RESEARCH ARTICLE

Effects of Vaccination on Sperm Attributes and Sperm DNA Fragmentation in Post-Thaw Bovine Semen

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Abstract

This study was conducted to evaluate the effects of HS-BQ and FMD vaccination on semen quality and sperm DNA fragmentation in Holstein Friesian bulls. Sperm motility was assessed using a sperm meter, while sperm vitality was evaluated by eosin-nigrosine staining. Morphological abnormalities were examined using a sperm morphology kit. Functional membrane integrity was determined through the hypoosmotic swelling test (HOST), and acrosome integrity was assessed using Giemsa staining. Sperm DNA integrity was evaluated by the sperm chromatin dispersion (SCD) test. Vaccination significantly affected most sperm quality parameters. HS-BQ vaccination exerted a greater adverse effect on sperm motility and HOST values, as the time required for these parameters to return to acceptable levels was longer than that observed following FMD vaccination. In contrast, FMD vaccination had a more pronounced negative effect on sperm morphology. However, both vaccines had a similar impact on sperm vitality. Likewise, the percentage of intact acrosomes did not decline below the acceptable threshold of 65% after either vaccination. The sperm DNA fragmentation index (DFI) remained within acceptable limits and did not exceed 8-10% following either HS-BQ or FMD vaccination. It was concluded that, in breeding bulls, a sexual rest of at least 3 weeks following HS-BQ vaccination and more than 4 weeks following FMD vaccination is recommended to allow seminal parameters to return to acceptable levels.

Key words: DNA fragmentation index, FMD, HS-BQ, Semen Quality, Vaccination.

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Introduction

India is the world's largest producer of milk. To further enhance milk production, artificial insemination (AI) has emerged as one of the most practical and cost-effective biotechnological tools, enabling the dissemination of superior genetics to farmers. The number of AI procedures performed in the country increased from 77.17 million in 2021-22 to 80.57 million in 2022-23 (BAHS, 2023). Haemorrhagic Septicaemia (HS), Black Quarter (BQ) and Foot-and-mouth disease (FMD) are among the most economically significant infectious diseases affecting dairy animals. Vaccination remains the primary preventive measure for controlling these diseases and reducing associated economic losses. Although vaccination is indispensable for disease prevention, the immune response induced following vaccination may result in transient physiological stress and pyrexia, which can adversely affect reproductive performance and semen quality in breeding males (Fernandez-Novo *et al.,* 2020; Gupta *et al.,* 2018). Post-vaccination elevations in body and testicular temperature may disrupt spermatogenesis, leading to temporary deterioration in semen quality, sperm morphology, and fertility-related parameters (Rahman *et al.,* 2018; Kastelic *et al.,* 2018).

Artificial insemination centres routinely evaluate semen quality using conventional parameters such as sperm motility, concentration, and morphology. These assessments help prevent the use of semen from subfertile bulls and the production of low-fertility semen doses. However, the relationship between conventional semen quality parameters and actual fertility remains inconsistent and continues to be debated (Holt, 2005; Lewis, 2007). In recent years, sperm DNA fragmentation (SDF) has emerged as a reliable indicator of male fertility potential (Bungum *et al.,*

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2011). The sperm chromatin dispersion (SCD) test and its subsequent refinements have been proposed as practical tools for differentiating spermatozoa with intact DNA from those exhibiting fragmented DNA based on chromatin decondensation patterns (Fernández *et al.,* 2003; Enciso *et al.,* 2006). Limited information is available regarding the effects of HS-BQ and FMD vaccination on sperm DNA integrity in breeding bulls. Evaluating the impact of these vaccinations on both conventional semen quality parameters and sperm DNA fragmentation may provide valuable insights into the optimal waiting period for semen collection following vaccination. Therefore, the present study was undertaken to assess the effects of HS-BQ and FMD vaccination on conventional sperm quality parameters and sperm DNA Fragmentation Index (DFI) in Holstein Friesian bulls, and to compare these parameters before and after vaccination of bulls.

Material and Methods

Frozen semen straws (0.25 mL) used in the present study were procured from the Frozen Semen Laboratory, Harsul, Sambhajinagar, Maharashtra, India, where they had been stored in liquid nitrogen. Six Holstein Friesian (HF) bulls maintained at the semen station were included in the study. The bulls were vaccinated against Haemorrhagic Septicaemia (HS) and Black Quarter (BQ) in June, followed by vaccination against Foot-and-Mouth Disease (FMD) one month later, in July. Frozen semen straws collected at 1, 2, 3, and 4 weeks following HS-BQ vaccination and at 1, 2, 3, 4, 6, and 8 weeks following FMD vaccination were used for evaluation of post-thaw seminal quality parameters. Semen samples collected prior to vaccination served as the control for both vaccination groups. Each bull was considered an experimental replicate for assessing the effect of HS-BQ and FMD vaccination on semen quality.

The seminal quality parameters evaluated included sperm motility, viability, morphology, plasma membrane integrity, and acrosome integrity. Sperm motility was assessed under a phase-contrast microscope using a 20× objective lens. During incubation, 10 µL of semen was loaded into a 10-µm depth sperm meter chamber (Sperm Processor, Aurangabad, Maharashtra, India) for motility assessment. Sperm viability was determined using eosin-nigrosine staining by counting live and dead spermatozoa. Total sperm morphological abnormalities were evaluated using a sperm morphology staining kit (Sperm Processor, Aurangabad, Maharashtra, India). Plasma membrane integrity was assessed using the hypo-osmotic swelling test (HOST) after exposing spermatozoa to a hypo-osmotic solution of 100 mOsm/L. Acrosome integrity was evaluated using Giemsa staining. The results for all these parameters were expressed as percentages based on the total number of spermatozoa counted.

The DNA Fragmentation Index (DFI) was determined using a sperm DNA fragmentation kit (Sperm Processor, Aurangabad, Maharashtra, India) based on the Sperm Chromatin Dispersion (SCD) test. During scoring of SCD-processed sperm nuclei under bright-field microscopy, dispersion patterns were classified according to the criteria described by Fernández *et al.* (2003). The observed patterns included: (i) nuclei with large DNA dispersion halos, where the halo width was equal to or greater than the diameter of the nucleoid core; (ii) nuclei with very small halos, where the halo width was equal to or less than one-third of the minor diameter of the core; and (iii) nuclei with no halo. Spermatozoa exhibiting fragmented DNA showed either no halo or very small halos, whereas spermatozoa with intact DNA displayed distinct peripheral halos surrounding the central core (Fig. 1).

Statistical analysis of the data was performed using the ICAR GOA-WASP 2.0 online software package. Data pertaining to sperm viability, functional plasma membrane integrity, morphology, acrosome integrity, and DNA Fragmentation Index were analyzed using a Completely Randomized Design (CRD).

Results and Discussion

The findings on the effects of HS-BQ and FMD vaccination on various post-thawed sperm quality parameters over a time period of 4 and 8 weeks in comparison to pre-vaccination values are presented in Table 1 and 2, respectively.

Sperm Motility

The mean total post-thaw motility decreased significantly during the first three weeks after HS-BQ vaccination as well

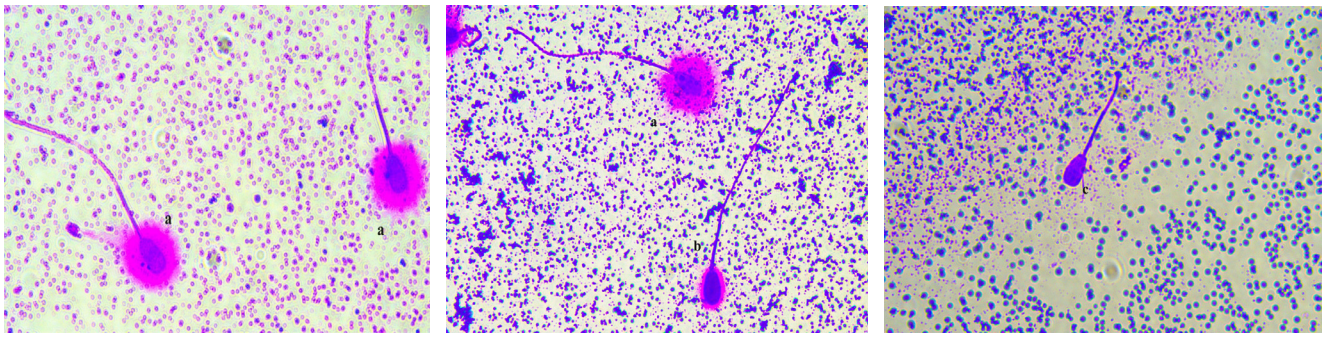


Fig. 1: DNA Fragmentation Index determined via Sperm Chromatin Dispersion test (SCD). **a)** Nuclei with large DNA dispersion halos- Non-fragmented DNA, **b)** Nuclei with very small sized halos- Fragmented DNA, and **c)** Nuclei with no halo-Fragmented DNA.

as FMD vaccination compared to the vaccination-free (control) period. The post-thaw motility returned to the acceptable range (≥50%) by week 4 after HS-BQ vaccination and by week 3 after FMD vaccination. These observations were in accordance with those of Reddy *et al.* (1991) and Kammar and Gangadhar (1998), who found a decrease in sperm motility after FMD vaccination in bovines. Mathur *et al.* (2003), Bhakat *et al*. (2015), and Rao *et al.* (2017) reported similar results following HS-BQ vaccination in bovines. Sperm motility decreases after vaccination. This might be because sperm cell motility develops as spermatozoa pass through the epididymis (Moulikrishnan and Rao, 1986). The substantial increase in body and testicular temperature during anaphylactic stress following immunization disrupts spermatogenesis (Reddy *et al.,* 1991) and epididymal function, which may result in vaccination-induced reductions in sperm motility.

Sperm Vitality

The mean live sperm percentage decreased significantly for first three weeks post HS-BQ and FMD vaccination compared to their controls. After HS-BQ vaccination, the live sperm percentage returned to the acceptance level at week 4 (71.00±1.57). Also, the values of live sperm per cent returned to the acceptance level (≥70) after FMD vaccination at week 4 (76.00±2.01). The present findings concurred with those published by Kumar (2004), and Singh *et al.* (2003) in Murrah buffalo bull. Saha *et al.* (2011) in HF demonstrated a significant decline in live sperm (%) during the post-FMD vaccination period. Pankaj *et al.* (2007) in Murrah and Rao *et al.* (2017) in Karan Fries bulls likewise documented a decline in live sperm per cent following Triovac (FMD+HS+BQ) vaccination. Increased in percentage of dead spermatozoa may be due to the negative effects of an increase in testicular temperature, there is a rise in aberrant and dead spermatozoa, which is followed by testicular degeneration and epididymal dysfunction.

Hypo-Osmotic-Swelling Test

The percentage of HOST-reacted spermatozoa decreased significantly during the first three weeks following HS-BQ and FMD vaccination compared with the respective control values. Following HS-BQ vaccination, the percentage of HOST-reacted spermatozoa returned to the acceptable level (≥40%) by week 4 (46.50 ± 0.92). In contrast, after FMD vaccination, the values returned to the acceptable level (≥40%) by week 3 (44.00 ± 1.96). The present findings are comparable with those reported by Gupta *et al.* (2018) in HF bulls, who observed a significant decline in HOST-positive spermatozoa following FMD vaccination. Similarly, Bhakat *et al.* (2015) in Murrah bulls and Rao *et al.* (2017) in Karan Fries bulls reported reductions in the percentage of HOST-reacted spermatozoa following HS-BQ and FMD+HS+BQ oil-adjuvant vaccination, respectively. The decline in HOST-reacted spermatozoa after vaccination may be attributed to impaired sperm plasma membrane integrity caused by increased production of reactive oxygen species (ROS). Mammalian spermatozoa are particularly susceptible to oxidative damage because their plasma membranes contain high levels of polyunsaturated fatty acids, making them vulnerable to lipid peroxidation (Griveau *et al.,* 1995).

Table 1: Effect of HS-BQ combine vaccination on different sperm quality parameters of post-thawed bovine semen (Mean ± SE, %)

Sperm parameter	pre-vaccination	Post-vaccination period			
		Week 1	Week 2	Week 3	Week 4
Motility (%)	58.17±1.37^{a}	34.67±2.67^{c}	38.67 ±2.29^{c}	44.83 ±1.49^{b}	58.33±1.60^{a}
Vitality (%)	72.67±0.55^{a}	48.17±2.53^{c}	51.83±2.12^{c}	62.50±1.52^{b}	71.00±1.57^{a}
HOST test (%)	49.33±1.05^{a}	32.67±2.34^{c}	35.00±2.29^{bc}	38.17±0.74^{b}	46.50±0.92^{a}
Morphological defects (%)	16.83±0.54^{d}	28.67±1.45^{a}	24.67 ±0.71^{b}	21.00±0.68^{c}	17.00±0.57^{d}
Intact acrosome (%)	75.00±1.46^{a}	68.83±1.40^{b}	70.33±1.11^{b}	75.17±0.60^{a}	76.17 ±1.19^{a}
DNA fragmentation Index (%)	2.64±0.36^{c}	7.28±0.93^{a}	4.58±0.52^{b}	3.02±0.58^{bc}	2.54±0.40^{c}

Means bearing different superscripts (a, b, c, d) differed significantly at p<0.05.

Table 2: Effect of FMD vaccination on different sperm quality parameters of post-thawed bovine semen (Mean ± SE, %)

Sperm parameter	Pre-vaccination	Post-vaccination period					
		Week 1	Week 2	Week 3	Week 4	Week 6	Week 8
Motility (%)	68.00±1.87^{a}	39.17±3.12^{d}	49.33±4.01^{c}	59.50±2.02^{b}	66.67±2.02^{ab}	69.83±2.32^{a}	70.67±1.35^{a}
Vitality (%)	77.83±1.74^{a}	48.50±3.47^{d}	58.67±3.77^{c}	66.83±2.56^{b}	76.00±2.01^{a}	79.83±1.57^{a}	80.17±1.42^{a}
HOST test (%)	51.50±2.15^{ab}	32.67±1.78^{d}	36.83±1.99^{d}	44.00±1.96^{c}	50.17±1.66^{b}	52.50±0.71^{ab}	56.17±1.53^{a}
Morphological defects (%)	18.67±0.55^{c}	31.50±1.14^{a}	28.50±2.18^{a}	23.50±1.05^{b}	20.67±1.22^{bc}	18.33±0.61^{c}	18.00±0.89^{c}
Intact acrosome (%)	76.50±0.95^{ab}	69.50±0.84^{c}	71.17±0.79^{c}	72.83±1.68^{bc}	76.50±1.43^{ab}	77.50±0.71^{a}	75.00±1.98^{ab}
DNA fragmentation Index (%)	1.36±0.21^{c}	3.96±0.51^{a}	2.67±0.39^{b}	1.53±0.29^{c}	1.39±0.33^{c}	1.38±1.38^{c}	1.34±1.34^{c}

Means bearing different superscripts (a, b, c, d) differed significantly at p<0.05.

Total Morphological Defects

The mean percentage of morphological defects increased significantly during the first three weeks following both HS-BQ and FMD vaccination compared to the pre-vaccination (control) period. However, the overall morphological defects returned to the acceptable limit (≤20%) by the 4th week after HS-BQ vaccination (17.00 ± 0.57%) and by the 6th week after FMD vaccination (18.33 ± 0.61%). The transient increase in sperm abnormalities observed during the early post-vaccination period may be associated with the physiological stress and rise in body and testicular temperature induced by vaccination. Since spermatozoa undergo essential maturation and functional modifications during epididymal transit, even a temporary disruption of testicular and epididymal function can adversely affect sperm morphology, leading to an increased incidence of tail and mid-piece abnormalities (Rahman *et al.,* 2018; Ozkocer and Konac, 2021). Elevated testicular temperature has been reported to impair spermatogenesis, epididymal sperm maturation, and overall sperm quality, which may explain the higher proportion of abnormal spermatozoa observed during the initial weeks following vaccination (Rahman *et al*., 2018; Shahat *et al.,* 2020; Morrell, 2020).

Intact Acrosome

The mean percentage of intact acrosomes decreased significantly during the first two weeks post-vaccination following both HS-BQ (68.83 ± 1.40 and 70.33 ± 1.11) and FMD vaccination (69.50 ± 0.84 and 71.17 ± 0.79). However, the values of intact acrosome percentage (PIA) remained within the acceptable fertility threshold (≥65%) throughout the study period after both vaccinations. These findings are in agreement with Gowda (1993) in Jersey bulls, Singh *et al*. (2003) in Holstein Friesian bulls, and Rao *et al*. (2017) in HF crossbred bulls, who also reported a transient decline in acrosomal integrity following FMD vaccination. Similar observations were made by Pankaj *et al*. (2007) in Murrah buffalo bulls and Rao *et al*. (2017), where acrosomal damage was significantly increased following multivalent (HS + BQ + FMD) vaccination.

The reduction in acrosomal integrity observed during the early post-vaccination period may be attributed to transient physiological stress and febrile response induced by vaccination, which can disrupt spermatogenesis and sperm membrane stability. Recent studies have shown that heat and immunological stress can induce oxidative damage to sperm plasma and acrosomal membranes, leading to premature acrosomal reaction or structural damage (Rahman *et al*., 2018; Shahat *et al*., 2020; Morrell, 2020). Oxidative stress-mediated membrane destabilization during spermatogenesis and epididymal maturation is a key mechanism affecting acrosomal integrity and fertilizing ability of spermatozoa (Aitken and Baker, 2020; Ozkocer and Konac, 2021). This may explain the transient reduction in intact acrosome percentage observed in the present study following vaccination.

DNA Fragmentation

The mean sperm DNA fragmentation index (DFI, %) increased significantly during the first two weeks following HS-BQ vaccination (7.28 ± 0.93 and 4.58 ± 0.52) as well as FMD vaccination (3.96 ± 0.51 and 2.67 ± 0.39) compared to the pre-vaccination control period. However, the DFI values remained within the acceptable fertility threshold (<10%) throughout the post-vaccination period, indicating that although a transient increase occurred, the overall genomic integrity of spermatozoa was not severely compromised (Fig 1). Similar findings have been reported in bovine and other mammalian species, where short-term physiological stress and elevated testicular temperature were associated with increased sperm DNA fragmentation (Banks *et al.,* 2005; Rao *et al.,* 2016).

The present observations are also consistent with Gosálvez *et al.* (2008), who reported a marked increase in sperm DNA fragmentation in rams following vaccination, suggesting that immune activation and associated febrile responses can temporarily impair chromatin stability. The underlying mechanism for increased DNA damage may be attributed to heat stress-induced disruption of spermatogenesis and epididymal sperm maturation, leading to incomplete chromatin condensation and increased susceptibility to strand breaks. Heat stress has been shown to suppress the expression of key DNA repair enzymes, including poly (ADP-ribose) polymerase (PARP), which plays a critical role in detecting and repairing DNA strand breaks (Rockett *et al.,* 2001; Tramontano *et al.,* 2000). This suppression may contribute to transient elevations in sperm DNA damage during periods of physiological stress.

In addition, oxidative stress is considered a major mediator of sperm DNA fragmentation, as reactive oxygen species (ROS) can induce base modifications, single- and double-strand DNA breaks, and chromatin instability in spermatozoa. Heat stress and immunological stimulation have been reported to reduce endogenous antioxidant defence systems in the testes and epididymis, thereby increasing sperm susceptibility to oxidative DNA damage (Aitken and Baker, 2020; Rahman *et al.,* 2018; Shahat *et al.,* 2020; Morrell, 2020). Nevertheless, the maintenance of DFI below the critical threshold (<10%) in the present study suggests that HF bull semen retains sufficient genomic stability despite transient post-vaccination stress, possibly due to effective antioxidative defence mechanisms and recovery of spermatogenic function over time.

Conclusions

HS-BQ and FMD vaccinations caused transient deterioration in semen quality of breeding bulls. HS-BQ vaccination had a greater impact on sperm motility and plasma membrane integrity, whereas FMD vaccination more adversely affected

sperm morphology. Sperm vitality was similarly influenced by both vaccines. However, acrosome integrity remained above the acceptable threshold (>65%) and DNA fragmentation index remained below critical levels (<8-10%) throughout the post-vaccination period. A sexual rest period of at least 3 weeks after HS-BQ vaccination and more than 4 weeks after FMD vaccination is recommended to allow recovery of semen quality to acceptable breeding standards.

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