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Identification of nucleotide variation in *CatSper2* gene in crossbred breeding bulls

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Abstract
Among the many genes governing sperm functions, a group of genes have been reported to control ion channels that play an important role in sperm motility. They were named the cation channel of sperm (*CatSper*) and further known to have five members, viz. *CatSper* 1, 2, 3, 4, and β (7). Any mutations in them were proposed as the cause of poor motility in sperms and infertility. Nucleotide alterations pertaining to *Catsper* gene cause poor motility in sperm production and infertility. Among the calcium-permeable channels present on sperm cells, only *CatSper*1 and 2 are known to be indispensable for male fertility. Exon 2 of *CatSper*2 gene was amplified and sequenced. There are two nucleotide changes observed, conversion of C nucleotide in 276th position to A nucleotide (g.276C>A) (Transversion) and conversion of A nucleotide to C in the 277th position (g.277A>C). In the translation process, the nucleotide changes at 276th and 277th position (g.276C>A and g.277A>C) does not reveal any amino acid change in the amino acid sequence, in both Jersey crossbred bulls and Holstein Friesian crossbred bull.

Keywords: crossbred bulls, *CatSper*2, spermiogenesis, sperm motility

Introduction
Crossbreeding introduced in India to improve milk production. However, they endure low breeding soundness, poor seminal quality, and repeat breeding compared to indigenous and exotic purebreds. The semen quality of the crossbred bull is unacceptable because of poor freezability, higher cryo-injuries, poor sperm motility and viability and a high percentage of dead/abnormal sperms that cause a decline in the fertility rate [1]. India has increased milk yield by 4 to 6 per cent /year over the past 20 years [2] primarily by mating high-quality dairy bulls to local cows to attain higher milk-yielding cows. The incidence of sub-fertility and poor semen quality is higher in crossbred bulls (offspring produced by crossing exotic bulls with Indigenous cows). Although the alterations in functional attributes of spermatozoa during cryopreservation have been reported to be associated with sub-fertility, the exact etiology remains elusive [3].
Among the many genes governing sperm functions, a group of genes has been reported to control ion channels that play an important role in sperm motility. They were named the cation channel of sperm (*CatSper*) and further known to have five members, viz. *CatSper* 1, 2, 3, 4, and β [7]. These genes had sperm-specific expression patterns and were localized primarily in the plasma membrane of the principal piece of sperm tail [4-6]. Any mutations in them were proposed as the cause of poor motility in sperms and infertility [7]. Among the calcium-permeable channels present on sperm cells, only *CatSper*1 and 2 are known to be indispensable for male fertility [8-10].
The role of *CatSper* genes in sperm motility is well recorded. Any mutation in these genes could be the causative factor of poor motility in sperms. Six novel SNPs in *CatSper*2 the gene of Vrindavani cattle was identified [11]. Hence this study formulated to identify nucleotide variation in *CatSper2* gene in Jersey and Holstein Friesian crossbred used for semen production.

Materials and Methods
The samples from Jersey crossbred (n=18) and Holstein Friesian crossbred 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 were 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 per cent and 50 per cent 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'-GACCTTGGGAGAAAGGGAG-3' and R: 5'-ACCTGGGAAGGGTGTGGAT-3') with the with the annealing temperature of 62.5 ºC. The amplification 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). Six samples from Jersey 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 sequences were aligned using CLUSTALW method of the MEGALIGN module of the DNASTAR suite (Lasergene V.7.2: DNAstar, 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 Friesian crossbred bulls mass motility, concentration and individual motility were assessed. The average values with standard error of the above parameter for selected animals were 2.25 ±0.22, 641.80 ± 95.60 million /ml, 49.09±4.05 percent respectively. Genomic DNA was successfully isolated from the above bull samples were amplified for the promoter and coding region of the *CatSper2* gene and the size of the amplified fragment was 288 bp in all the samples in 2 per cent agarose gel.

Amplified PCR product of exon 2 of *CatSper2* gene was observed in 228 bp of the agarose gel against the 50 bp ladder**.** The multiple nucleotide sequence alignment of breeding bulls pertaining to exon 2 of *CatSper*2 gene with the sequence available reference sequence KC493601and the variations are presented in chromatogram. There was nucleotide change CA>AC with respect to the reference sequence available in the Genebank and the same was submitted to GenBank (Accession no. MW972051). There are two nucleotide changes observed in this exon, the first one is a conversion of C nucleotide in 276th position to A nucleotide (g.276C>A) and conversion of A nucleotide to C in the 277th position (g.277A>C). In the translation process, the nucleotide changes at 276th and 277th position (g.276C>A and g.277A>C) does not reveal any amino acid change in the amino acid sequence. Both Jersey crossbred bulls and Holstein Friesian crossbred bull's expressed similar type of nucleotide variation in exon 2 of *CatSper2* gene.

In the present study, bidirectional (non freezable semen producing bulls) sequence was compared with the already available sequence in GenBank (Accession No: KC493601) belonging to Virdavani cattle. The results revealed that there were two nucleotide changes in the crossbred bulls compared with the reference sequence at 242nd position to produce a new CC geneotype variation with respect to exon2 of *Catsper*2.

In 122 randomly selected Vrindavani cattle, one SNP (C30G) in the exon 2 fragment were identified using PCR-SSCP and sequencing analysis (Sivakumar *et al*., 2018). Further, it was found that SNPs present in the sequence at the time of translation process did not produce any amino acid sequence variation.

Conclusion

In *CatSper*2 (exon 2) gene sequence assembly revealed that a nucleotide change CA>AC at a position of 276 and 277bp. A nucleotide at 276th position to A nucleotide (g.276C>A) and an another conversion of A nucleotide to C in the 277th position (g.277A>C) and the nucleotide changes does not reveal any amino acid change in the amino acid sequence on translation process. This infers that the *CatSper2* gene can be considered as a candidate gene for sperm motility and has to be further confirmed with more number of samples.

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