan native cows and Iranian Holstein cattle possessing a single 244 bp fragment indicating that none of the animals were carriers for FXI deficiency.

However, mutation for FXI deficiency has been reported by various investigators worldwide. Meydan *et al.,* (2009) screened 225 Holstein cows in Turkey and observed the frequency of the mutant FXI allele and prevalence of heterozygous cows as 0.09 and 8 per cent, respectively. Marron *et al*., (2004) observed a frequency of the mutated allele as 1.2 per cent in a contemporary population of the USA Holstein sires. Patel *et al*., (2007) from India reported two FXID carrier Holstein-Friesian bulls (frequency 0.6 per cent. The frequency for the FXI mutant allele and the FXI carrier prevalence were estimated at 0.06 and 1.17 per cent, respectively.

Conclusion

In present study, 70 blood samples collected from the HF crossbred cattle with history of reproductive disorders maintained at Bhagyalaxmi dairy farm, district Pune of Maharashtra State were screened for FXI. The PCR amplified 244 bp product revealed specific for normal animals. The carriers of the FXI deficiency exhibits two DNA fragments of size 320 bp and

244 bp, homozygous recessive animal exhibits only one fragment of size 320 bp. However further study in this aspect is needed to make realistic estimates of FXI allele frequency in Indian crossbred cattle population.

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