International Journal of Veterinary Sciences and Animal Husbandry 2024; 9(2): 695-698

ISSN: 2456-2912
VET 2024; 9(2): 695-698

www.veterinarypaper.com
Received: 08-01-2024
Accepted: 13-02-2024

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Seminal characteristics and oxidative stress in high-fertility versus low-fertility Mehsana buffalo bulls

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DOI: https://doi.org/10.22271/veterinary.2024.v9.i2j.1312

Abstract
The aim of the present study was to assess and compare semen parameters in Mehsana buffalo bulls classified as high-fertile and low-fertile based on their conception rates (CR). Twelve bulls were selected and divided into two groups (high-fertile with CR > 43% and low-fertile with CR < 35%). Frozen semen samples (n=72) were randomly chosen for analysis. Results showed that high-fertile bulls exhibited significantly (p<0.05) higher live sperm counts compared to low-fertile ones. Additionally, low-fertile bulls had significantly (p<0.05) higher rates of head abnormalities and total abnormalities. However, differences in mid-piece abnormalities, percent abnormal tail and tail less head were not statistically significant between the two groups. There were no significant variations in percent HOS reactive sperm and lipid peroxidation (MDA) between high-fertile and low-fertile groups. The study suggests a correlation between head morphology, sperm viability and fertility in Mehsana buffalo bulls.

Keywords: Conception rate, high-fertile bull, host, lipid peroxidation, live sperm, low-fertile bull

1. Introduction
Livestock plays a crucial role in India's agricultural industry, with a substantial presence in the country's economy. The country has the largest population of bovines including 192.50 million cattle and 109.9 million buffaloes (Anonymous, 2019) [4]. Despite the relatively low milk production per animal, India leads the world in milk output, contributing 20% of the global production (Anonymous, 2018) [3]. The dairy industry in India relies mainly on both cows and buffaloes, with domestic buffaloes making a significant contribution. According to the annual report (2019-20) of the Central Institute for Research on Buffaloes, domestic buffaloes alone contribute 91.82 million tonnes of milk, emphasizing their vital role in the agricultural economy.

To enhance the future productivity of buffalo herds, it's essential to employ high-quality genetic bulls for Artificial Insemination (AI). AI facilitates the widespread distribution of superior genetic material from a small number of genetically superior males to a large number of females in the population (Vishwanath and Shannon, 2000) [37]. Additionally, challenges such as infertility in buffaloes and the need for multiple insemination attempts contribute to frustration and financial losses for dairy farmers (Stevenson *et al.*, 1990) [34]. Buffalo spermatozoa exhibit lower cryotolerance compared to those of cattle and are more prone to hazards during cryopreservation (Andrabi, 2009) [2], which may contribute to suboptimal conception rates in buffalo herds.

The primary aim of sperm evaluation is to investigate the correlation between semen quality and fertility, with the goal of assessing the fertility potential of donors. Cryopreservation protocols involve several steps that can potentially harm sperm cells, diminishing their capacity to fertilize ova. Thus, it's essential to monitor the quality of cryopreserved semen to anticipate its fertility potential. Moreover, successful reproduction relies on various semen parameters. Therefore, establishing a fertility prediction system based on evaluating multiple semen quality parameters post-freezing and thawing could help exclude bulls with low fertility potential from breeding programs, saving significant time as well as resources.

Throughout cryopreservation, semen undergoes cold shock and exposure to atmospheric oxygen, increasing susceptibility to lipid peroxidation (LPO) due to increased reactive oxygen species (ROS) production (Nair *et al.*, 2006) [27]. This process generates malondialdehyde (MDA), which damages the structure and function of plasma membranes of bull spermatozoa. Freezing and thawing also induce ROS production, altering sperm membrane and antioxidant defence systems of sperm (Bilodeau *et al.*, 2000; Gadea *et al.*, 2013) [6, 15], ultimately resulting in decreased viability and fertility (Bailey *et al.*, 2000) [5].

The ability of spermatozoa to fertilize is closely linked to a range of sperm traits, assessed through diverse laboratory techniques. Numerous studies have indicated that factors such as viability and membrane integrity (Christensen *et al.*, 2005; Christensen *et al.*, 2011) [10, 11], morphology (Nagy *et al.*, 2013) [26], and lipid peroxidation status (Singh *et al.*, 2016) [33] are associated with bull fertility.

Considering the aforementioned facts, the objective of this study was to assess the correlations between fertility and semen parameters in Mehsana buffalo bulls with varying fertility levels.

2. Material and Methods

Twelve Mehsana buffalo bulls from Banas Dairy's Dama semen production unit were selected based on their conception rate (CR), evaluated through the field progeny testing program led by Banaskantha District Co-Operative Milk Producers' Union Ltd, Palanpur. These bulls were categorized into two groups: high-fertile (CR > 43%,) and low-fertile (CR < 35%), with equal representation in each group.

To compare the semen characteristics between high and low fertile bulls, frozen semen samples (n=72) with six different batches form each bull were included for further semen assessment. To ascertain the percentage of live spermatozoa and location based sperm morphology, a smear was prepared on a pre-warmed clean glass slide by gentle mixing of a drop of frozen-thawed semen and a drop of pre warmed (37 °C) eosin-nigrosin stain. After drying, the smear was examined under the microscope. The hypo-osmotic swelling test (HOST) was performed to assess the plasma membrane integrity of the sperm based on curled and swollen tails as described by Jeyendran *et al.* (1984) [19]. Membrane peroxidative damage in seminal plasma was determined in terms of malondialdehyde (MDA) using the method of Buege and Aust (1978) [7] with a few modifications.

The percentages were transformed into arcsine prior to analysis. Differences among groups in LPO, live sperm, HOS-reacted spermatozoa and sperm abnormality were assessed using a one-way independent t-test, with results presented as mean ± S.E. Significance was determined at the 95% level. Data analysis was conducted using SPSS 20 software.

3. Results and Discussion

In the present study, the percent live sperms were significantly (p<0.05) higher in high fertile (62.21±0.04) bulls as compared to low fertile (54.02±0.03) Mehsana buffalo bulls. In accordance with the present findings, Selvaraju *et al.* (2008) [32], Dogan *et al.* (2012) [14], Gliozzi *et al.* (2017) [18], Kumaresan *et al.* (2017) [21] and Narud *et al.* (2020) [28] observed significantly higher live sperm percentage in high fertile bull as compared to low fertile bull. Similarly, Gamal *et al.* (2010) [16] found significantly higher live sperm percentage in bull with higher conception rate as compared to lower conception rate. While, Mahmoud *et al.* (2013) [24] observed significantly higher live sperm percentage in highest fertile bull as compared to lowest fertile bull. Whereas, Kumar *et al.* (2016) [20] and Turri *et al.* (2021) [36] found non-significantly higher live sperm percentage in high fertile bull as compared to low fertile bull.

The overall mean value of percent live sperm (58.19) reported in present study was in accordance with Lone *et al.* (2018) [22]. The mean value of percent live sperm recorded in their experiment was 56.89%. However, Maurya and Tuli (2003) [25], Mahmoud *et al.* (2016) [23] and Ahmed *et al.* (2018) [1] found higher percent of live sperm (70.23, 65.3 and 64.41, respectively). While, Chaudhary *et al.* (2018) [9] observed lower percentage of live sperm (52.33) in Surti buffalo bull as compared to present study.

There was non-significant difference in percent HOS reactive sperm between high fertile (63.33±0.01) and low fertile (62.05±0.01) group. In agreement with the present findings, Oliveira *et al.* (2012) [29] found non-significant difference in the plasma membrane integrity between high conception rate bull and low conception rate bull. Further, similar to present findings they also found numerically lower HOST reacted sperm in bull with numerically lower conception rate. In contrary with the present findings, Correa *et al.* (1997) [12] reported significantly higher mean value of HOST reacted sperm in high fertile group as compared to low fertile group.

The overall mean value of HOST reacted sperm 62.69% reported in present study was almost at par with Chaudhari *et al.* (2007) [8] in 60% post thaw motility (65.11%) group and higher than in 50% post-thaw motility (55.81%) group in Mehsana buffalo bull. However, Dalal *et al.* (2019) [13] found lower percent of HOST reactive sperm in their experiment.

The mean percentage of head abnormalities and total abnormalities were significantly (p<0.05) higher in low fertile bulls (4.14±0.01 and 13.65±0.02, respectively) as compared to high fertile bulls (2.08±0.02 and 9.97±0.02, respectively). While, mid-piece abnormalities (2.41±0.01, 3.49±0.02), percent abnormal tail (3.00±0.02, 3.48±0.02) and tail less head (0.80±0.02, 0.93±0.01) differed non-significantly between high and low fertile buffalo bulls, respectively. The overall mean percentage of abnormal head, abnormal mid-piece, abnormal tail, tail less head and total abnormality were 3.02±0.01, 2.93±0.01, 3.24±0.01, 0.85±0.01 and 11.75±0.01, respectively in present study.

In accordance with the present findings, Garcia-Macias *et al.* (2007) [17] also found significantly higher sperm head abnormality and total abnormality in low fertile bull as compared to high fertile bull. Further, they also found non-significant difference in percentage tail abnormality between high fertile and low fertile bulls. Oliveira *et al.* (2012) [29] also found significantly higher major abnormality and total abnormality in low conception rate bull as compared to high conception rate bull which was in accordance with the present study. However, Mahmoud *et al.* (2013) [24] found non-significantly higher overall mean percentage of sperm abnormality in lowest fertile bull as compared to highest fertile bull.

The overall mean value of percent head abnormality (3.02 and 3.24, respectively) observed in present study was lower than the values reported by Patel *et al.* (2012) [30] in Mehsana (4.27 and 4.93, respectively) and Jafarabadi (4.27 and 4.70, respectively) buffalo bull. While, the overall percent total abnormality was slightly higher than the observation of Patel *et al.* (2012) [30] in Mehsana (10.83%) and Jafarabadi

(10.67%) buffalo bull. However, Swami *et al.* (2017) [35] observed higher value of sperm abnormality (13.55%) in their experiment as compared to present research work.

The mean values of Lipid Peroxidation (MDA) was non-significantly differed between high fertile (0.37±0.02) and low fertile (0.31±0.03) groups. In contrary with the present study, Singh *et al.* (2016) [33] and Saraf *et al.* (2021) [31] found significantly higher LPO in low fertile bull as compared to high fertile bull.

4. Conclusion

The findings of present study demonstrated a correlation between head abnormalities and sperm viability with fertility in Mehsana buffalo bulls, with significantly higher head abnormalities and lower live sperm percentages observed in low-fertility bulls compared to their high-fertility counterparts. However, there was no significant difference in sperm plasma membrane integrity as well as lipid peroxidation between high and low fertile Mehsana buffalo bulls.

5. Acknowledgements

Authors are thankful to the Principal, College of Veterinary Science and AH, Kamdhenu University, Sardarkrushinagar; management, Banaskantha District Co-Operative Milk Producers' Union Ltd, Palanpur and staff members of Dama Semen Production Unit, Banas Dairy for providing necessary help and facilities.

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