Journal of Cell and Tissue Research Vol. 21(2): 7095-7099 (2021)
(Available online at www.tcrjournals.com)
ISSN: 0973-0028; E-ISSN: 0974-0910

Original Article

NUCLEOTIDE VARIABILITY OF *PRM*1 GENE IN CROSSBRED CATTLE USED FOR SEMEN PRODUCTION

GANAPATHI, P.,[1*] MURALI, N.,[2] SARAVANAN, R.,[3] RAJA, A.[4] AND SELVARAJU, M.[5]

[1]Bargur Cattle Research Station, Bargur, Erode - 638 501; [2]Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Namakkal - 637 002; [3] Livestock Farm Complex, Veterinary College and Research Institute, Udumalpet – 642 122; [4]Education Cell, Veterinary College and Research Institute, Namakkal - 637 002; [5]Department of Veterinary Gynecology and Obstetrics, Veterinary College and Research Institute, Namakkal - 637 002; Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai – 600 051.
E. mail: ganagen@gmail.com, Cell: 090420 58592

Received: July 2, 2021; Accepted: July 15, 2021

Abstract: The protamines are the small arginine-rich protein that replaces the histone protein at the time of spermiogenesis. Nucleotide variability's in PRM1 and in PRM2 genes are used ad reliable candidate gene used to associated with the mass sperm motility, intial progressive sperm motility and post thaw sperm motility in crossbred cattle. This study was performed to identify the nucleotide variability and expression profile of PRM1 gene in the crossbred bulls. To identify the genetic variability of Bos indicus and Bos taurus crossbred animals, the nucleotide sequence alignment with respect to exon I of the PRM1 genes were assessed. The sequence alignment reports revealed nucleotide substitution of 'G' at 21st position (g.21delA) of the alignment, whereas deletion was reported in reference sequence and substitution of A with C in the 167th position of nucleotide alignment (g.167 A>C). It gave a new genotype BB pertaining to the PRM1 gene with a frequency of 100 per cent. One amino acid change viz a viz serine being converted into phenylalanine was observed. The sequence was submitted to NCBI and accession number MW835341 and MW835342 were obtained for Jersey cross bred and Holstein Friesian cross bred PRM1 gene.

Keywords: Nucleotide variability, *prm*1 gene, Crossbred cattle

Dr. P. Ganapathi is a basically animal breeder. His area of specialization is Conservation Genetics. He did pioneer work on conservation of Bargur Cattle and Buffalo in its breeding tract. He gave distinct effort for start a separate research station for Bargur Cattle and currently working as Assistant Professor and Head in Bargur Cattle Research Station under TANUVAS.

INTRODUCTION

The protamines are a diverse family of small arginine-rich proteins that are synthesized in the late-stage spermatids of many animals and plants and bind to DNA, condensing the spermatid genome into a genetically inactive state [1]. During spermigenesis, sperm DNA was packaged very tightly to protect the DNA during the transit that occurs before fertilization. However, this condensation cannot sacrifice chromosomal elements but they are essential for the embryo to access the correct sequences of the paternal genome for proper initiation of the embryonic development. The nuclear matrix organization is essential for DNA replication, and the histone-bound chromatin identifies genes essential for structural framework including molecular regulatory factors required for proper embryonic development [2]. During spermiogenesis, haploid spermatids undergo a series of changes in chromatin composition and density [3]. Meanwhile, in the round spermatid, the bond between deoxynucleic acid (DNA)-histone will be replaced by transition proteins, whereas in elongated spermatid, the transition protein will be replaced by protamine[4]. Transition nuclear proteins (TPs), the major proteins found in chromatin of condensing spermatids, are believed to be important for histone displacement and chromatin condensation during mammalian spermatogenesis [4]. Protamines have an important role in condensation of chromatin and complete inactivationof transcription [5,6].

During fertilization, spermatozoa plays an important role in the development of the embryo by providing oocyte activation factors, centrosomal components, and a paternal chromosome. Protamine (PRM) is a basic nucleus protein with small molecular weight contained in the head of spermatozoa [7,8], which is important in the packaging of sperm DNA and plays an important role in spermatogenesis and quality of sperm [9]. Protamine genes are integral in chromatin condensation during spermiogenesis and hence are reliable candidate genes as sperm motility markers in cattle. Nucleotide variability's in PRM1 and in PRM2 genes were identified.The SNPs in PRM2 as haplotype were found to be associated with mass sperm motility, initial progressive sperm motility and post thaw sperm motility in crossbred cattle. Knowledge of these SNPs/haplotypes, therefore, may be helpful in ascertaining bulls with efficient reproductive performance [10].

MATERIALS AND METHODS

The samples from Jersey crossbred (n=18) and Holstein freisiancross bred bulls (n=18) maintained in District Livestock Farm, Hosur; District livestock Farm, Ooty; Exotic cattle breeding farm,Echankottai were utilized for this study. Bulls were categorized as freezable semen producer and non freezable semen producer based on the Mass motility, individual motility and post thaw motility of the semen produced by the Individual bull.

Before collecting the blood samples semen variables like mass motility, individual motility, post thaw motility, semen volume, concentration and mass motility was assessed. Sperm of bulls were classified as having relatively greater motility (freezable semen) when they had Individual motility and post thaw motility values were greater than 60 percent and 50 percent respectively. Those bulls which had less than these were considered as non freezable. The bulls having motility impairments (non freezable) were selected for this study.

About 5 ml of blood were collected in EDTA vacutainer from external jugular vein of the bull and the genomic DNA was isolated from the blood using phenol-choloroform extraction method [11]. The good quality genomic DNA were amplified for coding region of PRM1 gene (Exon1) of crossbred cattle's with the suitable primer (F-5'-AGATACC-GATGCTGCCTCAC-3' and R: 5'-GTGGCAT-GTTCAAGATGTGG-3') with the annealing temperature of 60ºC. The ampification was verified by assessing PCR products on 2 per cent (w/v) agrose gel electrophoresis unit and evaluating the gels with a UV Gel documentation system (Biorad,USA). Nine samples from each crossbred were sequenced bidirectionally from Agri. Genome Labs. Pvt Ltd, Cochin, Kerala, India. The result FASTA files were used for contig formation using seqbuild software. The contig sequence were aligned using CLUSTALW method of the MEGALIGN module of the DNASTAR suite (Lasergenevrsion 7.2: DNA star, Madison, USA). The nucleotide was converted into amino acid and aligned using CLUSTALOMEGA software and aligned.

RESULTS AND DISCUSSION

Seminal parameters of Jersey crossbred and Holstein

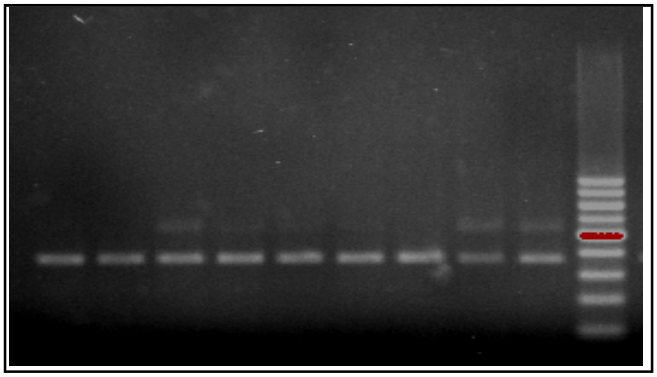
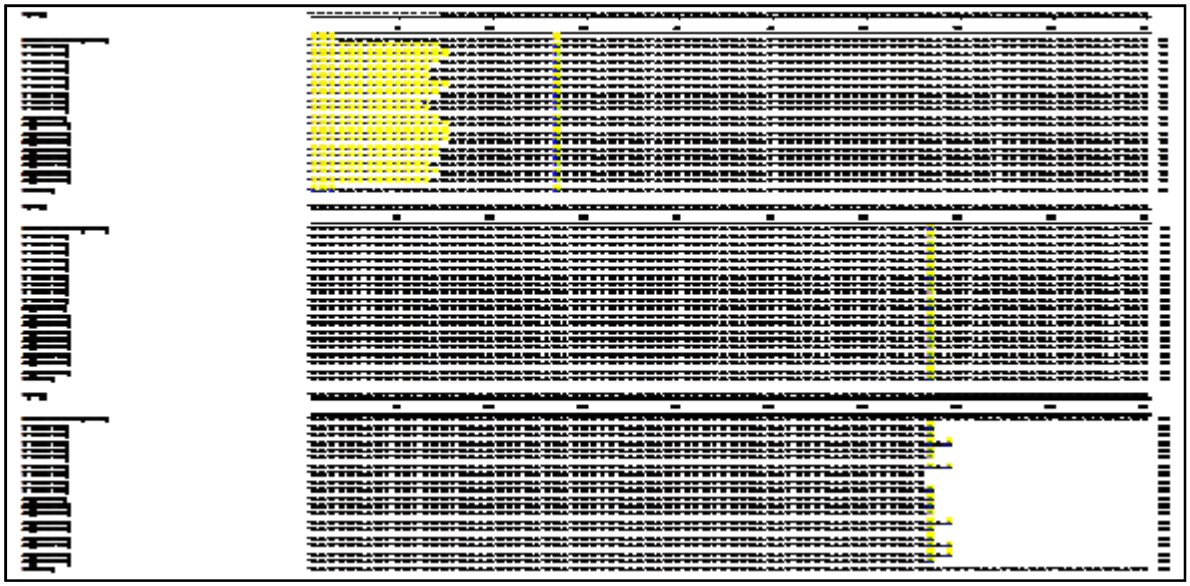


Fig. 1: Amplified PCR product of PRM1 gene in Agarose Gel Electrophoresis



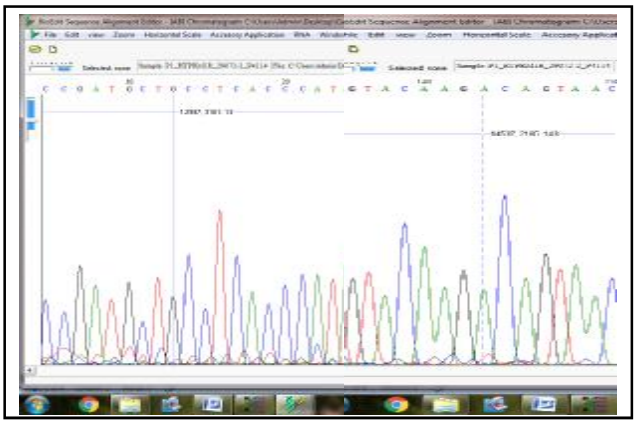


Fig 2: Nucleotide Sequences of Exon 1 of PRM1 Gene of crossbred bulls

Fig 3: Chromotogram of exon1 of PRM1 Gene

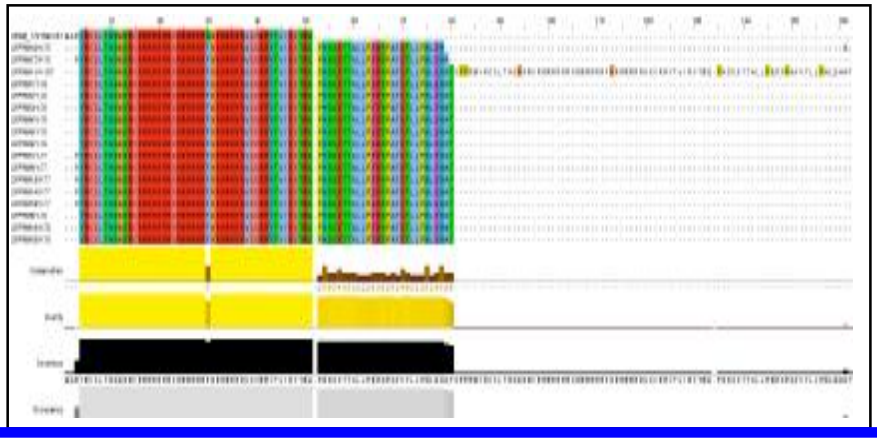


Fig 4: Multiple Sequence Alignment of PRM1 Proteins in Crossbred Bulls

Friesian cross bred bulls mass motility, individual motility and post thaw motility were assessed. The average values with standard error of above parameter for selected animals were 2.14 ±0.16,-25.83±1.84,12.5±1.40 respectively. Genomic DNA was successfully isolated from the above bull samples were amplified for the promoter and coding region of the PRM1 gene and the size of the amplified fragment was 234 bp in all the samples (Fig.1) in 2 per cent agarose gel.

Further, the amplified PCR product were sequenced to identify the nucleotide variability. The nucleotide sequences were aligned with already available sequence of *Bos taurus* species to identify the genetic variability (Gene Bank ID: BC 108207.1). Based on alignment report nucleotide insertion was noticed with G fragment of nucleotide in 27th position (g.26_27insG) and substitution of A with C in the 157th position (g.157C>A) of nucleotide alignment (Figs. 2,3). In both Jersey crossbred and Holstein Friesian crossbred similar type of nucleotide variability was observed and it gave a new genotype BB pertaining to the *PRM*1 gene. The same sequence of PRM1 gene was submitted to Gen Bank with accession numbers of MW835341 and MW835342. Further, the nucleotide variation was also given a amino acid change viz a viz serine being converted into Phenylalanine (Fig. 4).

The PRM1 sequences of Virdavani cattle have similar nucleotide variation and the author confirmed the presence of a 209 bp upstream region and 44 bp 5' UTR regions in 253 bp fragment I of the PRM1 gene [10]. The sequence analysis indicated that there were two SNPs at the 152nd and 179th nucleotide position (A152G and G179A transition) which were located in the upstream region and in the predicted promoter sequences, respectively. Sequences of allele A indicated that there was a complete homology to the *Bos taurus* sequence. The sequences of the A and B alleles of fragment I of the PRM1 gene were submitted to Gen Bank with accession numbers KU844285 through KU844287, respectively. The SSCP analysis of the fragment II and fragment III of PRM1 gene, however, indicated that there was one band pattern that was designated as AA [10]. Frequency of this pattern and allele 'A' was 100% in both crossbred and purebred cattle for both fragments II and III. Analysis of the nucleotide sequences for fragment II (188 bp) validated the presence of a 56 bp 5'UTR (partial), 112 bp CDS and 20 bp intron1 (partial) and for fragment III (163 bp) confirmed the presence of a 36 bp intron1 (partial), 44 bp complete CDS and 83 bp 3'UTR (partial) region. The sequence of fragment II and III of PRM1 gene in crossbred and purebred cattle was found to be identical to the *Bostaurus* sequence and there was no SNP identified in these regions. The nucleotide sequences of allele 'A' of fragment II and III of the PRM1 gene of cattle were submitted to Gene Bank with accession numbers KU844288 KU844291, KU844292 and KU844294 respectively. The*PRM1* gene from Aceh cattle is identical to that of *PRM1* gene from *Bos taurus* and *Bos indicus* both at the DNA sequence level and at the protein sequence [12].

The nucleotide variability was observed in PRM1 gene (exon1) at the nucleotide position 27 (g 26_27ins G) and at 157th position (g 157C >A) in Jersey and Holstein Friesian crossbred cattle with non freezable semen production characteristics. This infers that the PRM1 gene can be considered as a candidate gene for sperm motility and has to be further confirmed with more number of samples.

Acknowledgements: The first author thanks Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai for the financial assistance granted to carry out the study.

REFERENCES

[1] Balhorn, R.: The protamine family of sperm nuclear proteins. Genome biology., 9: 227. https://doi.org/10.1186/gb-2007-8-9-227 (2007).

[2] Ward, W.S.: Function of sperm chromatin structural elements in fertilization and development. Molecular Human Reproduction., 16(1):30-36 doi:10.1093/molehr/gap080 (2010).

[3] Steger, K.: Transcriptional and translational regulation of gene expressions in haploid spermatids. Anatomy and Embryology., 199: 471-478 (1999).

[4] Steger, K., Failing, K. and Klonisc, T.: Round spermatid from infertile men exhibit decreased protamine-1 and -2 mRNA. Human Reproduction., 16(4): 709-716 (2001).

[5] Aoki, V.W., Liu, L., Jones, K.P., Hatasaka, H.H., Gibson, M., Peterson, C.M. and Carrell, D.T.: Sperm protamine 1/protamine 2 ratios are related to in-vitrofertilization pregnancy rates and predictive of fertilization ability. Fertility and Sterility., 86: 1408–1415 (2006).

[6] Luke, L., Vicens, A., Tourmente, M. and Roldan, E.R.S.: Evolution of protamine genes and changes in sperm

head phenotype in rodents. Biology of Reproduction., 90:67 (2014).

[7] McKay, D.J., Renaux, B.S. and Dixon, G.H.: Human sperm protamines. Amino-acid sequences of two forms of protamine P2. European Journal of biochemistry., 156(1): 5–8. https://doi.org/10.1111/j.1432-1033.1986.tb09540.x (1986).

[8] Rooney, A.P. and Zhang, J.: Rapid evolution of a primate sperm protein, relaxation of functional constraint or positive Darwinian selection? MolBiolEvol., 16: 706-710 (1999).

[9] Dogan, S., Vargovic, P. and Oliveira, R.: Sperm Protamine-Status Correlates to the Fertility of Breeding Bulls. BiolReprod., 92(4):92 (2015).

[10] Yathish, H.M., Kumar, S., Chaudhary, R., Mishra, C., Sivakumar, A., Kumar, A., Chauhan, A., Ghosh, S.K. and Mitra, A.: Nucleotide variability of protamine genes influencing bull sperm motility variables. Animal Reproduction Science., 193: 126-139 (2018).

[11] Sambrook, J. and Russell, D.W.: *Molecular Cloning: A Laboratory Manual*. *Cold* Spring Harbor Laboratory Press, Cold Spring Harbor, NY. Pp. 415 (2001).

[12] Helmil, T.H., Hambal, M., Sugito, S., Zamzami, R.S., Rusli, R.R. and Akmal, M.: Identification and Characterization of Protamine1 Gene in Aceh Cattle. E3S Web of Conferences., 151: 01039. https://doi.org/10.1051/e3sconf/202015101039 (2020).