Association of single nucleotide polymorphisms of PPP1R11 gene with conception rate in Karan Fries bull

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ABSTRACT

Bull fertility is an important component of reproductive efficiency in dairy cattle. PPP1R11 is a potent candidate gene for bull fertility being involved in the pathway of spermatogenesis. Present investigation was carried out in twenty Karan Fries (KF) bull to identify SNPs of PPP1R11 gene and their association with conception rate. Genomic DNA was extracted from frozen semen straws by phenol-chloroform method. Primer pair targeted to intron II region of PPP1R11 gene was designed using Primer 3.1 software. PCR reactions were carried out in thermal cycler after optimizing. The PCR product was checked on 1.5% horizontal agarose gel electrophoresis to verify the amplification of target region. Samples were sequenced and SNPs were identified using multiple alignment programme with respect to reference sequence and visual inspection of chromatograms. Sequencing results revealed only two SNPs [G28708846A, T28708860C] at intron II region of PPP1R11 gene in KF bulls. Each bull was genotyped with respect to each of the two identified SNPs. Association of SNP genotype with conception rate was analyzed using least square ANOVA taking genotypes as fixed effect. The result indicated that, only one SNP [T28708860C] had significant (p<0.01) effect on conception rate. The bulls with TT genotype revealed significantly (p<0.01) highest conception rate (53.20±2.96%) followed by bulls with TC (43.12±2.39%) and the lowest in bulls with CC (34.59±2.28%) genotypes. The results of present finding suggested that association of SNPs with conception rate after validation on large population might be useful in marker assisted selection for selection of bull with high fertility in future.

Key words: Bull fertility, Conception rate, Karan Fries, Single Nucleotide polymorphism.

INTRODUCTION

Crossbred cattle population contribute more than 50 per cent (54.14%) of the total milk produced by cattle with only 20.81 per cent population which play an important role in white revolution in India (19th Livestock Census, 2012). Crossbreeding programmes were mainly focused towards milk production traits and not much emphasis was given toward reproductive traits which led to decline in fertility traits (Lucy, 2001). Bull fertility is a complex trait, having relatively low heritability and difficult to improve through traditional phenotypic selection (Li et al., 2012; Mahmoud and Nawito, 2012). Demand of genetically elite bulls’ semen with high fertility has increased in recent years to meet the projected demand of 191.3 million tones of milk by 2020 in the country (Kumar et al., 2016). Approximately 20 to 40% crossbred bulls are culled due to poor libido, semen quality and freezability, which lead to decline in conception rate, adversely affect the crossbreeding policy and incur huge economic losses to dairy enterprises (Mukhopadhyay et al., 2010; Panmei et al., 2016). Advancement of molecular genetics tools now easily enables us to explore individual genes in animals. Failure of spermatogenesis, widespread loss of post-meiotic germ cells and abnormalities in the mitochondrial sheath was reported due to various mutants of protein phosphatase gene (MacLeod et al., 2014). Researchers also reported significant association of PPP1R11 gene with sire conception rate in cattle (Li et al., 2012; Khatib, 2014). Protein phosphatase 1 gene regulatory subunit 11 (PPP1R11) is a potent candidate gene for bull fertility being involved in the pathway of spermatogenesis. Identification of such genetic markers and understanding of molecular mechanism involved in bull fertility would help in selection for better reproductive performance. Therefore, the present investigation was undertaken to identify SNPs of intron II region of PPP1R11 gene and their association with conception rate in crossbred Karan Fries bull.

MATERIALS AND METHODS

Twenty Karan Fries bulls were selected on the basis of conception rate which were calculated as a proportion of confirmed pregnancies to total insemination by a particular bull as per Naha et al. (2015). Five to ten semen straws were procured from twenty Karan Fries bulls (KF) maintained at Artificial Breeding Research Centre, ICAR-NDRI, Karnal. These semen straws were carefully collected and transported.
to laboratory, in a liquid nitrogen container (Cryocan) and stored till DNA isolation. Genomic DNA was extracted from four frozen semen straws by Phenol-Chloroform extraction method (Sambrook and Russell, 2001). The quality of DNA was assessed using 0.8% agarose gel electrophoresis and purity and quantity by NanoDrop spectrophotometer (NanoDrop Technologies, USA). The DNA samples having intact band and optical density ratio (260/280nm) within normal range, 1.7-1.9, were subjected to PCR amplification. Primer pair (forward 5’-ACCA TCAAACTTCGGAAACG-3’ reverse 5’-TTCCA TTCCCACTGGATCTC-3’) of partial exon II and intron II region of PPP1R11 were designed to amplify the target regions of PPP1R11 gene using Primer3.1 software by taking *Bos taurus* as reference sequence (AC_000180.1). The primers were inspected for their properties using oligoanalyzer software and got synthesized from Eurofins Genomics Indian Pvt. Ltd., Bangalore (India). PCR reactions were carried out in 25µl reaction mix containing 12.5µl of 2X Dream Taq® Master Mix, 1µl (10 pM) each of forward and reverse primers, and 50-100ng genomic DNA as template in nuclease free water. Amplification was carried out in programmable thermal cycler (BioRad T100™) using PCR programme consisting of initial denaturation at 94°C for 5 min, followed by 35 cycles of (i) denaturation at 94°C for 1 min. (ii) annealing at 58°C for 45 s and (iii) extension at 72°C for 45 s followed by final extension at 72°C for 5 min. After PCR amplification, the PCR product was checked by 1.5% horizontal agarose gel electrophoresis to verify the amplification of target region.

**Nucleotide sequencing of the PCR product:** The amplified PCR products of all bulls were sequenced at Eurofins sequencing (Eurofins Genomics India Pvt Ltd.), Bangalore, from both the ends. Nucleotide sequence of every animal was checked and sequences were aligned to get a consensus sequence using Mega5 software. Each edited sequence was aligned with corresponding reference sequence using MegAline Multiple Sequence Alignment Program of DNA STAR to identify SNPs. Each bull was genotyped with respect to each of the identified SNPs markers. The gene and genotype frequencies were estimated by standard procedure.

**Statistical analysis:** Association of SNPs markers with conception rate were analyzed by least squares analysis of variance (Harvey, 1990) by taking SNPs genotype as fixed effects in the statistical model. Where,

\[ Y_{ij} = \mu + S_i + e_{ij} \]

\[ Y_{ij} \] Observation at i\(^{th}\) genotype measured on j\(^{th}\) individual,

\[ \mu \] Overall mean,

\[ S_i \] Fixed effect of i\(^{th}\) genotype of a particular SNP,

\[ e_{ij} \] Random error associated with mean zero and variance \(\sigma^2\).

Modified Duncan multiple range test at 5% level of probability of significance was performed for assessing critical differences among the least squares means of conception rate in different genotypes groups (Kramer, 1957).

**RESULTS AND DISCUSSION**

**Estimation of conception rate:** The conception rate ranged from 27.64 % to 58.75 % with overall estimates of 43.64 \(\pm1.62\) % in Karan Fries bulls. Present finding of average conception rate of Karan Fries bulls were in accordance with the earlier report of Vineeth (2015) in Sahiwal (43.62\(\pm1.31\)%) and Karan Fries (40.91\(\pm1.65\)%) bulls, respectively. Naha et al. (2015) reported overall conception rate as 46.38\(\pm1.55\)% in Sahiwal breeding bulls. Similar conception rate was also reported by Singh and Pant (1999) in Jersey and Jersey× Red Sindhi bulls. A lower conception rate (35%) of field data (Kuhn et al. 2008) and higher conception rate (54.55%) using A.I. programme were reported than the present findings in Holstein Friesian bulls (Davidson and Farver1980).

**Analysis of sequence data of target region of PPP1R11 gene:** Target region of PPP1R11 gene was successfully amplified with the length of 498bp ampollic, which covers the partial exon II and intron II regions of PPP1R11 gene in Karan Fries crossbred cattle (Fig.1). The amplified fragments were sequenced (Eurofins Genomics India Pvt Ltd.) and results of respective region were deduced from the raw sequences by using BioEdit software. Each edited sequence was aligned with corresponding reference *Bos taurus* sequence using MegAline Multiple Sequence Alignment Program of DNA STAR (ver.1.00) for determining the

![Fig 1: Gel electrophoresis indicating amplified PCR product (498bp) of PPP1R11 gene in Karan Fries bulls.](image-url)
polymorphism at target region of PPP1R11 gene in Karan Fries bull.

Detection of SNP at target region of PPP1R11 gene:
Alignment of comparative sequence data analysis revealed that, there were two transitional mutations at G28708846A and T28708860C position in intorn II regions of PPP1R11 gene as compared to Bos taurus (NCBI GenBank AC_000180.1). Different chromatograms showing polymorphism (SNPs) as compared to Bos taurus are presented in Fig. 2 and 3. Sequencing data analysis showed three genotypes (GG, GA and AA) and consequently two alleles (G and A) at first SNP [G28708846A]. The frequencies of GG, GA and AA genotypes were obtained as 0.35, 0.50 and 0.15, respectively, while the frequencies of G and A allele were 0.60 and 0.40, respectively. Second SNP [T28708860C] also revealed 3 genotypes CC, TC and TT and consequently, 2 alleles C and T at this locus. The frequencies of CC, TT and TC genotypes and C and T alleles were estimated as 0.40, 0.25, 0.35, 0.57, 0.43 respectively for genotypes and alleles. A and C alleles of PPP1R11 gene were predominant in Karan Fries crossbred bulls. Chi-square test revealed that the calculated values was less than chi square tabulated values, indicating no significant difference between observed frequencies and expected frequencies (Table 1). The gene and genotypic frequency observed in present population was found to be in Hardy Weinberg equilibrium.

Nucleotide substitution at both positions did not show any amino acid substitution and therefore results in silent mutation. The piece of information generated is very important since the silent mutated site relatively more prone to future mutation or indirectly affects the process of transcription and translation during the expression of protein (Dayal et al., 2016).

Association of SNPs genotype with bull conception rate
The SNPs wise least square means of conception rate are presented in Table 2. Analysis revealed significant (p<0.01)
effect of SNP \([T28708860C]\) genotypes on conception rate. The animal with TT genotype (53.20 ± 2.96%) were having significantly (p<0.01) higher conception rate followed by TC genotype (43.12 ± 2.39%) and CC genotypes (34.59 ± 2.28) in Karan Fries bulls. Present findings are in accordance with the earlier report of Li et al. (2012) who identified three SNPs at intronic region and one SNP at 5'UTR of PPP1R11 gene in US Holsteins bulls and reported only one SNP at 5'UTR \([T28710268G]\) were significantly associated with sire conception rate. PPP1R11 gene play an important role in spermatogenesis in mouse and reported different isoforms of gene were expressed in most of the tissues with high expression in testis, epididymis, head and tail regions of spermatozoa (Han et al., 2007). Han et al. (2008) reported that mutations in the long transcript of PPP1R11 were associated with normal sperm function. Cheng et al. (2009) reported that an isofrom of protein phosphatase 1 (PP1) has essential role in spermatogenesis, which forms a complex with PPP1R11 in the testis and expressed more in testis compared to other tissues of mice, these reports further support the idea that PPP1R11 has important functions in spermatogenesis.

Present investigation suggested that, bulls with TT genotype of PPP1R11 gene had significantly (p<0.01) higher conception rate compared to other genotypes. This SNP can be used as fertility marker for selecting breeding bulls to be incorporated into breeding programme as fertility marker for improvement of reproductive performance after validation on large numbers of samples and in different population to reach a definite conclusion.

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