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THERMAL STRESS AND ITS CONSEQUENCES ON BULL SPERMATOGENESIS AND SEMEN QUALITY

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Abstract

Heat stress is a significant environmental stressor with detrimental effects on the male reproductive system in cattle, but the physiological, endocrine, oxidative, and sperm functional responses have not been fully elucidated. Spermatogenesis and semen quality were studied in 30 bulls exposed to heat stress, using a within-subject paired design. The animals were evaluated under thermoneutral (THI ≈ 67) and heat stress (THI ≈ 83) conditions, and the physiological and hormonal profiles, oxidative stress biomarkers, and semen quality traits were recorded in both periods. Heat stress resulted in higher scrotal and internal temperatures and heart rate, along with increased cortisol concentration and reduced testosterone levels in endocrine analysis. The activities of antioxidant enzymes in seminal plasma (SOD, CAT, and GPx) were markedly decreased, and malondialdehyde levels were significantly increased, suggesting oxidative stress. Semen analysis revealed significant decreases in ejaculate volume, sperm concentration, motility, and morphology, hematocrit, viability, acrosome integrity, and membrane integrity under HS, but an elevation in the DNA fragmentation index. A negative regression between THI and total and progressive motility, lipid peroxidation, and sperm functional tests was corroborated by means of regression models. These results substantiate earlier studies showing an association between heat stress and reduced bull fertility and further elucidate the mechanistic pathways involved in endocrine disruption, oxidative stress, and sperm DNA

damage. These findings underscore the susceptibility of bull spermatogenesis to elevated environmental heat loads and the importance of applied management, nutrition, and genetic strategies for maintaining reproductive efficiency in cattle under global warming conditions.

1. Introduction

Heat stress is widely accepted as the most severe challenge that impairs livestock productivity and fertility in relation to global climate change. The susceptibility of bull spermatogenesis to thermal variations is relatively higher than that of other species, since spermatogenesis in bulls relies on testes temperature being several degrees Celsius lower than the body core temperature for normal germ cell development. Transient disruption of testicular thermoregulation impairs the survival of germ cells, sperm mobility, and fertilizing potential (Hansen, 2009; Kastelic et al., 2021). Seasonal and climatic effects on bull fertility have been extensively described, and semen quality is reduced during hot seasons in both temperate and tropical climates (Netherton et al., 2022; Sabés-Alsina et al., 2019). The temperature–humidity index (THI) has become a standard measure of environmental thermal load, and values surpassing thresholds are invariably correlated with lower semen quality and fertility (Alves et al., 2025). Therefore, understanding how environmental heat stress affects sperm development is crucial for maintaining reproductive efficiency in dairy and beef production ecosystems.

Contemporary empirical research has thoroughly elucidated the etiology of the multifactorial effects of heat stress on bull reproductive function. Capela et al. (2022) found that high THI caused lower sperm motility, abnormal structure, and damaged membranes in bulls; however, Vanselow et al. (2024) showed that natural summer heat during spermatogenesis reduced blastocyst development rates and increased sperm DNA damage. Similarly, Celeghini et al. (2024) also pointed out that warmer ambient temperatures due to climate change interfere with spermatogenesis and Leydig cell steroidogenesis, in which a decrease in testosterone levels and damage to sperm production occurs. Molecular studies have shown that heat stress leads to an oxidative imbalance and decreased antioxidant enzyme activity, with increased lipid peroxidation in germ cells (Chitkara et al., 2025). This oxidative damage is highly associated with diminished sperm viability and fertilization efficiency (Hussain et al., 2023). In addition, environmental factors interact with genetic and physiological factors. Popović et al. (2024) reported that age, breed, and seasonal effects were significant for semen traits, whereas Dipaz Berrocal et al. (2024) found that heat and humidity tolerances differed between breeds. Overall, these results highlight the intricate nature of heat stress and call for well-controlled experimental designs to control confounders.

Despite emerging evidence, significant knowledge gaps persist in understanding the causal links between thermal stress, endocrine disruption, and oxidative imbalance, combined with sperm function integrity. Most of the studies were cross-sectional or observational, which limited the ability to establish within-bull effects of heat exposure. In addition, a limited number of studies have been conducted to compare hormones, oxidative stress, and DNA integrity factors with semen quality. Treatment strategies, including antioxidant supplementation, have exhibited some degree of advantage in reducing the negative impact of heat on sperm (J. Wang et al., 2025). However, more robust baseline data are necessary to understand which physiological pathways are most susceptible to thermal exposure. To fill this gap, the current study employed a paired experimental design to compare semen ejaculates collected from each bull under both thermoneutral and heat stress conditions. Through the measurement of physiological parameters, endocrine responses, oxidative stress biomarkers, and specific semen quality traits, we studied a detailed characterization of how heat stress compromises spermatogenesis. Ultimately, the results of this study are intended to inform better management, breeding, and mitigation

strategies for protecting bull fertility in a world of increasing climate change.

2. Methodology

2.1 Study Design

This study was conducted at University of Agriculture, Faisalabad, Pakistan as a within-subjects prospective experimental trial to assess the impact of heat stress on spermatogenesis and semen quality in bulls. Each bull was sampled under two environmental conditions: thermoneutral (TN) and high heat stress (HS). Although there are still limitations to the design, including between-animal differences not controlled in comparison with animals across hot and cold environments and animal adaptation, this approach reduces inter-animal variability. It increases confidence in causal inferences about heat stress impacts.

2.2 Animals and Management

A sample of thirty adult breeding bulls aged 2.5 to 7.5 years representing Bos taurus, Bos indicus, and crossbred genotypes selected from an artificial insemination (AI) center was studied. All bulls were clinically healthy and exhibited a body condition score ranging from 2.5 to 4.0 on a five-point scale. Animals were raised under conventional management and feeding conditions during the experimental period. Men provided semen samples on a regular collection schedule to eliminate potential bias related to the degree of abstinence or the frequency of collection.

2.2 Environmental Monitoring

The ambient temperature and relative humidity were monitored using computer-controlled sensors installed at the animal level. These data were later converted to the temperature-humidity index (THI) using an established equation as a measure of thermal stress. Thermoneutrality was defined as a THI ranging from 60 to 68, and thermal stress when THI values were greater than or equal to 76. Before each semen collection, the rams were exposed to the respective thermal environments for a minimum of 72 h.

2.3 Physiological Assessments

Physiological responses to heat stress were measured at each sampling site. A digital thermometer and infrared thermography were used to measure the rectal temperature and scrotal surface temperature, respectively. Heart rate auscultation was performed for 1 min using a stethoscope. These measurements objectively confirmed heat stress in pigs.

2.4 Blood and Seminal Plasma Collection

Blood was obtained via jugular venipuncture within 30 min of semen collection. Serum was collected, and samples were stored at −20°C until further analysis. Cortisol and testosterone levels were assessed using enzyme-linked immunosorbent assay (ELISA). Seminal plasma was extracted from the semen samples by centrifugation and evaluated for oxidative stress markers such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and malondialdehyde (MDA).

2.5 Semen Collection and Evaluation

Semen was collected from each bull with an artificial vagina twice per estrus period at least 48 h apart. Ejaculatory volumes were directly quantified, and sperm concentration was calculated using hemocytometry, which was confirmed by spectrophotometry. Sperm mobility and kinematics were evaluated by Computer-Assisted Sperm Analysis (CASA). The parameters analyzed were total motility, progressive motility, curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAC), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF). The morphology of the stained smears was determined using laser photometry under oil immersion (at least 200 sperms/slide). Sperm viability was evaluated by eosin–nigrosin staining, whereas membrane integrity was tested using the hypo-osmotic swelling test (HOS). The integrity of the acrosome was determined using fluorescein isothiocyanate–peanut agglutinin (FITC-PNA). DNA integrity was analyzed using the sperm chromatin structure assay (SCSA) as a DNA fragmentation index (DFI).

2.6 Laboratory Analyses

All laboratory protocols adhered to standardized methodologies based on internal and external quality controls. Spectrophotometry was used to analyze enzyme activity in the seminal plasma. Cortisol and testosterone levels were measured in duplicate with an assay variability of < 10%. The CASA was standardized before each session, and all samples were scored by technologists who were blinded to the experimental conditions.

2.7 Statistical Analysis

The data were analyzed using linear mixed-effects models that included the bull as a random factor to control for repetition of measures. The fixed effects were thermal period (TN or HS), THI, breed, age, and body condition score. Normality and homoscedasticity of residuals were checked, with percentage outcome variables logit-transformed if necessary. The primary semen parameters (progressive motility, sperm concentration, and morphology) between TN-HS were compared in pairs with $p<0.05$, representing significance. Secondary outcomes were corrected for multiple testing using false discovery rate (FDR) correction.

2.8 Ethical Considerations

The Institutional Animal Ethics Committee approved all the experiments for Animal Ethics. Animals were handled and sampled in compliance with the international ethical guidelines for animal research and were kept to a minimum during sampling.

3. Results

3.1 Environmental and Physiological Responses

Marked differences in environmental conditions were recorded between the thermoneutral (TN) and heat stress (HS) periods. Mean ambient temperature increased from 24.6 ± 1.5 °C in TN to 35.4 ± 1.8 °C in HS, with relative humidity rising from 55 ± 7% to 65 ± 8%. Consequently, the mean Temperature–Humidity Index (THI) increased significantly from 67.3 in TN to 82.6 in HS. Physiological measures followed a similar pattern: scrotal temperature rose from 33.2 ± 0.5 °C in TN to 35.1 ± 0.5 °C in HS, rectal temperature increased from 38.6 ± 0.3 °C to 39.5 ± 0.3 °C, and heart rate elevated from 55 ± 4 bpm to 68 ± 4 bpm. These findings confirm that bulls experienced a clear shift in thermal load and physiological strain when exposed to HS. The detailed values are presented in **Table 1**.

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Table 1: Environmental and Physiological Indicators of Bulls During Thermoneutral and Heat Stress Conditions

Parameter	Thermoneutral (TN)	Heat Stress (HS)
Ambient temperature (°C)	24.6 ± 1.5	35.4 ± 1.8
Relative humidity (%)	55 ± 7	65 ± 8
THI	67.3 ± 2.4	82.6 ± 2.8
Scrotal temperature (°C)	33.2 ± 0.5	35.1 ± 0.5
Rectal temperature (°C)	38.6 ± 0.3	39.5 ± 0.3
Heart rate (bpm)	55 ± 4	68 ± 4

3.2 Hormonal and Oxidative Stress Biomarkers

Exposure to HS significantly altered endocrine and oxidative profiles. Serum cortisol concentrations nearly doubled, increasing from 12.0 ± 3.0 µg/dL in TN to 22.0 ± 3.0 µg/dL in HS. In contrast, testosterone declined from 6.2 ± 0.8 ng/mL to 4.8 ± 0.8 ng/mL. Markers of oxidative stress further demonstrated the adverse effects of HS. Antioxidant enzyme activities were significantly lower during HS, including SOD (1250 ± 120 U/L vs. 1500 ± 120 U/L in TN), CAT (14 ± 2 kU/L vs. 18 ± 2 kU/L), and GPx (6.9 ± 0.9 U/mL vs. 8.5 ± 0.9 U/mL). Malondialdehyde (MDA), a key indicator of lipid peroxidation, rose from 1.9 ± 0.4 nmol/mL in TN to 3.0 ± 0.4 nmol/mL in HS, signifying increased oxidative damage. These comparisons are summarized in **Table 2**.

Table 2: Hormonal and Oxidative Stress Biomarkers in Bulls During Thermoneutral and Heat Stress Conditions

Parameter	Thermoneutral (TN)	Heat Stress (HS)
Cortisol (µg/dL)	12.0 ± 3.0	22.0 ± 3.0
Testosterone (ng/mL)	6.2 ± 0.8	4.8 ± 0.8
SOD (U/L)	1500 ± 120	1250 ± 120
CAT (kU/L)	18 ± 2	14 ± 2
GPx (U/mL)	8.5 ± 0.9	6.9 ± 0.9
MDA (nmol/mL)	1.9 ± 0.4	3.0 ± 0.4

3.3 Semen Characteristics

Heat stress had a pronounced impact on semen production and quality. Ejaculate volume declined from 5.0 ± 0.6 mL in TN to 4.6 ± 0.6 mL in HS. Sperm concentration fell from 1.2 ± 0.15 ×10^9/mL to 1.0 ± 0.15 ×10^9/mL, reducing total sperm output per ejaculate. Motility parameters were significantly impaired. Progressive motility decreased from 58 ± 6% in TN to 48 ± 6% in HS, while total motility dropped from 70 ± 6% to 60 ± 6%. CASA-derived kinematic parameters (VSL, VCL, VAC) also declined under HS conditions. Functional integrity of spermatozoa showed a consistent downward trend. Normal morphology decreased from 83 ± 4% in TN to 75 ± 4% in HS. Viability declined from 78 ± 5% to 68 ± 5%, acrosome integrity from 82 ± 4% to 73 ± 4%, and membrane integrity from 80 ± 5% to 70 ± 5%. DNA fragmentation index (DFI) increased sharply from 9 ± 2% in TN to 15 ± 2% in HS, indicating genomic instability. These differences are detailed in **Table 3**.

Table 3: Semen quality and functional parameters of bulls during thermoneutral and heat stress conditions

Parameter	Thermoneutral (TN)	Heat Stress (HS)
Ejaculate volume (mL)	5.0 ± 0.6	4.6 ± 0.6
Sperm concentration (×10^9/mL)	1.2 ± 0.15	1.0 ± 0.15
Progressive motility (%)	58 ± 6	48 ± 6
Total motility (%)	70 ± 6	60 ± 6
VSL (µm/s)	75 ± 8	68 ± 8
VCL (µm/s)	120 ± 12	110 ± 12
VAC (µm/s)	40 ± 6	35 ± 6
Normal morphology (%)	83 ± 4	75 ± 4
Viability (%)	78 ± 5	68 ± 5
Acrosome intact (%)	82 ± 4	73 ± 4
Membrane intact (%)	80 ± 5	70 ± 5
DNA Fragmentation Index (%)	9 ± 2	15 ± 2

3.4 Relationship Between Heat Load, Oxidative Stress, and Sperm Function

Regression analysis demonstrated a significant negative association between THI and progressive motility, confirming that sperm motility declines with increasing heat load (**Figure 1**).

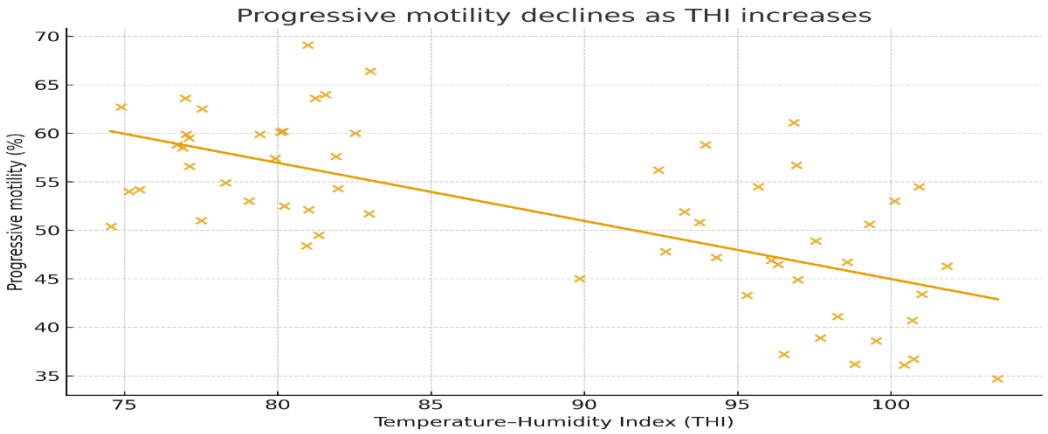


Figure 1: Relationship between thermal load (THI) and progressive sperm motility in bulls. A significant negative slope indicates declining motility with increasing THI.

Comparisons of DFI between periods revealed a sharp increase under HS, as shown in **Figure 2**, supporting the conclusion that heat stress compromises sperm DNA integrity.

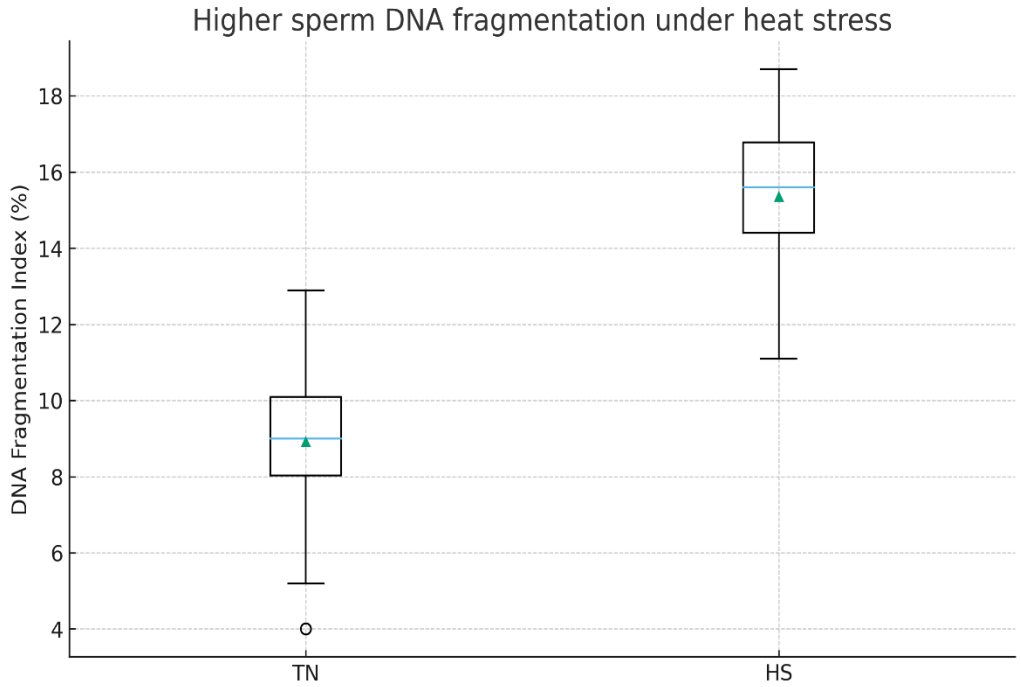


Figure 2: Comparison of sperm DNA fragmentation index (DFI, %) between thermoneutral (TN) and heat-stress (HS) periods. Boxplots show medians, interquartile ranges, and mean markers.

Within-bull analysis further confirmed these findings: most bulls exhibited a decline in progressive motility when moving from TN to HS **(Figure 3)**.

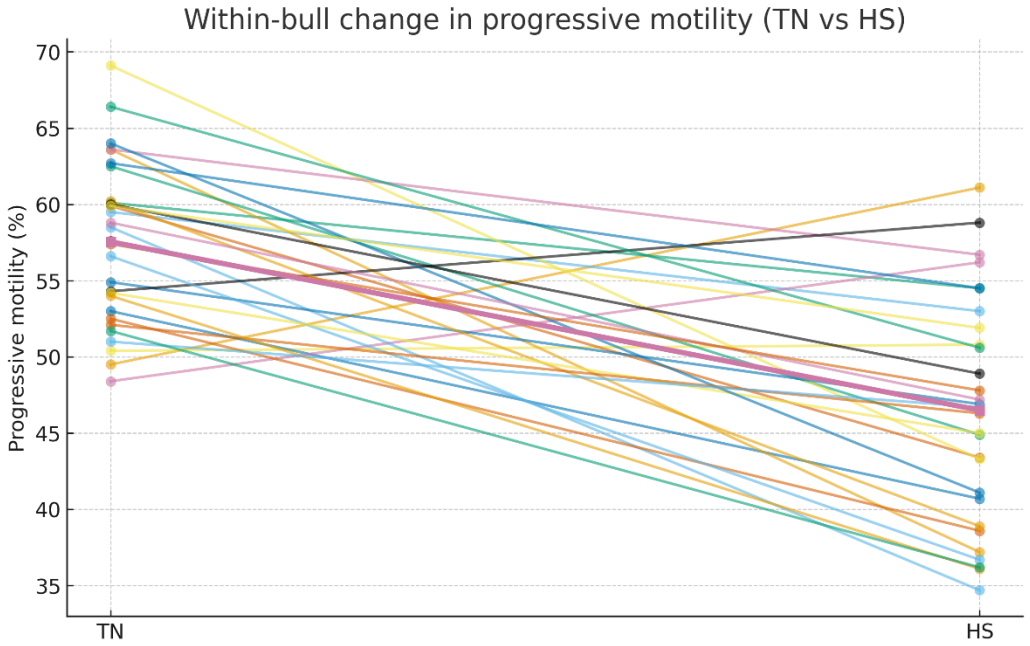


Figure 3: Paired trajectories of progressive motility for individual bulls across TN and HS periods. Most lines trend downward under HS, with a bold line representing the group mean.

Oxidative stress markers were closely linked to semen quality. A negative relationship between seminal plasma MDA and progressive motility was observed (**Figure 4**), highlighting the role of lipid peroxidation in impairing sperm function.

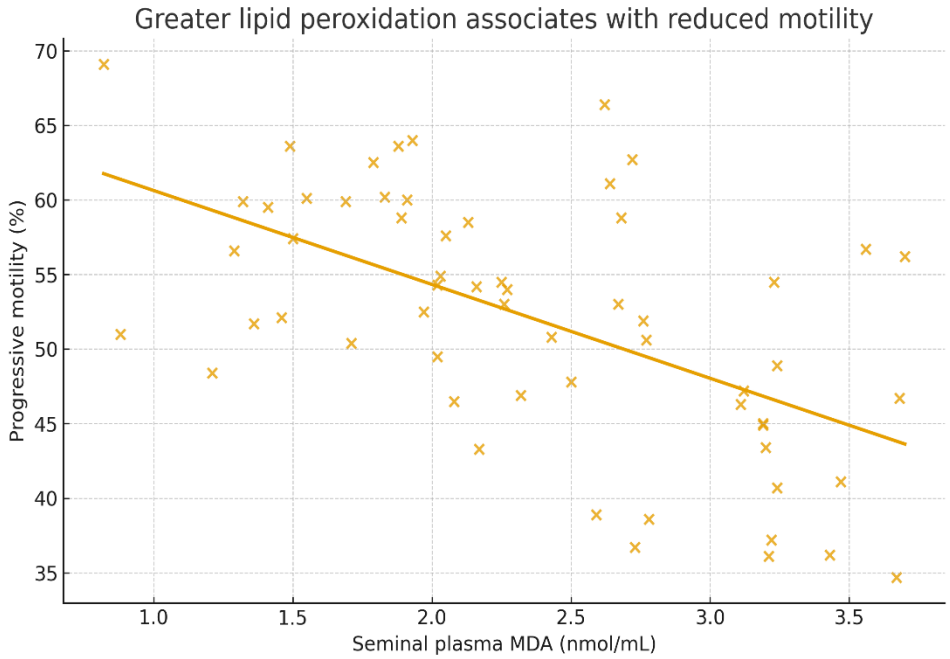


Figure 4: Association between seminal plasma malondialdehyde (MDA) concentration and progressive motility. Greater lipid peroxidation is associated with reduced motility.

Finally, key semen quality indicators, including progressive motility, morphology, and sperm concentration, were consistently lower in HS compared with TN. These comparisons are visualized in **Figure 5**, which reinforces the negative impact of heat stress on reproductive potential.

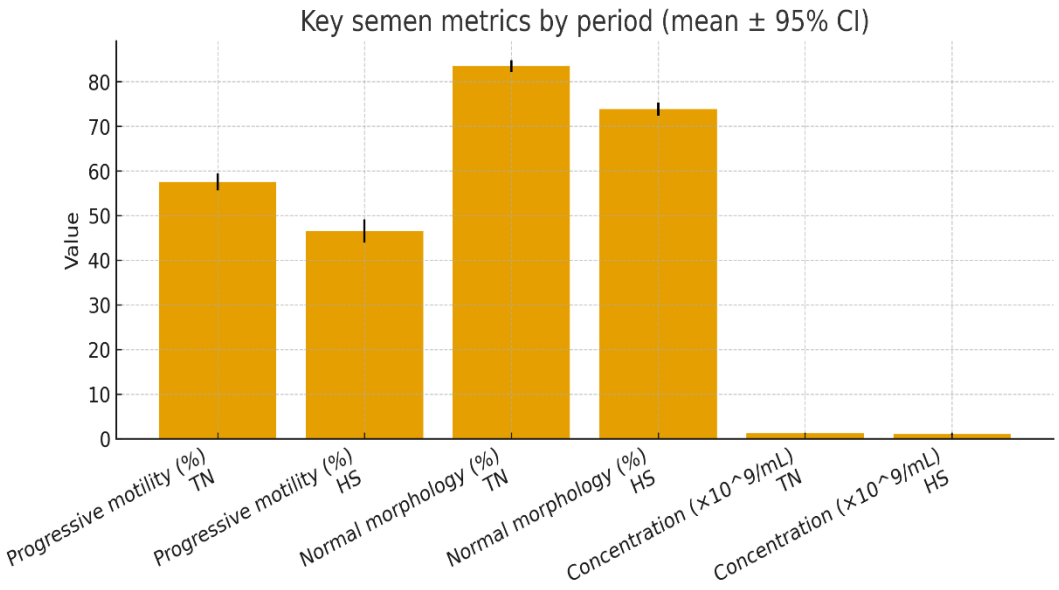


Figure 5: Mean values (±95% CI) for progressive motility, normal morphology, and sperm concentration by period, highlighting consistent reductions under heat stress.

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4. Discussion

In the present study, high thermal load (high THI and physiological strain) had deleterious effects on spermatogenesis and semen quality in bulls. These results are consistent with prior literature showing that environmental heat stress increases both rectal and scrotal temperatures, heart rate, and endocrine disruption. For instance, Capela et al. (2022) and Llamas-Luceño et al. (2020) observed that severe THI in Holstein bulls was associated with a decline in progressive motility, abnormal sperm morphology, and fertility. In the present study, it was clearly observed that there were high cortisol levels and low testosterone concentrations under heat-stress conditions, like the endocrinological responses analyzed by Celeghini et al. (2024), who reported the germ cell-stimulating action of testicular temperature elevation in Leydig cells and testosterone production. These physiological data validate that pervasive thermoregulatory failure is the cause of impaired reproductive performance.

Moreover, these findings emphasize the importance of oxidative stress in the adverse effects of heat on sperm function. The activities of SOD, CAT, and GPx decreased significantly with increasing MDA content in HS bulls. Hussain et al. (2023) have also shown the same oxidative imbalance of male infertility among species, and by Y. Wang et al. (2025), who underlined the cause-and-effect relationship between peroxidative injury and decreased sperm motility. Consistent with our results, Chitkara et al. (2025) employed omics studies and found that heat stress disrupted antioxidant defense mechanisms in bovine spermatozoa. According to Y. Wang et al. (2025), the harmonic correlation between MDA and progressive motility further supports an association between oxidative damage and functional sperm impairment. This similarity implies that oxidative imbalance is not only a

biomarker but also plays an important role in heat-induced reproductive dysfunction.

At the semen level, our work described significant reductions in progressive motility, concentration, morphology, viability, and membrane integrity with a substantial elevation of the DNA fragmentation index. These findings agree with those of Vanselow et al. (2024), who observed lower blastocyst rates and higher levels of sperm DNA damage in bulls challenged by summer heat. Netherton et al. (2022) and Alves et al. (2025) also found seasonal decreases in motility and morphology even in temperate environments, which suggests that increased THI is negatively associated with semen quality. The significant increase we detected in DFI is of particular importance, given that DNA damage strongly predicts impaired fertilization and embryonic development (Vanselow et al., 2024). The within-bull paired analysis in this study underlines these observations by presenting uniform individual declines and removing a large part of the between-animal variation presented in seasonally wider studies, including Popović et al. (2024). Altogether, these findings provide evidence that heat stress decreases sperm number and jeopardizes fertility outcomes by influencing both the genomic and functional integrity of gametes.

Finally, our results must be considered in the context of potential treatments and broader applications. The linear decrease in semen quality of bulls of all breeds in our data corroborates some earlier statements by Wettemann and Boehmer (2014) that heat stress induces damage to the spermatogenesis process across genetic backgrounds. However, Bos indicus would present partial resistance. The potential for genetic variation in thermal tolerance was also indicated by Raz et al. (2025), who presented gene–environment interactions that shaped the effect of oxidative stress responses. Although interventions were not investigated in our study, the oxidative stress proxies suggested that antioxidant supplementation might provide benefits, as demonstrated by J. Wang et al. (2025), who demonstrated that astaxanthin can also protect against heat-induced deterioration of sperm quality. The comparative approach in dogs (Burgio et al., 2024) and other species also confirms that the mechanisms we observe, oxidative stress, DNA damage, and decreased motility, are widely conserved, unequivocally underlining the translational impact of our data. Altogether, our results add to the accumulating evidence for heat stress as a significant problem in bovine fertility from a breeding perspective, in which antibiotics are potential breeding and nutritional therapies.

5. Conclusion

This study showed the detrimental effects of heat stress on spermatogenesis and semen quality in bulls, and such effects are commensurate with physiological, endocrine, and oxidative pathways. High THI exposure increased scrotal, testicular, and rectal temperatures, heart rate, and hormonal imbalance by increasing cortisol and reducing testosterone levels. At the same time, antioxidant defenses were significantly inhibited, and lipid peroxidation increased, which verified oxidative stress as a primary pathogenic mechanism. These metabolic adaptations are associated with severe defects in sperm quality, including decreased ejaculate volume, concentration, motility, shape, gut-quality parameters, reduced intrinsic and total sperm viability and membrane integrity, and a high DNA fragmentation index. Crucially, the analysis of within-bull paired samples identified consistent reductions in all males, which increased confidence in causality and decreased the potential for confounding. The current results confirm previous reports that seasonal heat stress negatively influences sperm function and fertility, but further correlate oxidative biomarkers with sperm quality outcome per se. The associations detected between MDA and motility, as well as between THI and sperm function, highlight the negative impact of increased environmental heat loads on bull fertility. From an applied perspective, our findings underscore the importance of targeted strategies to alleviate HS, such as environmental cooling, antioxidant feeding, and genetic selection, for thermotolerance in bulls. Given the risk of climate change, the preservation of male fertility is vital to secure cattle production, rendering the results of such studies crucial for breeding, animal welfare, and global food security.

6. Funding
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