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Breed-wise comparison of semen quality parameters for bulls having polymorphism for TNP 2 gene

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Abstract

Present study was conducted for breed-wise comparison of semen quality traits for bulls of Sahiwal, Karan Fries, and Tharparkar breed having different genotypes for g.480 C>T locus of TNP2 gene. Semen quality parameters, namely semen volume, mass motility and post-thaw sperm motility percentage (PTM) record of 593 ejaculates of 18 Sahiwal bulls, 554 ejaculates of 14 Karan Fries (KF) bulls, and 305 ejaculates of 9 Tharparkar bulls having different genotype were used for study. Among Karan Fries (KF) and Sahiwal bulls having the CC genotype bulls (n = 5) showed the highest mean semen volume (4.00 ± 0.63 mL, 4.07 ± 0.41 mL). The mean mass motility differed among bulls with different genotypes within each breed. The highest average mass motility (3.50 ± 0.29) was observed in Sahiwal bulls carrying the CT genotype, whereas the lowest (1.80 ± 0.58) was recorded in Karan Fries bulls having the CC genotype. The highest post-thaw motility was recorded for bulls carrying the CT genotype in both Karan Fries and Sahiwal breeds (55-60), whereas the lowest mean value was observed in Tharparkar bulls with the CC genotype (45-50). Numerical differences in semen volume, mass motility, and post-thaw motility were observed among bulls of studied breed but statistical analysis revealed that these differences were not significant.

Keywords: Semen parameter, breed, genotype, TNP2 gene

Introduction

Transition Nuclear Proteins plays a major role in sperm DNA condensation and in turn have impact on sperm motility which is an important semen quality parameter which affects the conception rate (Shirley *et al.*, 2004) [7]. TNP2 involved in chromatin condensation (Baskaran and Rao, 1990) [1]. In *Bos taurus*, the TNP2 gene is localized at protamine gene cluster on chromosome 25q12-q13 (Acc.No-BK_006514), highlighting its close association with genes involved in sperm chromatin remodeling (Reinhart *et al.*, 1991) [10]. The expression of TNP2 for both the mRNA and protein levels varies across species. Studies in mice have demonstrated that inactivation of the TNP2 gene disrupts the he PRM2 transduction, resulted into a subfertile phenotype characterized by impaired sperm chromatin condensation, reduced nuclear compaction and increased DNA fragmentation (Engel *et al.*, 1992; Yu *et al.*, 2000) [4, 12].

Gao *et al.* (2014) [5] reported three SNPs namely, g.269 G>A (exon 1), g.480 C>T (intron 1), and g.1536 C>T (3'-UTR) in the TNP2 gene of Chinese Holstein bulls (Gao *et al.*, 2014) [5].

Gao *et al.* (2014) [5] reported that bulls carrying the TT genotype at locus g.480C>T exhibited lower sperm deformity rates than the animal with the CC and CT genotypes. In contrast, for g.1536C>T locus, animals with the CT genotype showed greater ejaculate volume and higher post-thaw sperm motility compared with bulls carrying the CC genotype.

Panigrahi and Yadav (2009) [9] identified two single nucleotide polymorphisms (SNPs) within the intronic region of the TNP2 gene in Murrah buffalo bulls but their effects on mass activity, individual motility were non-significant.

For g.480 C>T locus different genotypes were observed in Sahiwal, Tharparkar and Karan Fries cattle. Therefore, the present study was carried out with the objective for breed-wise comparison of semen quality parameters for bulls having polymorphism for TNP 2 gene.

Materials and Methods

For the study, bulls of Indian cattle breeds having different genotypes for g.480 C>T locus of TNP2 gene, semen quality parameters record of each male animal were obtained from the Animal Breeding Research Centre (ABRC), ICAR–National Dairy Research Institute (NDRI), Karnal. The recorded information included breed, individual animal identification number, date of birth, and date of semen collection. In addition, semen quality parameters, namely semen volume, mass motility and post-thaw sperm motility percentage (PTM), were documented for each semen sample. These phenotypic records of semen, along with the bulls of corresponding genotype, were used to study the animal for semen parameters, as well as post-thaw semen quality parameters in the sampled population. Statistical analyses were performed using semen quality records obtained from 593 ejaculates of 18 Sahiwal bulls, 554 ejaculates of 14 Karan Fries (KF) bulls, and 305 ejaculates of 9 Tharparkar bulls. The compiled data was subsequently used to examine the relationship between bulls of different breeds having genetic polymorphism and the semen quality parameters in the

present study.

The effect of bulls having SNP variants on semen quality traits was analysed using the following models:

$$Y_{ij} = \mu + G_i + e_{ij}$$

Where,

Y_{ik} = Observed value of studied semen quality parameter of j^{th} animal belonging to i^{th} genotype

μ = Overall mean

G_i = Effect of bulls having i^{th} genotype on semen quality parameters

e_{ij} = Random error associated with Y_{ij} observation and assumed to be NID ($0, \sigma^2_e$)

Statistical analyses were performed using Microsoft Excel software running on the Windows 10 operating system.

Results and Discussion

The mean semen volume for animal having different genotypes within each breed is presented in Table 1.

Table 1: Mean semen quality traits of bulls having different TNP2 (g.480 C>T) genotype in Sahiwal, Karan Fries and Tharparkar breed

S. No.	Breed	Genotype	Number of animals	Volume (ml)		MM		PTM (%)
				Mean	SD	Mean	SD	Range
1	Karan Fries	CT	7	3.57	1.40	3.00	0.58	45-50
		CC	5	4.00	1.41	1.80	1.30	50-55
		TT	2	2.25	0.35	3.25	0.35	45-50
2	Tharparkar	CT	3	5.83	2.75	3.00	1.73	55-60
		CC	6	4.00	1.14	3.20	1.04	50-55
3	Sahiwal	CT	3	3.33	0.58	3.50	0.50	55-60
		CC	15	4.07	1.60	3.13	0.64	50-55

Among Karan Fries (KF) bulls, the CC genotype bulls ($n = 5$) showed the highest mean semen volume (4.00 ± 0.63 mL), followed by bulls having the CT genotype ($n = 7$) with a mean of 3.57 ± 0.53 mL. Bulls possessing the TT genotype ($n = 2$) had the lowest mean semen volume (2.25 ± 0.25 mL). For Sahiwal and Tharparkar bulls have two types (CT and CC) of genotype in the present sampled population. Sahiwal bulls, having CC genotype ($n = 15$), have a higher mean volume (4.07 ± 0.41 mL) than those with bulls with CT genotype ($n = 3$), which had a mean semen volume of 3.33 ± 0.33 mL, while, for Tharparkar bulls having CT genotype ($n = 3$) shows a higher mean semen volume (5.83 ± 1.59 mL) than the bulls having CC genotype ($n = 6$), which had a mean semen volume of 4.00 ± 0.47 mL.

Gao *et al.* (2014) [5] reported that the Chinese Holstein bulls having TT genotype at the g.480 C>T locus exhibited higher ejaculate volume (6.78 ± 0.73 mL). In contrast, the present study observed lower ejaculate volume for bulls having the TT genotype and was non-significant.

The mean mass motility (MM) of semen for bulls belonging to different breeds and for bulls having different genotypes are presented in Table 1. In Karan Fries (KF) bulls, the mean mass motility varied across the bulls of three genotypes. Bulls with the TT genotype ($n = 2$) exhibited the highest mean mass motility (3.25 ± 0.25), followed by the bulls having CT genotype (3.00 ± 0.22 ; $n = 7$), whereas the bulls with CC genotype ($n = 5$) recorded the lowest mean value (1.80 ± 0.58). For Tharparkar (TP) bulls, the mean mass motility was 3.20 ± 0.46 for bulls carrying the CC genotype ($n = 6$) and 3.00 ± 1.00 for those bulls with the CT genotype ($n = 3$). Although the mean values for the mass motility for Tharparkar bulls were similar, greater variability was

observed among bulls with CT genotype, as indicated by its higher standard deviation (± 1.73). Among Sahiwal bulls with the CT genotype ($n = 3$) showed a slightly higher mean mass motility (3.50 ± 0.29) than the bull having CC genotype (3.13 ± 0.17 ; $n = 15$).

Overall, the mean mass motility differed among bulls with different genotypes within each breed. The highest average mass motility was observed in Sahiwal bulls carrying the CT genotype, whereas the lowest was recorded in Karan Fries bulls having the CC genotype. These differences suggest that the effect of bull on sperm motility may be breed-specific and influenced by the genetic background of individual breeds (Coleman *et al.*, 1993; Nikitkina *et al.*, 2022; Zhang *et al.*, 2015) [2, 8, 13]. However, the small number of bulls with different genotype groups required validation with larger populations for selection bull on semen parameters.

Gao *et al.* (2014) [5] reported that the Chinese Holstein bulls having TT genotype at the g.480 C>T locus exhibited higher initial sperm motility ($74.13 \pm 2.88\%$). The present result is in agreement with for KF bulls having TT genotypes for which has non-significant difference was observed for mass motility. The post-thaw motility (PTM) of semen for bulls of different breeds grouped according to genotype are presented in table 1. In Karan Fries (KF) bulls, the highest mean post-thaw motility was observed in animals with the CT genotype (55-60; $n = 7$), followed by bulls having CC genotype (50-55; $n = 5$). Bulls having TT genotype ($n = 2$) recorded the lowest mean post-thaw motility (45-50). In Tharparkar (TP) bulls, animals with the CT genotype ($n = 3$) exhibited a higher mean post-thaw motility (50-55) than those bulls with the CC genotype (45-50; $n = 6$). Among Sahiwal bulls, the mean post-thaw motility was slightly higher in animals carrying the

CT genotype (55-60; n = 3) than in those with the CC genotype (50-55; n = 15). Overall, differences in mean post-thaw motility were observed among genotypes within each breed. The highest range post-thaw motility was recorded for bulls carrying the CT genotype in both Karan Fries and Sahiwal breeds (55-60), whereas the lowest mean value was observed in Tharparkar bulls with the CC genotype (45-50). Gao *et al.* (2014) [5] reported that the Chinese Holstein bulls having TT genotype at the g.480 C>T locus exhibited higher post-thaw sperm motility ($40.66 \pm 2.53\%$), compared with bulls possessing the CC and CT genotypes. In contrast, the present study non-significantly observed lower post-thaw motility in bulls with the TT genotype than the bulls having CT genotype. These contrasting results may reflect differences in breed, genetic background, environmental conditions, management practices, or sample size. These observations imply that the effect on semen parameters may be breed-dependent and influenced by the underlying genetic background (Dementieva, *et al.*, 2024; Zhang *et al.*, 2015) [3, 13]. However, owing to the limited number of bulls in certain genotype groups, further studies with larger populations are required to validate this effect on semen parameters before considering for selection of bulls with superior semen freezability and fertility.

Conclusion

Although numerical differences in semen volume, mass motility, and post-thaw motility were observed among bulls of Sahiwal, Karan Fries, and Tharparkar bulls but statistical analysis revealed that these differences were not significant. Thus, no significant association was detected between the semen quality traits evaluated and the bulls of different breeds having different genotype for TNP2 (g.480C>T) polymorphism in the present study.

Ethical Approval

The study protocol involving animals was approved by the Institutional Animal Ethics Committee (IAEC) of ICAR-National Dairy Research Institute, Karnal, India

Conflict of Interest

The authors declare that there are no conflicts of interest with respect to the research, authorship and publication of this article

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