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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(11): 1923-1927

www.thepharmajournal.com
Received: 16-11-2023
Accepted: 27-11-2023

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DMXL1 could be a potential candidate gene for semen quality in Holstein Friesian bulls

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DOI: https://doi.org/10.22271/tpi.2023.v12.i11Sv.24251

Abstract
The herd's overall financial success is closely tied to the reproductive efficiency of the breeding bulls. Reduced reproductive efficiency in dairy cattle often arises due to the subfertility or infertility of the breeding bulls. In this study, our main focus is on identifying candidate genes containing SNPs within the QTL region, with the goal of using them as markers to predict semen quality. Our study reports the relationship between DMXL1 gene and sperm motility in cattle bulls. Diverse studies emphasized the significance of DMXL1 in various cellular processes and pathways, encompassing cell cycle regulation, cell proliferation, and signal transduction. Further investigation into the DMXL1 gene could offer valuable insights and potentially position it as a candidate gene for semen quality in breeding bulls.

Keywords: Fertility, DMXL1, breeding bulls, semen quality, QTL

Introduction
The primary cause of reproductive challenges in dairy cattle often stems from the subfertility or infertility of the breeding bulls (Fair & Lonergan, 2018, Taylor *et al.*, 2018) [5, 24]. The overall profitability of a herd is directly linked to the reproductive efficiency or inefficiency of the bulls (Krpalkova *et al.*, 2016) [9]. Thus, it is essential to assess semen quality before performing Artificial Insemination (AI) to prevent reproductive failures, especially considering that semen from one bull is used for inseminating multiple cows. However, numerous bulls that appear phenotypically healthy end up producing unsatisfactory ejaculates (Kumaresan *et al.*, 2021) [20], resulting in the rejection of the ejaculates for AI. These issues could potentially arise from molecular defects within the DNA, RNA, and proteins of the spermatozoa (Prakash *et al.*, 2021; Talluri *et al.*, 2022; Elango *et al.*, 2023; Kumaresan *et al.*, 2023) [20, 23, 4, 11]. The existing method for selecting bulls does not provide a comprehensive evaluation of semen quality since it primarily concentrates on physical characteristics and neglects genetic traits. Thus, through a deeper comprehension of cattle genetics, we can acquire a more profound understanding of semen quality and also facilitate the identification of genetic markers for improved bull selection.
The utilization of genome-wide association studies (GWAS) has been successful in employing abundant genetic markers, specifically single-nucleotide polymorphism (SNP) markers (Höglund *et al.*, 2019) [6]. Utilizing single-nucleotide polymorphism (SNP) markers is considered one of the dependable methods for identifying genetic markers (Liu, 2007). Consequently, examining the variations resulting from SNPs can provide a distinct perspective for pinpointing potential biomarkers. The identification of SNP markers allows for the creation of a comprehensive genetic map and the recognition of quantitative trait loci (QTLs), (Mora *et al.*, 2016) [16]. The QTL associations for cattle traits can be located in the animal QTL database. There are several techniques available for SNP identification within species, with RNA sequencing-next-generation sequencing (NGS) being the most commonly employed method. NGS produces substantial volumes of sequencing data, facilitating the identification of SNPs within a species (Podnar *et al.*, 2014; Sinha *et al.*, 2022) [19, 23]. Additionally, it allows for the determination of the SNP's location within the coding region associated with the gene trait. In this research, our primary emphasis is on the identification of potential genes with SNPs within the QTL region, with the aim of utilizing them as markers for predicting semen quality.

2. Materials and Methods

2.1 Ethical approval statement

The current study was carried out at the Theriogenology laboratory, Southern Regional Station of ICAR-National Dairy Research Institute, Bengaluru, Karnataka. All the experiments and procedures adopted in this study were approved by the Institute Animal Ethical Committee (CPCSEA/IAEC/LA/SRS-ICAR-NDRI-2019/No.04).

2.2 Sample Collection and QTL mapping

For this investigation, we used 5 good quality semen producing Holstein Friesian (HF) breeding bulls and 5 poor quality semen producing bulls. RNA extraction and cDNA synthesis was done by method described by Parthipan *et al.* (2015) [18] with sligh adapations. Methods described by H. Li *et al.*, 2009 [12], Danecek *et al.*, 2011 [2], Cingolani *et al.*, 2012 [1] and Nayeri & Stothard, 2016 [17] were used for Library preparation and SNP identification to map with the Cattle QTL region (https://www.animalgenome.org/). The semen quality trait comprises a total of 15 different trait attributes. The identified SNPs were mapped to the cattle QTL database to identify genes with SNPs.

2.3 DEG and Karyoplot

We performed an enrichment analysis using the ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/) tool. To visualize the data, we generated a Karyoplot using Plotly (https://plotly.com/). In the plot, the genes are denoted by red dots, and the purple lines signify regions where these genes exhibit statistical enrichment compared to the background gene density. We employed a sliding window approach to analyse the genome, where each window is subdivided into equal-sized segments for continuous scanning. In each window, we applied the hypergeometric test to assess whether there is a significant overrepresentation of specific genes. Essentially, the genes within each window form a gene set or pathway, and we conducted an enrichment analysis. It's worth noting that only a portion of the chromosomes may be visible, with the line drawn using the location of the last gene. You can hover over to reveal gene symbols and zoom in on regions of interest for a closer examination.

3. Results

Data obtained from both the good and poor-quality semen samples underwent variant analysis to be correlated with the Cattle QTL database. These SNPs were cross-referenced with the Cattle QTL database for the semen quality trait to pinpoint SNPs located within the QTL region. The distinct SNPs identified in the good and poor-quality semen samples were subsequently mapped with the QTL database to pinpoint genes containing SNPs associated with the semen quality trait.

The poor-quality sample, mapped to the Cattle QTL database for the semen quality trait revealed variations in the genes DMXL1. DMXL1 had a SIFT deleterious score of 0 indicating a high likelihood of protein alteration.

Differential gene expression (DEG) analysis was conducted for the genes identified with unique SNPs in both good and poor-quality semen samples that had been mapped to the QTL database. Figure 1 illustrates the heatmap depicting the comparison between good and poor-quality semen samples associated with the differentially expressed genes (DEG).

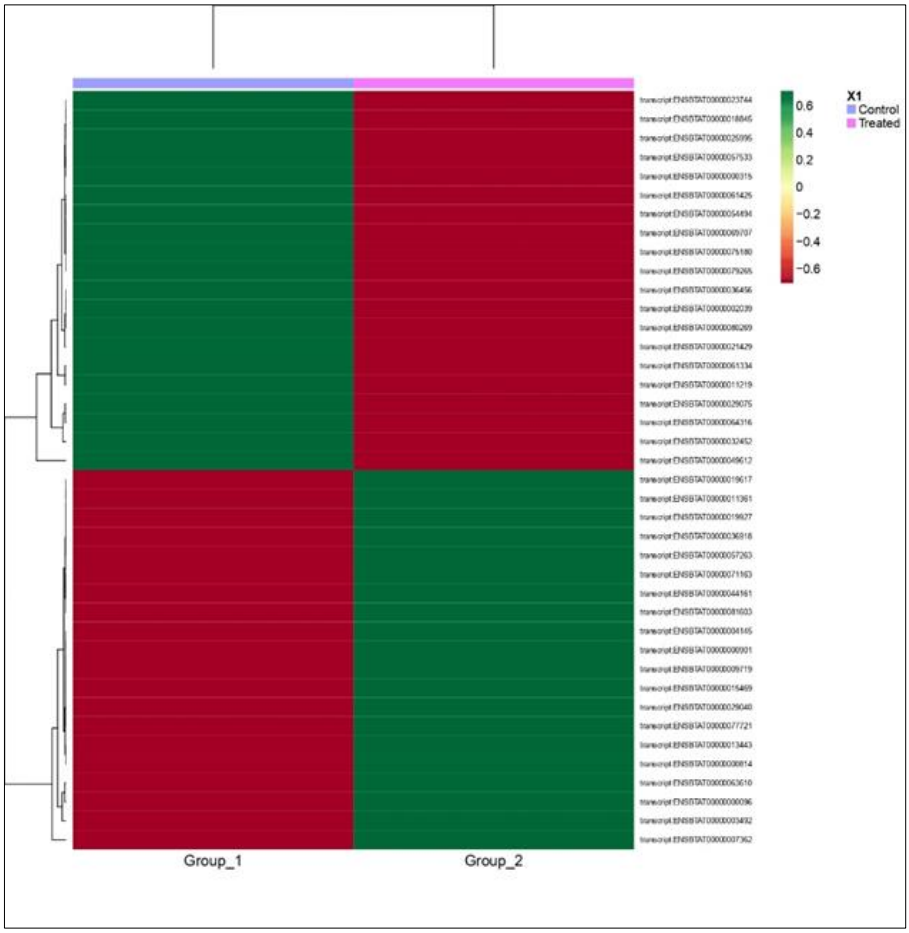

Fig 1: Heat map between top 20 genes of good and poor quality semen sample (Group 1 represents good and group 2 represents poor quality samples)

A Karyoplot was generated to visualize the chromosomal distribution of the identified DEGs and their associated SNPs in the good and poor-quality samples. This allowed for the representation of SNPs within the genes on a chromosome-by-chromosome basis. Figure 2 and Figure 3 display karyoplots for the good and poor-quality semen samples, respectively. The Karyoplot for the poor-quality semen sample revealed that the DMXL1 gene displayed both differential expression and a missense mutation. This dual observation suggests a strong potential for DMXL1 to serve as a marker for semen quality.

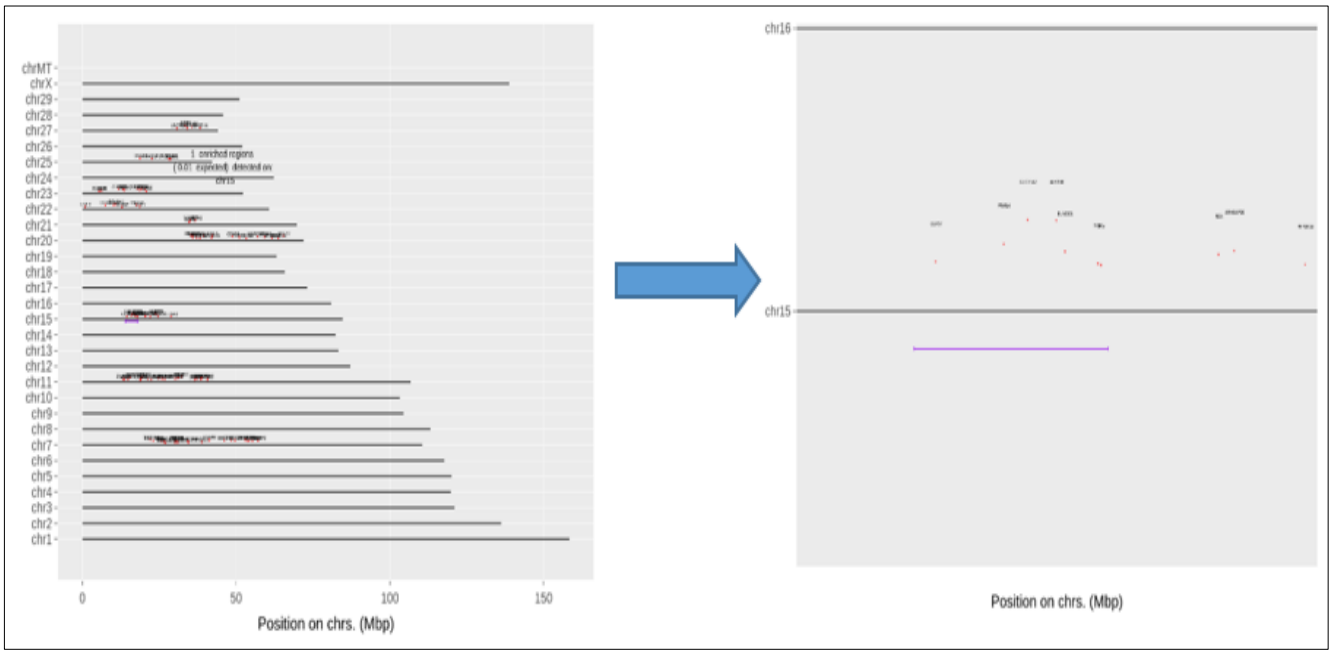

Fig 2: Karyoplot of DEG genes mapped to QTL region in good quality samples (figure on the right side show the magnified region of DEG)

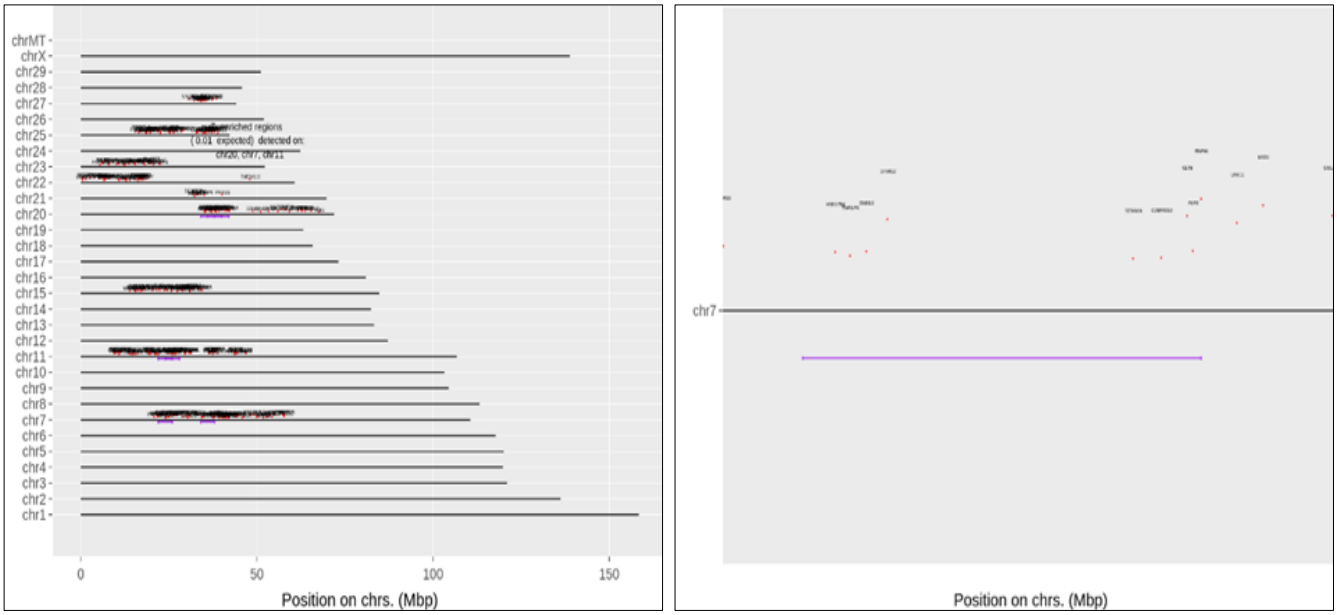

Fig 3: Karyoplot of DEG genes mapped to QTL region in poor quality samples (figure on the right side show the magnified region of DEG)

4. Discussion

In our previously published research (Ebenezer Samuel King *et al.*, 2022) [22], we identified a notable missense mutation in the DMXL1 gene. Consequently, we aimed to investigate whether the DMXL1 gene exhibited any variations in expression associated with semen quality differences between good and poor semen samples. Hence, we embarked on this investigation due to the established association of DMXL1 with sperm motility, a connection previously documented in our prior study. This particular single nucleotide polymorphism (SNP) within the DMXL1 gene was exclusively detected in semen samples of poor quality and was absent in samples of good quality. Consequently, this SNP appears to be a promising candidate for serving as a reliable biomarker to assess semen quality.

V-ATPases, also known as H+ ATPases, are complex, ATP-dependent proton pumps that play a key role in maintaining pH balance in nearly all eukaryotic organisms. Additionally, they have vital functions in male fertility. When proteins that interact with V-ATPases, such as DMXL1, are knockdown, it results in the inhibition of intracellular vesicle acidification mediated by V-ATPases (Merkulova, M *et al.*, 2015) [14]. In

Iranian Holstein heifers, it was observed that DMXL1 exhibited a single nucleotide polymorphism (SNP) associated with reproductive traits. The SNPs found within the DMXL1 gene were linked to the process of proteolysis in cellular protein catabolism, specifically in relation to gestation length, suggesting its influence on the animal's fertility (Mohammadi *et al.*, 2022) [15].

The rabconnectin-3 complex plays a role in the regulation of Notch signaling. The Notch signaling pathway is a crucial mechanism in cell-cell communication. The pathway regulates cell proliferation, differentiation, and apoptosis. Both DMXL1 and DMXL2 isoforms are essential for the proper function of rabconnectin-3, as any alterations in these isoforms can impact cell-cell communication through changes in the assembly of V-ATPase (Jaskolka *et al.*, 2021) [7]. Research conducted in human subjects revealed the absence of the DMXL1 gene in individuals with azoospermia, attributed to an autosomal deletion on the chromosome (Shi *et al.*, 2023) [21]. WD or beta-transducin repeat proteins play a multifaceted role in various cellular processes. It is involved in signal transduction, ensuring the accurate processing of RNA, facilitating vesicular trafficking, contributing to the assembly of the cytoskeleton, regulating the cell cycle, and participating in the formation of the transcription initiation complex, among many other essential functions. Research conducted in Drosophila melanogaster has revealed that the DMXL1 gene exhibits an unusually high number of WD repeat units. These WD repeat units are structural motifs within the gene that are repeated extensively, and their abundance in DMXL1 is noteworthy in this particular species (Kraemer *et al.*, 2000) [8]. Therefore, a modification in the DMXL1 gene, characterized by its abundant WD repeat units, has the potential to influence the previously mentioned functions within spermatozoa.

5. Conclusion

Our research reveals an association between DMXL1 and sperm motility, a significant factor in fertility. Similarly, an earlier study on Iranian Holstein heifers also indicated a connection between the DMXL1 gene and fertility. Collectively, these studies underscore the importance of DMXL1 in various cellular processes and pathways, including cell cycle regulation, cell proliferation, and signal transduction. It's conceivable that these functional changes could also occur in poor-quality sperm, potentially affecting motility. Further investigation of the DMXL1 gene could provide valuable insights and potentially establish it as a promising candidate gene for assessing semen quality.

6. Acknowledgments

Authors thank Director of ICAR – National Dairy Research Institute for funding and providing facilities for conducting this study

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