Short Communications

Indian Journal of Animal Sciences **81** (9): 981–983, September 2011

Sequence variability in CatSper1 gene in Vrindavani crossbred cattle

GEETHA T[1], SUBODH KUMAR[2*], P P DUBEY[3], SIVAMANI B[4], S K GHOSH[5], ABHIJIT MITRA[6], A K S TOMAR[7] and ARJAVA SHARMA[8]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243122 India

Received: 7 March 2011; Accepted: 21 April 2011

Key words: AluI, CatSper1, Crossbred Cattle, Exon 5, PCR-RFLP, TaqI

The damage to the semen largely refers to post thaw motility (PTM, Ravimurugan *et al.* 2007) which again depends on initial/prefreeze motility and is the ultimate trait which qualifies the semen for artificial breeding. A fairly good number of crossbred bulls donate semen of poor quality leading to high rejection rate of the bulls in breeding programmes (Chacon *et al.* 1999, Kumar 2006, Tyagi *et al.* 2006). Poor sperm motility and freezability of semen were reported in Vrindavani, a crossbred stock developed at Indian Veterinary Research Institute (Ghosh *et al.* 2007). Many genes control sperm motility but recently, a calcium selective ion channel gene called CatSper (cation channel of sperm) has received much attention (Quill *et al.* 2001, Ren *et al.* 2001). No report on CatSper gene is available either in crossbred or in zebu cattle. The present study is an attempt to ascertain sequence variability and polymorphism in the exon 5 of CatSper1 gene of Vrindavani cattle so as to initiate a step in searching the promising DNA markers that could be developed to improve sperm motility of crossbred cattle by assisting in bull selection process.

Randomly selected Vrindavani crossbred cattle (100) (crosses of Holstien Frisian, Brown Swiss, Jersey; with Hariana as indigenous stock and having exotic inheritance level in between 50-62.5%) developed at the Indian Veterinary Research Institute, Izatnagar and maintained at Livestock Production Management Section (LPM) as well as Germplasm Centre (GPC) of the institute were included in the present investigation. Venous blood (10 ml) was collected from each animal in sterile 15 ml polypropylene centrifuge tube containing 0.5 ml of 2.7% EDTA as anticoagulant. DNA isolation was done by phenol chloroform extraction method (Sambrook and Russel 2001) and the precipitated DNA was dissolved in 200µl of TE buffer. The quality and concentration of genomic DNA was evaluated by spectrophotometer. The samples that showed an optical density ratio (optical density 260/optical density 280) in the range of 1.7–1.9 were assessed to be of good quality. The 282 bp fragment comprising of exon 5 and with flanking partial intron 4 and intron 5 of CatSper1 gene in the genomic DNA of crossbred cattle was amplified by using a pair of self designed primers (5' CCT TTC TGG CCC CCT TAC A 3' as forward primer 5' ACC AAC ATC AAC GGC CTT CTCTAC 3' as reverse primer) on the basis of predicted CatSper sequence in the GenBank (NC_007330). The primers were stored at –20°C. The stock solution was prepared by diluting the same with DNase free water in such a way that each had a concentration of 300 pmoles/µl. This was kept at 4°C for 2–3 days for allowing complete dissolution of primers. The working primer solution was further prepared by 10 fold dilution of stock primer solution in DNAse free water so that each has a concentration of 30 pmoles/µl. The reaction mixture and PCR programme were optimized to achieve the satisfactory level of amplification in a final volume of 25µl containing genomic DNA (60–100ng), 2.5µl of 10xPCR (1.5 mM), 2.5 µl of dNTPs mix (0.2 mM), 1.5 µl of $MgCl_2$ (1.5 mM), 1µl each forward and reverse primers and 0.2 µl of Taq DNA polymerase (5U/µl). Samples were amplified for 35 cycles with initial denaturation at 94°C for 3 min, cyclic denaturation 94°C for 1 min, annealing at 55°C for 1 min, cyclic extension 72°C for 1 min., final extension 72°C for 10 min. The 282 bp PCR products comprising of exon 5 and with flanking partial intron 4 and intron 5 of CatSper1 gene of crossbred cattle were digested with AluI as well as TaqI restriction enzymes as per manufacturer protocol. The digested products were electrophoresed in 2.5% w/v agarose gel which was stained with ethidium bromide, at 100 V for 5 min and then 90V for 1 h in 1 × TBE buffer and visualized under UV light. The amplicon of CatSper1 gene was eluted, cloned in pGEMT vector and sequenced in both orientations. The same primer pair was used to amplify the gDNA of some other domestic animals, viz. zebu cattle, mithun, buffalo, sheep and goat. The obtained sequence

Present address: [1]Ph D Scholar, [2*]Senior Scientist (e mail: subkum@yahoo.com), [3]Research Associate, [4]Scientist, [5, 6, 7]Senior Scientists, [8]Head, Division of Animal Genetics.

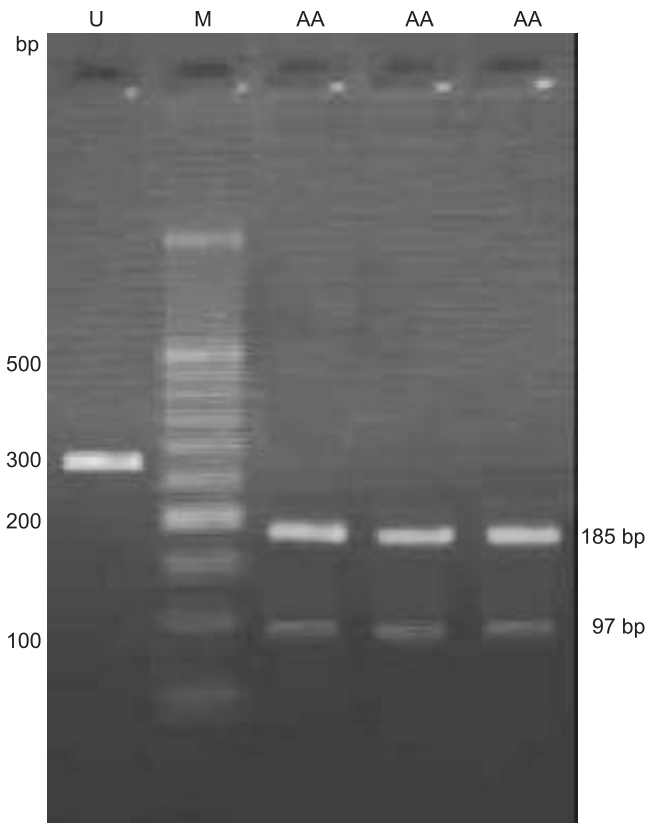

Fig 1. Digestion of 282 bp amplicon encompassing exon 5, with *AluI* restriction enzyme.U, Undigested product; M, 50bp marker; AA, *Alu*I genotype.

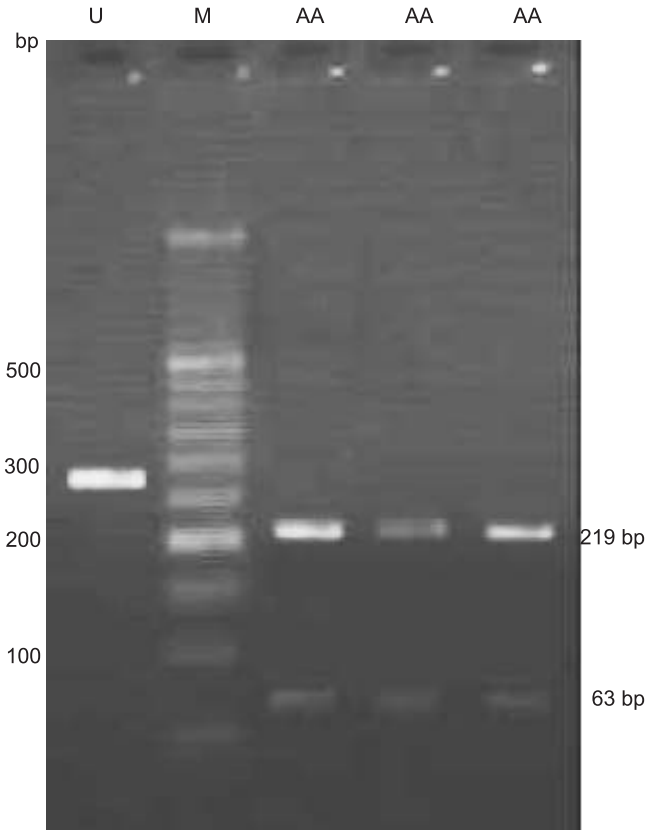

Fig 2. Digestion of 282 bp amplicon encompassing exon 5, with *Taq* I restriction enzyme. U, Undigested product; M, 50bp marker; AA, *Taq*I genotype.

was aligned the sequences of these domestic animals and all the sequences were submitted to the GenBank (www.ncbi.nlm.nih.gov: JK737763 -67).

The amplified products on digestion with AluI (AG↓CT) produced 2 bands of 185 bp and 97 bp (Fig. 1), which was confirmed by the presence of restriction site at 97th position in the sequence. Similarly, TaqI (T↓CGA) had restriction site at 219th position which gave two bands of 219 bp and 63 bp (Fig. 2). All the bands were distinctly visible. This suggested that amplified fragment of CatSper1 gene contained one RE cutting sites each for AluI and TaqI restriction enzymes. No polymorphism was found with respect to these restriction enzymes, hence the gene and genotype frequency of these fragments were 1.00. No reports are available to compare with the present findings. The nucleotide sequence analysis revealed that only one nucleotide variation existed in zebu cattle in exon 5, when compared to that of crossbred cattle which was identified to be C→T at 33rd position. This was the first nucleotide difference observed so far with respect to crossbred cattle. Similarly, when crossbred cattle sequences were compared to buffalo, 6 nucleotide difference were noticed in latter, which were found in exon 5. On comparison of cattle with goat 12 nucleotide variation were noticed. The phylogenetic tree revealed crossbred cattle closest to zebu cattle for this amplicon as both were located in the same cluster. Analysis of deduced amino acid revealed that exon 5 coded for 70 amino acids having strongly basic (6), strongly acidic (5), hydrophobic (21) and polar (13) amino acids. The deduced amino acid sequence of exon 5 of CatSper1 of crossbred cattle was compared with similar sequence of other species. Only one amino acid change in crossbred cattle compared to zebu cattle, at fourth (proline to leucine) position was noticed. When compared to buffalo, two amino acid change at 13th (serine to glycine), 57th (methionine to leucine) and when compared to goat seven amino acid change at 6th (threonine to alanine), 15th (phenylalanine to leucine), 17th (tyrosine to cystine), 31st (arginine to glutamine), 60th (alanine to aspargine), 63rd (phenylalanine to proline), 64th (alanine to proline) positions were noticed. The sequence homology of crossbred cattle was highest (100%) with mithun and it was distant with goat (94.7%). This appears to be the first report of CatSper1 gene in cattle as there is dearth of literature to compare the results.

Genetic characterization and polymorphisms identification of CatSper1 gene is prerequisite for finding a genetic marker of this gene which may help to improve the sperm motility and freezability in crossbred cattle. The

monomorphic pattern of exon 5 of CatSper1 gene with respect to different enzymes indicated the conserveness of this exon and suggested to explore polymorphism in other coding regions of this gene as well as other genes responsible for sperm motility. Further, if any polymorphism in this gene is identified in future, then association of these polymorphs with sperm motility could be used as a genetic marker for selection programme

SUMMARY

A 282 bp fragment encompassing exon 5 of CatSper1 gene was amplified to characterize and identify the polymorphism in Vrindavani cattle. The PCR- RFLP analysis showed the absence of polymorphism in this fragment with respect to AluI and TaqI restriction enzymes. The fragment was sequenced in both zebu (Tharparker) and crossbred (Vrindavani) cattle. The nucleotide sequence analysis revealed that only one nucleotide variation existed in zebu cattle in exon 5 when compared to that crossbred cattle. This was the first nucleotide difference observed so far with respect to crossbred cattle. The gene sequence of the wild allele, was submitted to GenBank (JF737763).

REFERENCES

Chacon J, Perez E, Muller E, Soderquist L and Martinez H R. 1999. Breeding soundness evaluation of extensively managed bulls in Costa Rica. *Theriogenology* **52**: 221–31.

Ghosh S K, Singh S K, Singh L P, Tripathi R P and Tumnyak L. 2007. Rejection rate in crossbred bull semen. A paper in Compendium of XXIII Annual Convention and National Symposium on *Challenges in improving reproductive efficiency of farm and pet animals*. December 7–9, Bhubaneshwar, Orissa, India.

Kumar S. 2006. Advances in assessment of frozen semen of crossbred bulls. National Seminar on *Artificial Insemination*: *Acceptability, impact, constraints and solutions* Pp. 106–16.

Quill T A, Ren D, Clapham D E and Garbers D L. 2001. A voltage-gated ion channel expressed specifically in spermatozoa. *Proceedings of National Academy of Science U S A* 98: 12527–31.

Ravimurugan T, Kanakaraj P and Thangaraju P. 2007. Frozen semen production potential of murrah bulls. *Tamilnadu Journal Veterinary and Animal Science* **3**(5): 269–71.

Ren D, Navarro B, Perez G, Jackson A C, Hsu S, Shi Q, Tilly J L and Clapham D E. 2001. A sperm ion channel required for sperm motility and male fertility. *Nature* **413**: 603–09.

Sambrook J and Russel D W. 2001. *Molecular cloning- A Laboratory Manual*. 3rd edn. Cold Spring Harbor Laboratory Press. New York, U S A.

Tyagi S, Mandal D K, Kumar M and Mathur A K. 2006. Reproductive wastage rate of crossbred dairy bulls with reference to level of exotic inheritance and number of breed component. *Indian Journal of Animal Reproduction* **27**(1): 27–30.