Indian Journal of Animal Sciences **90** (4): 658–660, April 2020/Short communication
https://doi.org/10.56093/ijans.v90i4.104227

Genetic polymorphism in *MAP1B* gene associated with conception rate in Holstein Friesian crossbred breeding bulls

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Received: 22 April 2019; Accepted: 20 September 2019

Keywords: Conception rate, Karan Fries bulls, *MAP1B* gene, SNP, T-ARMS PCR

The current cattle and buffalo breedable female population is 118.59 million in India (BAHS 2017), however, the country is producing around 85 million semen doses (NDDB 2017), which is sufficient to cover only around 42 million breedable female population (assuming the conception rate of 50% in cattle). To bring a large proportion of breedable female population under artificial insemination (AI) coverage, India needs to produce more number of breeding bulls. However, it is observed that many bulls after attaining the age of 2.5 to 3 years are culled in progeny testing programmes due to poor fertility (Panmei *et al*. 2016). This leads to the unavailability of the required number of bulls in the progeny testing programmes. It is desirable to select breeding bull based on fertility at a very young age to economize the breeding programme at any herd. Selection based on genetic information has made it possible to select animals at an early age. Male fertility is governed by genetic disposition of animals along with environmental factors. The microtubule associated protein 1 B (MAP1B) gene is one of the candidate genes which was found to be strongly associated with sire conception rate (Li *et al*. 2012). Bovine *MAP1B* gene is located on *Bos taurus* autosome 20 (Gene ID 514739), comprising 7 exons and 6 introns and conferring a total length of 93117 bp. Recent reports on the expression of MAP1B in the male reproductive tract in both rat and human (Queiróz *et al*. 2006) and in testis of fruit fly and mouse (Bonilla and Xu 2008) suggest important functions of this gene in the regulation of male fertility. The identification of polymorphism in the *MAP1B* gene will assist in the early evaluation of bulls based on fertility.

The present study was carried out on Karan Fries (KF) cattle kept under the progeny testing program at National Dairy Research Institute (NDRI), Karnal, Haryana, India. The KF is an *inter-se* mated crossbred population developed as a milch breed by crossing Holstein Friesian (*Bos taurus*) and Tharparkar (*Bos indicus*) cattle at NDRI farm. Cows were provided with *ad lib.* seasonal green fodder and roughages, and an additional amount of 1.0 concentrate mixture (20% CP and 3400 kcal/kg DE) for every 2.5 kg milk produced above 5.0 kg daily milk yield.

Genomic DNA was isolated from the frozen semen straws of 74 KF bulls. Three mini semen straws (0.25 ml) from each bull were used for DNA isolation and the protocol used for DNA isolation involved two steps: Lysis and extraction. The lysis of spermatozoa was done as per Hossain *et al*. (1997) with some modifications while the extraction step was done using a standard Phenol Chloroform extraction. The SNP, g.3,066A>G, located on intron 1of *MAP1B gene* (NCBI Reference Sequence: AC_000177.1) was genotyped using Tetra-Primer Amplification Refractory Mutation System Polymerase Chain Reaction (T-ARMS PCR) technique. Primers were designed using Primer 1 software. The sequence of the primers used in the present study was as follows: outer forward, 5'-AACTCTCTTGGGTCCTGGGGTC-3'; outer reverse, 5'-TGCTTCACACAACTGGTCCCAT-3'; inner forward, 5'-GATGCTTCCTCAGCTCTCCGA-3'; and inner reverse, 5'-AGGAGGCCCTGCTGGCAC -3'. The expected polymerase chain reaction (PCR) fragment size for g.3,066A>G mutation were 105 bp for A allele, 178 bp for G allele, and 249 bp for the common outer fragment.

The PCR was performed in a total volume of 25 µL containing approximately 100 ng DNA, 2.5 µL of 10× buffer, 2 mM of $MgCl_2$, 0.1 mM dNTPs, 10 pM of each of outer and inner pair of primer and 1 U of Taq Polymerase (Sigma-Aldrich, USA). The amplification conditions (Touchdown reactions) were: Initial denaturation at 95°C for 2 min, 5 cycles of denaturation at 95°C for 15 sec, with annealing temperature of 60°C for the first cycle, decreasing by 1°C per cycle until annealing temperature of 56°C was reached for 15 sec and extension at 72°C for 20 sec, followed by 25 cycles of denaturation at 95°C for 15 sec, with annealing temperature of 55°C for 15 sec and extension at 72°C for 20 sec, and final extension at 72°C for 5 min. The accuracy and efficiency of the T-ARMS PCR assay were evaluated by DNA sequencing (M/s First BASE Laboratories Sdn Bhd, Seri Kembangan, Selangor, Malaysia) of outer band product of three samples for each genotype.

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Table 1. Allele and genotype frequency, and Chi-square test for Hardy-Weinberg equilibrium for the SNP locus g.3,066 A>G of the *MAP1B* gene in Karan Fries bulls

SNP	Allele	Frequency	Genotype	Frequency	Chi-square (χ^2) value	P value	Interpretation
g.3,066 A>G	A	0.37	AA	0.11 (8)	1.10	0.29^{NS}	H-W equilibrium
	G	0.63	AG	0.53 (39)			
			GG	0.36 (27)			
Total				74			

A total of 6,595 fertility records of 74 KF breeding bulls and bred on 1989 through 2015 were analyzed in the present study. The number of AI per bull were ranged between 12 and 243 with an average being 89.12. The conception rate (CR) of KF bulls were taken in term of probability of conceiving the cow through particular AI, a threshold trait approach, thereby coded as 0 and 1 for the successful AI and unsuccessful AI. Successful AI was defined as the AI that led to pregnancy in a cow.

The relationship between the CR and Polymorphic locus of MAP1B gene was analyzed by the logistic regression model by using Statistics Analysis System (SAS version 9.3) programme. The following dichotomous logistic regression model was used:

$$\ln\left[\frac{p}{1-p}\right] = \beta_0 + \sum_{j=1}^{c}\beta_j X_j$$

$$\ln (p/1-p) = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4$$

where, ln, natural logarithm; p, probability that the dependent variable (probability of conceiving the cow) equals a case; β_0, intercept from the linear regression equation (the value of the criterion when the predictor is equal to zero); $\beta_j X_j$ (j= 1 to 4), regression coefficient multiplied by some value of the predictor; X_1, Year of successful insemination (1989 to 2015); X_2, Season of successful insemination [summer (April to June), rainy (July to September), autumn (October to November) and winter (December to March)]; X_3, Age at first calving groups (≤30, 31–36 and ≥37 months) and X_4, Stage of lactation (0–90, 91–180 and 181–305 days) of cows.

The allelic and genotypic frequencies, and their accordance with Hardy-Weinberg equilibrium were studied using POPGENE software package (Yeh *et al*. 1999).

The genotype profiling of KF breeding bulls using T-ARMS PCR technique revealed three kinds of genotype, viz. AA (105 and 249 bp), AG (178, 105 and 249 bp) and GG (178 and 249 bp) with respect to the SNP locus, 3,066 A>G. Furthermore, the presence of SNP scored using T-ARMS PCR assay was confirmed by sequencing the outer band product (249 bp) of each genotype and it revealed that the genotypes scored by the assay were 100% similar to the DNA sequencing. The results of present study are in accordance with the findings of Li *et al*. (2012), who also reported 3,066 A>G SNP in *Bos taurus* crossbred populations.

The allele and genotype frequencies, and chi-square test for Hardy-Weinberg equilibrium for the SNP locus, 3,066 A>G of the *MAP1B* gene are presented in Table 1. The frequencies of A and G alleles were 0.37 and 0.63; and the frequencies of AA, AG, and GG genotypes were 0.11, 0.53 and 0.36, respectively. The results of the chi-square test revealed that the KF bull population was in Hardy-Weinberg equilibrium.

Table 2. Effect of MAP1B genotypes on conception rate of KF bulls, in terms of odds values

Effect	df	Wald Chi-Square	P value	Genotype	Odds value
g.3,066A>G	2	13.96	0.0009	AA	1.296
				AG	1.191
				GG	1.000

Identification of genotypes significantly associated with the CR will help in the evaluation of bulls at a younger age. The relationship between the CR of KF bulls and SNP locus, 3,066 A>G of *MAP1B* gene was analyzed. The CR of KF bulls with respect to different genotypes are presented in Table 2. The results revealed that SNP locus, 3,066 A>G had a highly significant (P=0.0009) influence on the CR. Bulls with AA genotype had the highest CR, followed by bulls with genotype AG and GG. Likelihood of conceiving the cow was 1.3 times higher in bulls with AA genotype than the bulls with GG genotype. The findings of present study were in the concordance of Li *et al*. (2012), who also reported a significant effect of SNP locus g.3,066 A>G of *MAP1B* gene on sire conception rate of Holstein bulls.

The findings of the present study suggest that the identified SNP can be used as a molecular marker for developing marker-assisted selection strategy. The "A" allele of SNP locus, g.3,066 A>G of *MAP1B* gene may be propagated by mating bulls with the "AA" genotype for enhancing the CR in the herd.

SUMMARY

The microtubule associated protein 1 B (*MAP1B*) gene is one of the candidate genes which was found to be strongly associated with the sire conception rate. In the present study, identification of single nucleotide polymorphisms (SNPs) in the targeted region of *MAP1B* gene and their effect on conception rate (CR) in Karan Fries (KF) breeding bulls were sought. A tetra-primer ARMS PCR technique was adapted to genotype an SNP, g. 3,066A>G located on intron 1 of *MAP1B* gene. The relationship between the CR of KF breeding bulls and polymorphic locus of *MAP1B* gene was assessed. Genotyping of the targeted region

revealed three kinds of genotype (AA, AG and GG) with respect to g. 3,066A>G SNP locus. The identified SNP had highly significant (P=0.0009) influence on CR. The KF bulls with AA and AG genotype had comparatively higher CR than the bulls with GG genotype. The identified SNP was significantly associated with CR, thus it may be utilized as a molecular marker for developing marker-assisted selection strategy to enhance the CR in KF cattle population.

ACKNOWLEDGEMENT

The authors are indebted to Director, ICAR-NDRI, Karnal, Haryana, India for providing infrastructure, facilities, and support for conducting the work.

REFERENCES

Basic Animal Husbandry Statistics. 2015. Ministry of Agriculture and Farmers Welfare, Department of Animal Husbandry, Dairying and Fisheries, New Delhi, Government of India.

Bonilla E and Xu E Y. 2008. Identification and characterization of novel mammalian spermatogenic genes conserved from fly to human. *Molecular Human Reproduction* **14**: 137–42.

Li G, Penagaricano F, Weigel K A, Zhang Y, Rosa G and Khatib H. 2012. Comparative genomics between fly, mouse, and cattle identifies genes associated with sire conception rate. *Journal of Dairy Science* **95**(10): 6122–29.

NDDB. 2017. National Dairy Development Board, Anand, Gujarat, INDIA (https://www.nddb.coop/services/animal breeding/animalreproduction/semenproduction)

Panmei A, Gupta A K, Shivahre P R, Bhakat M and Dash S K. 2016. Culling pattern of Karan Fries (crossbred) males in an organised herd. *Indian Journal of Animal Research* **50**(6): 1005–08.

Queiroz D B, Silva A M, Gutierrez-Ospina G, Porto C S, Grossman G, Petrusz P and Avellar M C. 2006. Cells positive for microtubule associated protein 1B (MAP 1B) are present along rat and human efferent ductules and epididymis. *Cell Tissue Research* **325**: 125–33.

SAS Institute 2012. SAS statistics, Cary, NC. Version 9.3.

Yeh F C, Yang R C and Boyle T. 1999. POPGENE VERSION 1.31: Microsoft Window-based free Software for Population Genetic Analysis, 1999. https: //sites.ualberta.ca/~fyeh/ popgene_download.html.