Curcumin and vitamin E in combination improves scrotal temperature and semen traits in FMD-vaccinated Murrah bulls

M. Sethi[1], N. Kumar*[1], T.K. Mohanty[1], N. Shah[2], D.K. Yadav[3]

[1]Animal Reproduction, Gynaecology and Obstetrics, Artificial Breeding Research Centre (ABRC), ICAR-National Dairy Research Institute, Karnal -132001, [2]Sardar Vallabhbhai Patel University of Agriculture & Technology, Meerut-250110, [3]Department of Instructional Livestock Farm Complex (ILFC), Ranchi College of Veterinary Science and Animal Husbandry, BAU, Ranchi -834006 India
Corresponding Author: drnishantvet78@gmail.com

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Abstract

Foot-and-Mouth Disease (FMD) vaccination, administered biannually in dairy bulls, is known to transiently compromise semen quality by disturbing testicular thermoregulation. The present study evaluated the efficacy of combined dietary supplementation with curcumin and vitamin E, provided at two different doses, in mitigating these adverse effects in Murrah buffalo bulls. Eighteen sexually mature bulls were randomly allocated into three groups (n=6 each): Control (C), Supplemented I (SI), and Supplemented II (SII). The control group bulls received a standard basal diet (ICAR, 2013), while SI and SII group bulls were fed with vitamin E (3500 IU/day and 4500 IU/day, respectively) along with curcumin (150 mg/kg DM and 250 mg/kg DM, respectively). Supplementation began six weeks prior to and continued for six weeks following FMD vaccination. Scrotal surface temperatures (PPT, MPT, DPT, ET, and TG) were evaluated before and after vaccination. In addition, semen quality parameters such as hypo-osmotic swelling (HOS) response and acrosome integrity were assessed at post-dilution, pre-freeze, and post-thaw stages, both before and after vaccination. Results revealed that dietary supplementation significantly ($p<0.05$) reduced vaccination-induced elevations in scrotal surface temperatures and preserved sperm quality compared with controls. However, no significant differences were observed between SI and SII groups. In conclusion, curcumin and vitamin E supplementation effectively attenuates vaccine-induced thermal stress and sperm impairment, thereby supporting the sustained production of superior-quality germplasm in Murrah buffalo bulls.

Keywords: Bull; Curcumin; Cytokines; Rectal Temperature; Vaccination; Vitamin E

Introduction

Routine vaccination is crucial for herd health management since dairy animals, especially buffaloes, are extremely vulnerable to infectious illnesses (Martinez-Burnes et al., 2024). Foot and Mouth Disease (FMD) is one such highly contagious and economically significant viral infection (Orsel and Bouma 2009). To stop the spread of FMD, dairy bulls receive booster doses of the vaccine twice a year. Although vaccination is essential for preventing disease, it can temporarily impair physiological processes and productivity (Knight-Jones and Rushton 2013).

Testicular health is very important for productivity of breeding bulls (Mohammadi et al., 2019). Fever is a frequent systemic reaction after vaccination, triggered by pro-inflammatory cytokines acting on the hypothalamus to raise core body temperature (Dinarello 2004; Evans et al., 2015). The scrotal skin, vascular cone, and counter-current heat exchange system of the spermatic cord all work together to maintain the testicular temperature 4–5°C below the core body temperature, which is necessary for optimal sperm production (Kastelic et al., 2001; Brito et al., 2004). Hyperthermia brought on by vaccination might increase oxidative stress and testicular metabolism, which can affect sperm motility, viability, and morphology (Setchell 2006). It has been demonstrated that the FMD vaccine causes a brief rise in rectal temperatures, which has a detrimental effect on buffalo bulls' semen quality, especially during the first week after vaccination (Perumal et al., 2013; Pankaj et al., 2007; Rao et al., 2017a). Despite this, fluctuations in scrotal surface temperatures, which are crucial for spermatogenesis, have received less attention.

Antioxidants like vitamin E and curcumin have been shown to improve semen quality when given as dietary supplements to male animals (Rao et al., 2017a; El-Sherbiny et al., 2022). However, their combined effect on reducing vaccination-induced thermoregulatory and seminal changes has not been studied. The purpose of this study was to determine the effect of combined dietary supplementation with curcumin and vitamin E, administered both before and after FMD vaccination, on scrotal surface temperatures, including proximal, middle, and distal poles, epididymis, and testicular thermal gradient, as well as post-thaw semen quality parameters such as HOS response and acrosome integrity in Murrah buffalo bulls.

Materials and Methods

Ethics approval

The experiment was conducted with prior approval from the Institutional Animal Ethics Committee (IAEC) of ICAR–National Dairy Research Institute, Karnal, Haryana, India (https://ndri.res.in) (Reg. No. 1705/GO/ac/13/CPCSEA; Approval No. 50-IAEC-23-08). All procedures strictly followed the ethical standards and guidelines prescribed by the IAEC.

Experimental bulls, supplement mixture and feeding protocol

The experiment was conducted at the Artificial Breeding Research Centre (ABRC), ICAR–National Dairy Research Institute, Karnal, Haryana, India during the month of October, when the monthly average THI remained consistently below the heat stress threshold (THI < 75) for Murrah buffaloe. The institute is located 250 meters above the mean sea level at 29.43°N latitude and 72.2°E longitude. A total of 18 healthy (N=18), sexually mature Murrah buffalo bulls with normal libido were randomly allocated into three groups (n = 6 per group): Control, Supplemented I (SI), and Supplemented II (SII). Bulls in the control group were maintained on the standard ICAR diet (ICAR, 2013). In addition to the basal diet, bulls in SI group received Vitamin E (3,500 IU/bull/day) and Curcumin (150 mg/kg DM), while the SII group bulls were provided with higher levels of Vitamin E (4,500 IU/bull/day) and Curcumin (250 mg/kg DM). Dietary supplementation was initiated six weeks before Foot-and-Mouth Disease (FMD) vaccination and continued for six weeks post-vaccination. Vitamin E was provided in the form of dry Vitamin E-Acetate (50% DC; BASF SE, Germany; CAS No. 30041051), and Curcumin (95%) was sourced from Maxima Nutrition (LOT No.: TTLGL/74621).

FMD Vaccination

In this study, a commercially available oil-adjuvanted FMD vaccine, Raksha-Ovac Trivalent (Indian Immunologicals Limited, Hyderabad, India), was employed. The vaccine consists of inactivated antigens from three FMD virus serotypes (O, A, and Asia-1), formulated with mineral oil as the adjuvant and thiomersal (0.01% w/v) as a preservative. It was stored under refrigeration at 4 °C, protected from light and radiation, until use. Each bull received a single 2 ml intramuscular injection in the neck region.

Scrotal temperature evaluation via infrared thermography (°C)

The thermal profile of the scrotum was recorded by positioning the infrared (IR) camera (Darvi DTL007, TAK Technologies Pvt. Ltd.) with a resolution of 384 × 288 pixels at a distance of 1 meter perpendicular to the scrotal surface. Scrotal surface temperature was assessed at four anatomical points: proximal pole (PPT), mid pole (MPT), distal pole (DPT) of the testes, and the epididymis (ET) as illustrated in Figure1.The testicular gradient (TG) was calculated as the difference between PPT and DPT (TG = PPT − DPT). A drawing pad was used to mark and analyze the specific points on thermal images. The constant area of sharp and focused IR images of particular points were selected, interpreted and analysed by the Darvi TI analysis software. Recordings were obtained three

Fig. 1: Scrotal image of Murrah buffalo bull showing the anatomical sites for scrotal surface temperature measurement. PPT = Proximal pole (cranial region, closest to the abdomen; normally the warmest area due to higher proximity to the body. Testicular vascular cone (TVC) is concentrated at the proximal region of the testis, which helps in counter-current heat exchange and thermal regulation, MPT = Middle pole (central region representing the average thermal status of the testicular parenchyma), DPT = Distal pole (caudal region, normally the coolest area indicating the efficiency of scrotal cooling), and ET = Epididymis (cauda, site of mature sperm storage where temperature directly influences sperm motility and fertility).

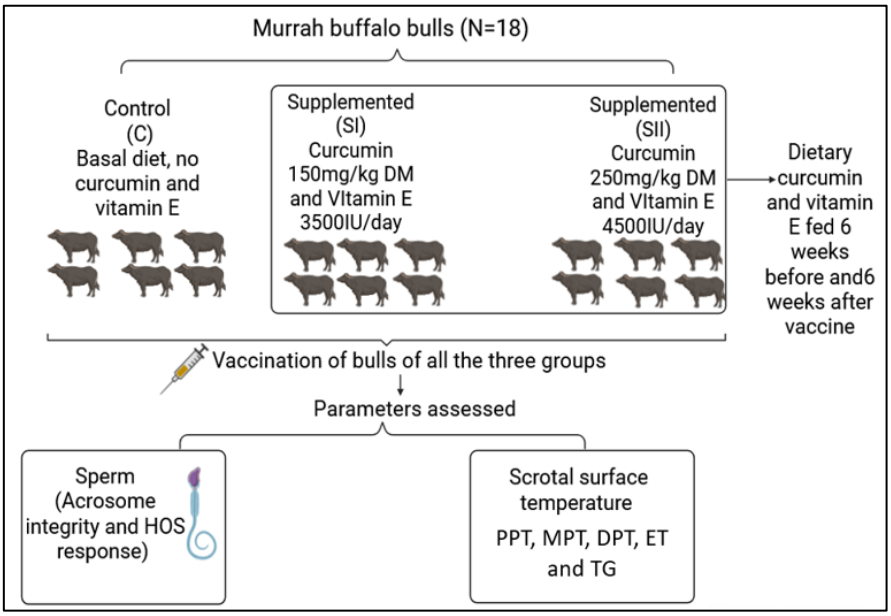

Fig.2: Methodological framework of the study showing dietary supplementation provided to bulls and the type of sample collection performed at pre- and post-vaccination stages.

weeks prior to vaccination and six weeks following FMD vaccination. Pre and post vaccination measurements were taken on days 1, 3, 5 and 7 of every week and the mean of these readings was used to represent the weekly value for each bull (N = 18).

Semen collection, cryopreservation, and thawing

Semen was collected once weekly from all eighteen bulls across the three groups (C, SI, and SII) for six weeks before and six weeks following vaccination, using a bovine artificial vagina (IMV model-005417). Following collection, semen was extended with a Tris–egg yolk–glycerol extender composed of Tris (30.28 g/L), fructose (1% w/v; 10 g/L), citric acid monohydrate (1.675% w/v; 16.75 g/L), supplemented with 20% (v/v) fresh

egg yolk (200 mL/L), 6.4% (v/v) glycerol (64 mL/L), penicillin G sodium (1,000 IU/mL), and streptomycin sulfate (1 mg/mL). The extended semen was adjusted to a final concentration of 80 million spermatozoa/mL. Samples were filled and sealed in 0.25 mL French mini straws (IMV, France).The straws were equilibrated for 4 h at 4 °C in a cold-handling cabinet, then transferred to an IMV programmable biological freezer, where cooling to −140 °C was achieved within approximately 7 minutes. Straws were subsequently plunged into liquid nitrogen (−196 °C) for long-term storage. Thawing was performed after 24 h of cryopreservation by immersing straws in water at 37 °C for 30 s using a CITO warm water thaw unit. Thawed straws were wiped, cut, and the semen was transferred into microcentrifuge tubes maintained at 37 °C in a dry bath for further evaluation. Methodology of the experiment is described in Figure 2.

Semen evaluation

Semen was evaluated for its HOS response and acrosome integrity at post-dilution, pre-freeze and post-thaw stages of semen cryopreservation.

HOS Response

The functional integrity of the sperm plasma membrane was evaluated using the hypo-osmotic swelling (HOS) test, following the method described by Correa et al., 1994. For this, 900 µL of hypo-osmotic solution (150 mOsmol/L) was combined with 100 µL of semen and incubated at 37 °C for 1 h. A drop of the well-mixed suspension was then placed on a clean glass slide, covered with a coverslip, and observed under a phase-contrast microscope at 400× magnification. Spermatozoa showing tail curling, indicative of swelling, were recorded, and a total of 200 cells were counted across different microscopic fields.

Sperm Acrosome Integrity

Giemsa staining as per Watson (1975), was used for the assessment of sperm acrosome integrity. Sperm acrosome integrity was assessed post-dilution, pre-freeze and post thaw stages. A thin smear was prepared on a clean, grease-free slide and was air-dried. The smear was fixed with Hancock's fixative for 15 minutes, followed by washing in slow-running water for 15 minutes. The slide was then air-dried and dipped in Giemsa working solution for 120-150 minutes. The slides were washed adequately with distilled water, then air-dried and observed slide under a microscope in the oil immersion objective (100X). At least 200 sperm in two other smears, i.e., 400 sperm, were counted to estimate intact acrosome percentage**.**

Statistical analysis

Each scrotal surface temperatures (PPT, MPT, DPT, ET and TG) was recorded three times from each bull (N = 18). All statistical analyses were performed using SPSS software, version 25 (IBM, Armonk, NY, USA). A probability (p) value of ≤ 0.05 was considered statistically significant. Since all variables were continuous, results are presented in tables as Mean ± SE. The effects of treatment and time (before and after vaccination) on scrotal surface temperatures i.e proximal pole temperature (PPT), mid pole temperature (MPT), distal pole temperature (DPT), epididymal tail temperature (ET), and the temperature gradient (TG) were assessed using repeated measures two-way ANOVA. The same statistical method was applied for sperm HOS response and acrosome integrity. Post hoc comparisons between the control and supplemented groups were conducted using Tukey's test. The data obtained from statistical analysis were graphically represented using the free trial version of GraphPad Prism 8.1.2 (332) (San Diego, USA), following APA style guidelines

Results and Discussion

Scrotal surface temperatures

The week-wise effect of dietary supplementation with Curcumin and Vitamin E on scrotal surface temperatures (PPT, MPT, DPT, ET and TG) of the testis in FMD-vaccinated Murrah buffalo bulls, measured via infrared thermography (IRT), is presented in Figure3.

A significant ($p<0.05$) increase in proximal scrotal temperature (PPT) was observed in all groups during the first week post-vaccination (C: 35.40 ± 0.29 °C; SI: 35.88 ± 0.29 °C; SII: 35.62 ± 0.29 °C) compared to their respective pre-vaccination values (C: 34.58 ± 0.06 °C; SI: 34.55 ± 0.06 °C; 95% CI: 34.42–34.68; SII: 34.57 ± 0.06 °C). Recovery to baseline occurred by week 3 in all the groups (Table1).

A significant ($p < 0.05$) rise in MPT was observed during the first week post-vaccination (C: 33.65 ± 0.18 °C; SI: 33.62 ± 0.18 °C; SII: 33.57 ± 0.18 °C) in all groups as compare to their prevaccine values (C: 33.25 ± 0.14 °C; SI: 33.30 ± 0.14 °C; SII: 33.21 ± 0.14 °C). However, differences between the groups remained non-significant. Temperatures gradually normalized from week 2 onward in all the groups (Table 2).

All groups showed a significant ($p<0.05$) increase in scrotal temperature at the distal pole during the first week post-vaccination (C: 32.93 ± 0.23 °C; SI: 32.77 ± 0.23 °C; SII: 32.63 ± 0.23 °C;) compared to their respective pre-vaccination values (C: 30.91 ± 0.16 °C; SI: 30.96 ± 0.16 °C; SII: 30.88 ± 0.16 °C). By week 3, DPT in Groups SI and SII returned to baseline, while the control group remained elevated. However, no significant difference was observed between the SII and SI groups, nor between the SI and control groups (Table 3).

Epididymal temperature significantly ($p<0.05$) increased during the first week post-vaccination (C: 31.37 ± 0.18 °C; SI: 31.40 ± 0.18 °C; SII: 31.32 ± 0.18 °C) compared to pre-vaccination values (C:

30.22 ± 0.13 °C; SI: 30.28 ± 0.13 °C; SII: 30.25 ± 0.13 °C) in all three groups. Full recovery was observed in Groups SI and SII by week 3, and in the control group by week 4 (Table 4).

Before vaccination, all groups showed similar baseline values for scrotal temperature (testicular gradient), with no significant differences (C: 3.68 ± 0.18 °C; SI: 3.59 ± 0.18 °C; SII: 3.69 ± 0.18 °C). Following vaccination, a significant ($p<0.05$) decrease in the testicular gradient was observed in the first week (C: 2.47 ± 0.40 °C; SI: 3.12 ± 0.40 °C; SII: 2.98 ± 0.40 °C). Early recovery was observed in Groups SI and SII by week 2, while the control group recovered by week 3(Table 5). A thermal image of testis captured by IRT camera has been provided in Figure 4.

Table 1: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on proximal pole temperature (PPT) of testis in FMD-vaccinated Murrah buffalo bulls

Scrotal temperature (PPT) (˚C)				
Groups		**C (n=6)**	**S I (n=6)**	**S II (n=6)**
Pre vaccination		34.58^{CD}± 0.05	34.55^{D} ±0.06	34.57^{B} ±0.05
Post vaccination	**1st week**	35.50^{A}± 0.36	35.88^{A}±0.17	35.62^{A} ±0.31
	2nd week	35.15^{AB}± 0.27	35.45^{B}±0.18	35.27^{A} ±0.28
	3rd week	34.80^{BC} ±0.24	34.78^{C}±0.05	34.73^{B}±0.20
	4th week	34.62^{CD} ±0.11	34.52^{D} ±0.14	4.53^{B} ±0.24
	5th week	34.52^{D} ±0.09	34.55^{D} ±0.08	34.58^{B} ±0.10
	6th week	34.55^{CD}± 0.10	34.58^{D} ±0.11	34.57^{B} ±0.11

Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$).

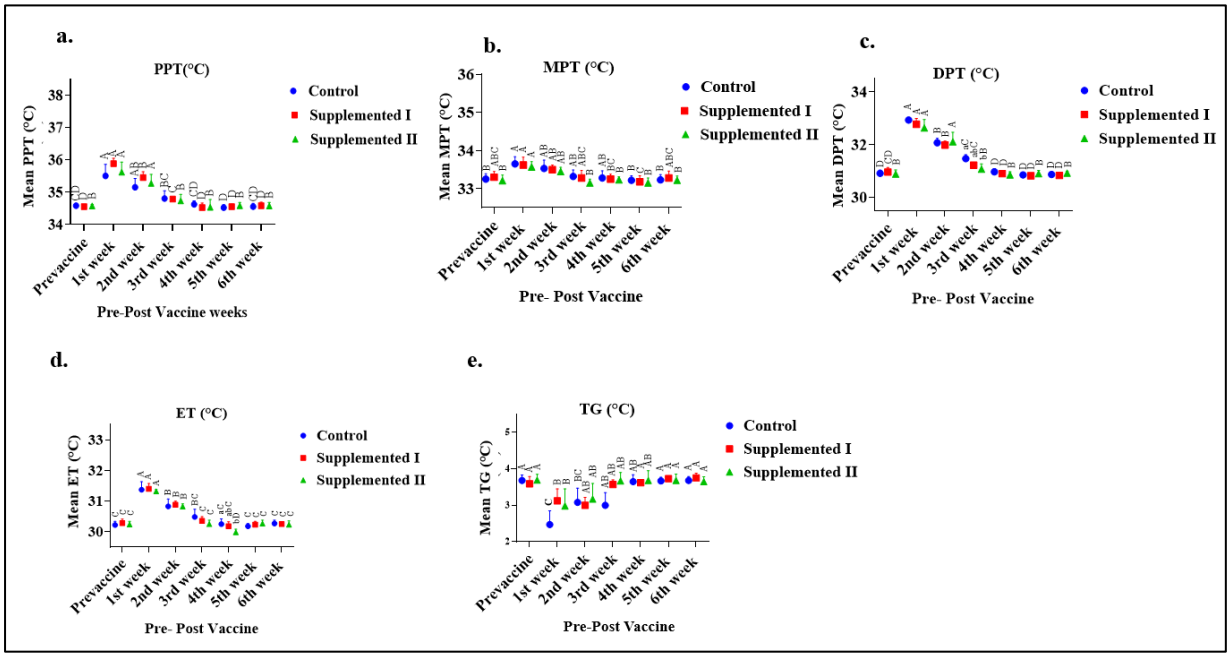

Fig 3: Effect of dietary supplementation with curcumin and vitamin E on scrotal surface temperatures of bulls before and after vaccination. (a) Proximal Pole Temperature (PPT), (b) Middle Pole Temperature (MPT), (c) Distal Pole Temperature (DPT), (d) Epididymal Temperature (ET), and (e) Temperature Gradient (TG) were recorded at pre-vaccination and up to six weeks post-vaccination. Data are presented as mean ± SE. Different superscripts denote significant differences at $p < 0.05$, where capital letters (A, B, C) represent differences across time points, and lowercase letters (a, b, c) represent differences among groups (Control, Supplemented I, and Supplemented II).

Table 2: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on middle pole temperature (MPT) of testis in FMD-vaccinated Murrah buffalo bulls

Scrotal temperature (MPT) (˚C)				
Groups		**C (n=6)**	**S I (n=6)**	**S II (n=6)**
Pre vaccination		33.25^{B}± 0.14	33.30^{ABC} ±0.15	33.21^{B} ±0.15
Post vaccination	**1st week**	33.65^{A}± 0.19	33.62^{A} ±0.21	33.57^{A}±0.14
	2nd week	33.53^{AB}± 0.22	33.50^{AB} ±0.12	33.45^{AB}±0.11
	3rd week	33.32^{AB} ±0.17	33.28^{ABC} ±0.20	33.15^{B} ±0.10
	4th week	33.28^{AB} ±0.19	33.25^{BC}±0.13	33.23^{B} ±0.08
	5th week	33.22^{B} ±0.12	33.18^{C} ±0.16	33.15^{B} ±0.13
	6th week	33.23^{B}± 0.14	33.28^{ABC} ±0.18	33.22^{B} ±0.11

Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$).

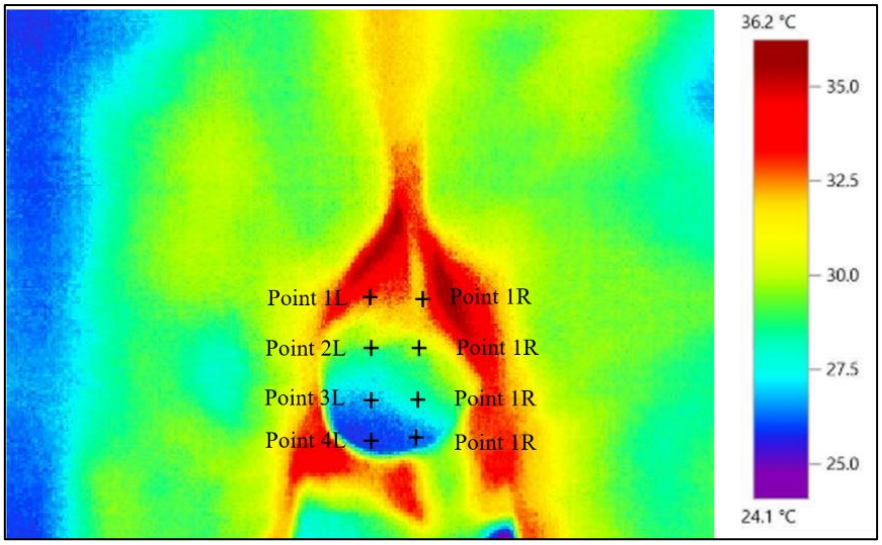

Fig 4: Infrared thermographic image of the testes showing temperature measurements at different anatomical sites: Point 1 (left and right proximal poles), Point 2 (left and right mid poles), Point 3 (left and right distal poles), and Point 4 (left and right epididymides).

Table 3: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on distal pole temperature (DPT) of testis in FMD-vaccinated Murrah buffalo bulls

Scrotal temperature (DPT) (˚C)				
Groups		**C (n=6)**	**S I (n=6)**	**S II (n=6)**
Pre vaccination		$30.91^{D}\pm 0.12$	$30.96^{CD}\pm 0.17$	$30.88^{B}\pm 0.16$
Post vaccination	**1st week**	$32.93^{A}\pm 0.06$	$32.77^{A}\pm 0.22$	$32.63^{A}\pm 0.32$
	2nd week	$32.07^{B}\pm 0.17$	$31.98^{B}\pm 0.15$	$32.10^{A}\pm 0.37$
	3rd week	$31.47^{aC}\pm 0.15$	31.22^{abC} 0.09	$31.07^{bB}\pm 0.20$
	4th week	$30.97^{D}\pm 0.12$	$30.90^{D}\pm 0.07$	$30.85^{B}\pm 0.12$
	5th week	$30.85^{D}\pm 0.06$	$30.82^{D}\pm 0.07$	$30.90^{B}\pm 0.12$
	6th week	$30.87^{D}\pm 0.06$	$30.83^{D}\pm 0.10$	$30.92^{B}\pm 0.07$

Different lowercase superscripts (a, b, c) in the same row indicate significant differences between groups ($p < 0.05$). Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$).

Table 4: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on epididymal temperature (ET)of testis in FMD-vaccinated Murrah buffalo bulls

Scrotal temperature (ET) (Epididymis temperature) (˚C)				
Groups		**C (n=6)**	**S I (n=6)**	**S II (n=6)**
Pre vaccination		$30.22^{C}\pm 0.12$	$30.28^{C}\pm 0.13$	$30.24^{C}\pm 0.10$
Post vaccination	**1st week**	$31.37^{A}\pm 0.26$	$31.40^{A}\pm 0.17$	$31.32^{A}\pm 0.06$
	2nd week	$30.83^{B}\pm 0.24$	$30.88^{B}\pm 0.12$	$30.82^{B}\pm 0.09$
	3rd week	$30.48^{BC}\pm 0.25$	$30.35^{C}\pm 0.14$	$30.25^{C}\pm 0.13$
	4th week	$30.25^{aC}\pm 0.17$	$30.18^{abC}\pm 0.14$	$29.98^{bD}\pm 0.10$
	5th week	$30.18^{C}\pm 0.09$	$30.23^{C}\pm 0.11$	$30.27^{C}\pm 0.11$
	6th week	$30.27^{C}\pm 0.11$	$30.25^{C}\pm 0.08$	$30.23^{C}\pm 0.12$

Different lowercase superscripts (a, b, c) in the same row indicate significant differences between groups ($p < 0.05$). Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$).

Table 5: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on testicular gradient temperature of testis in FMD-vaccinated Murrah buffalo bulls

Scrotal temperature (Testicular gradient) (˚C)				
Groups		**C (n=6)**	**S I(n=6)**	**S II (n=6)**
Pre vaccination		$3.68^{A}\pm 0.15$	$3.59^{A}\pm 0.20$	$3.69^{A}\pm 0.16$
Post vaccination	**1st week**	$2.47^{C}\pm 0.38$	$3.12^{B}\pm 0.33$	$2.98^{B}\pm 0.47$
	2nd week	$3.08^{BC}\pm 0.39$	$3.47^{AB}\pm 0.22$	$3.17^{AB}\pm 0.43$
	3rd week	$3.33^{AB}\pm 0.35$	$3.57^{AB}\pm 0.13$	$3.67^{AB}\pm 0.23$
	4th week	$3.65^{AB}\pm 0.19$	$3.62^{A}\pm 0.09$	$3.68^{AB}\pm 0.27$
	5th week	$3.67^{A}\pm 0.10$	$3.73^{A}\pm 0.08$	$3.68^{A}\pm 0.18$
	6th week	$3.68^{A}\pm 0.11$	$3.75^{A}\pm 0.13$	$3.65^{A}\pm 0.13$

Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$).

The improved scrotal surface temperatures observed in the supplemented groups might be due to the beneficial effect of feeding curcumin and vitamin E in combination before and after vaccination. Vaccination is known to cause pyrexia, which elevates both body and testicular temperatures in bulls (Gupta et al., 2025). Literature also reveals that vaccination increases the levels of pro-inflammatory cytokines, which act as endogenous pyrogens and induce fever in animals (Dinarello, 2004). The resulting rise in body temperature increases the testicular temperature post-vaccination, ultimately impairing semen quality. Curcumin has been reported to reduce the levels of these pro-inflammatory cytokines (Nimiya et al., 2016). Therefore, feeding curcumin to the bulls before and after vaccination might have helped to decrease pyrogenic cytokines, which eventually led to less increase of scrotal surface temperature in the supplemented groups in our study as compared to the control. Furthermore, vitamin E stabilizes curcumin under oxidative conditions, while curcumin can regenerate and prolong vitamin E activity, suggesting potential synergism between the two (Gupta et al., 2013; Nimiya et al., 2016; Kharat et al., 2020; Anas et al., 2024). Hence, this synergism might have contributed to a faster recovery from vaccination-induced increase in scrotal temperature in the supplemented groups relative to the control.

Semen quality parameters

HOS Response

Prior to vaccination, no significant differences in HOS response were observed among the groups (C, SI, and SII) at any stage of cryopreservation (post-dilution, pre-freeze, and post-thaw). Following vaccination, a significant ($p < 0.05$) decline in HOS response was observed across all groups, with the maximum reduction recorded during the second week post-vaccination (Table 6). In the first week, HOS response decreased significantly ($p < 0.05$) at all stages of cryopreservation in all groups, with no significant inter-group differences. By the second week, the lowest HOS response values were recorded. At the pre-freeze and post-thaw stages, both supplemented groups (SI and SII) maintained significantly higher values compared to the control group, although no significant difference was noted between SI and SII. From the third week onward, gradual recovery of HOS response was observed in the supplemented groups. At the post-dilution stage, SII recovered by the fifth week, while SI reached baseline levels by the sixth week. In contrast, the control group failed to recover fully even by the sixth week. At the pre-freeze stage, SI and SII recovered by the sixth week, with SII showing significantly higher HOS response than its pre-vaccination value, whereas C remained impaired. Post-thaw, recovery in SI and SII was evident by the fifth week, while the control group continued to exhibit significantly lower values. Across multiple time points, particularly from the third week onward, SI and SII consistently showed significantly higher HOS responses than the control group, with SII maintaining the highest values overall.

Table 6: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on sperm HOS Response at post dilution, prefreeze and post thaw stage of semen cryopreservation in FMD-vaccinated Murrah buffalo bulls

HOS Response (%)										
Stage of cryopreservation		**Post dilution**			**Pre freeze**			**Post thaw**		
Groups		**C (n=6)**	**S I (n=6)**	**S II (n=6)**	**C (n=6)**	**S I (n=6)**	**S II (n=6)**	**C (n=6)**	**S I (n=6)**	**S II (n=6)**
re vaccination		70.47^{A} ±0.40	69.76^{A} ±0.34	70.03^{A} ±0.37	63.86^{A} ± 0.46	63.06^{A} ± 0.42	63.28^{A} ± 0.53	51.88^{A} ± 0.58	51.61^{B} ± 0.49	52.39^{B} ± 0.59
Post Vaccination	**1st week**	49.33^{D} ±1.01	50.58^{D} ±0.57	49.92^{D} ± 0.40	40.42^{E} ± 1.77	42.08^{D}± 0.51	41.50^{D} ± 0.68	30.75^{D} ± 1.88	32.67^{E} ± 0.70	31.75^{D} ± 0.69
	2nd week	44.92^{E} ±1.18	44.58^{E} ±1.04	45.25^{E} ± 0.53	34.00^{bF} ± 1.34	37.25^{aE} ± 1.21	38.50^{aE} ± 0.70	26.00^{bE} ± 1.35	29.00^{aF} ± 1.28	30.25^{aD}± 0.72
	3rd week	50.67^{bD} ±0.68	52.50^{aC} ±0.74	52.92^{aC} ± 1.03	43.25^{bD} ± 0.96	46.08^{aC} ± 0.55	47.67^{aC} ± 1.00	35.25^{bC} ± 0.96	40.08^{aD} ± 0.68	41.92^{aC} ± 1.34
	4th week	58.42^{C} ±1.35	59.50^{B} ±1.25	59.25^{B} ± 1.33	52.42^{C} ± 1.74	54.25^{B} ± 1.35	54.75^{B} ± 1.51	46.42^{bB} ± 1.86	48.58^{aC}± 1.36	50.50^{aB} ± 1.10
	5th week	65.83^{bB} ±0.45	69.50^{aA} ±1.12	70.42^{aA} ± 0.76	59.42^{bB} ± 0.20	62.75^{aA} ± 0.82	63.67^{aA} ± 0.84	47.25^{bB} ± 0.93	52.33^{aA}± 0.94	53.17^{aB}± 1.28
	6th week	67.58^{bB} ±0.76	69.00^{aA} ±0.76	70.75^{aA}± 0.50	60.08^{cB} ±0.42	62.50^{bA}± 0.63	64.33^{aA}± 0.33	51.67^{bA} ± 0.58	54.83^{aA}± 0.49	55.67^{aA}± 0.65

Different lowercase superscripts (a, b, c) in the same row indicate significant differences between groups ($p < 0.05$). Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$).

Table 7: Effect of pre- and post-vaccination feed supplementation with combined curcumin and vitamin E at two different dose levels on sperm Acrosome Integrity at post dilution, prefreeze and post thaw stage of semen cryopreservation in FMD-vaccinated Murrah buffalo bulls

Acrosome Integrity (%)										
Stage of cryopreservation		**Post dilution**			**Prefreeze**			**Post thaw**		
Groups		**C (n=6)**	**S I (n=6)**	**S II (n=6)**	**C (n=6)**	**S I (n=6)**	**S II (n=6)**	**C (n=6)**	**S I (n=6)**	**S II (n=6)**
Pre vaccination		86.25^{A} ±0.36	86.18^{A} ±0.41	86.53^{A} ±0.44	81.25^{A} ±0.42	81.54^{A} ± 0.43	81.99^{A} ±0.50	71.22^{A}±0 .49	72.01^{A} ±0.59	71.36^{A} ±0.63
Post Vaccination	**1st week**	66.50^{bE} ± 0.75	68.17^{bE} ±1.00	71.75^{aC} ±0.86	53.67^{bE} ±0.65	57.67^{aE} ±0.75	58.58^{aD}± 0.60	44.67^{bE} ±1.12	48.50^{aF} ±0.68	49.25^{aD} ±0.46
	2nd week	64.00^{bE} ± 1.18	65.58^{abE} ± 1.18	66.92^{aD} ±0.62	48.33^{bF} ±0.98	52.50^{aF} ±1.28	53.33^{aE} ±0.85	40.42^{bF} ±1.15	44.25^{aG} ±1.22	45.33^{aE} ±0.68
	3rd week	71.25^{D} ± 1.05	73.08^{D} ±1.09	72.58^{C} ±0.57	61.42^{bD} ±0.60	66.42^{aD} ±0.85	65.25^{aC} ±0.56	52.92^{bD} ±.54	59.42^{aE} ±0.72	59.75^{aC} ±0.69
	4th week	76.50^{C} ± 1.08	78.00^{C} ±1.09	78.42^{B} ±1.15	69.08^{bC} ±0.77	72.25^{aC} ±1.11	73.17^{aB} ±1.33	58.50^{bC} ±0.41	65.17^{aD} ±1.47	66.08^{aB} ±0.75
	5th week	78.75^{bC} ± 0.62	83.83^{aB} ±1.51	84.83^{aA} ±1.15	72.25^{bB} ±0.92	78.67^{aB} ±1.67	79.58^{aA} ±1.53	62.92^{bB} ±1.31	69.08^{aB} ±1.59	69.75^{aA} ±1.17
	6th week	81.58^{bB} ±1.02	85.58^{aAB} ±1.06	86.42^{aA} ±0.94	74.50^{bB} ±1.39	80.08^{aA} ±1.04	81.00^{aA} ±1.04	65.08^{bB} ±0.68	71.00^{aAB} ±1.01	72.50^{aA} ±1.22

Different lowercase superscripts (a, b, c) in the same row indicate significant differences between groups ($p < 0.05$). Different uppercase superscripts (A, B, C) in the same column indicate significant differences over time ($p < 0.05$)

Acrosome Integrity

Prior to vaccination, no significant differences in sperm acrosome integrity were observed among the groups (C, SI, and SII) at any cryopreservation stage (post-dilution, pre-freeze, post-thaw). In the first week post-vaccination, a significant ($P < 0.05$) decline in acrosome integrity was recorded across all groups. At the post-dilution stage, integrity decreased to 66.50 ± 0.75% in C, 68.17 ± 1.00% in SI, and 71.75 ± 0.86% in SII compared to pre-vaccination values (~86%). Among the groups, SII maintained significantly higher values than C and SI, while no significant difference was noted between the latter two. Similar declines were observed at the pre-freeze and post-thaw stages, with SII showing the highest integrity, followed by SI and C. By the second week, the lowest acrosome integrity values were recorded across all cryopreservation stages. At post-dilution, integrity dropped to 64.00 ± 1.18% in C, 63.92 ± 0.91% in SI, and 66.92 ± 0.62% in SII, with SII significantly higher than C and SI. At the pre-freeze and post-thaw stages, SII again maintained significantly higher values, while no difference was detected between C and SI. Between the third and sixth weeks post-vaccination, gradual recovery trends were observed. At the post-dilution stage, acrosome integrity in C remained significantly lower than pre-vaccination values throughout, whereas SI and SII recovered by the fifth and sixth weeks, respectively. At the pre-freeze stage, SII began to recover by the fifth week and SI by the sixth week, while C failed to regain pre-vaccination levels even at week six. Post-thaw recovery was fastest in SII (by the fifth week), followed by SI (sixth week), whereas C showed only partial improvement by the sixth week (Table 7).

Our findings are consistent with prior reports in Murrah (Pankaj et al., 2007), Sahiwal bulls (Bhakat et al., 2008), Karan Fries and Murrah buffalo bulls (Bhakat et al., 2010) and Mithun bulls (Perumal et al., 2013) which also reported significant decline in semen quality parameters ~1-2 months post vaccination. However no study till date has reported amelioration of vaccination induced decline in semen quality post vaccination via dietary feed supplements. We for the time time saw that feeding of bulls with curcumin and vitamin E roughly one month prior vaccine and even after vaccine helps in early recovery of vaccination induced decline in semen quality. We saw that dietary supplementation with curcumin and vitamin E facilitated earlier recovery of post-thaw HOS response and acrosome integrity in supplemented group by approximately one week as compared to non-supplemented group.

The likely mechanism underlying the protective effects of curcumin and vitamin E involves their complementary antioxidant and anti-inflammatory properties. Vaccination induces oxidative stress by increasing ROS, leading to lipid peroxidation (LPO) of sperm membranes. LPO progresses through initiation (alkyl radical formation), propagation (peroxyl radical chain reactions with polyunsaturated fatty acids), and termination (formation of stable non-radical products) (Girotti, 1998; Yin et al., 2011). Vitamin E directly interrupts this chain reaction by donating hydrogen atoms to lipid radicals, stabilizing sperm membranes, inhibiting phospholipase A2, and protecting mitochondrial structures essential for motility (Niki, 2014). Curcumin acts indirectly by upregulating endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) via activation of the Nrf2 signaling pathway, enhancing long-term cellular defense against oxidative stress (Rahban et al., 2020). While scrotal surface temperatures returned to baseline relatively quickly, sperm HOS response and acrosome integrity showed delayed recovery. Given that epididymal transit of

sperm typically spans 9–14 days, recovery restricted to epididymal sperm would be expected within 3–4 weeks. The sustained impairment up to six weeks suggests that vaccination-induced hyperthermia during the first week adversely affected spermatogenesis itself, rather than only epididymal sperm reserves.

In conclusion, dietary supplementation with curcumin and vitamin E effectively counteracts vaccination-induced thermal stress in bulls, helping maintain scrotal temperatures and preserving sperm functionality, including HOS response and acrosome intregrity.

Author contribution

Nishant Kumar and Manisha Sethi conceptualized and designed the experiment; Manisha Sethi conducted the research experiment. Nadeem Shah and Dileep Kumar Yadav helped in data analysis. Manisha Sethi interpreted the findings and wrote the manuscript under the guidance of Nishant Kumar and Tushar K Mohanty proofread the manuscript. All authors reviewed the results and approved the final version of the manuscript.

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Data availability

Upon request from the corresponding authors.

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Ethics approval

The ethical principles outlined by the journal have been upheld, and the study has obtained the necessary ethical approval. Additionally, the research complies with the EU standards regarding the protection and utilization of animals for scientific purposes and/or feed legislation. The study has been approved by the Institutional Animal Ethical Committee of ICAR-National Dairy Research Institute, Karnal-132001, Haryana, India (https://ndri.res.in) (Reg. no.1705/GO/ac/13/CPCSEA) with the approval number 50-IAEC-23-08. The study adhered to all the ethical guidelines set by IAEC.

Conflicts of interest

The authors disclose no conflicts of interest

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