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Seasonal Variation in Sperm Quality of Brahman Bulls under Consistently High Temperature-Humidity Index Conditions

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ABSTRACT

Bulls in humid tropical climates are constantly exposed to heat stress, affecting their reproductive performance. This study evaluated the effect of variables associated with season, especially temperature and humidity, on semen quality of Brahman bulls. A total of 39 ejaculates were analyzed from 12 Brahman bulls with ages at collection between 24 and 30 months. The temperature-humidity index (THI) was modeled as a continuous predictor variable; whereas, ejaculates were grouped into two THI cohorts for secondary exploratory analyses. These were based on sample medians of ≤80.1 and >80.1 points in a consistently high-THI tropical environment. Season was associated with variation in total motility (TM), progressive motility (PM), and sperm morphology. TM and PM were highest during the transitional season, whereas the percentage of normal sperm morphology was highest during the rainy season. Sperm curvilinear velocity (VCL), straight-line velocity (VSL), average path velocity (VAP), linearity index (LIN), straightness index (STR), and wobble (WOB) were significantly higher ($p<0.05$) during the dry season. Sperm viability did not differ significantly among seasons. Under the THI cohort comparison, significant differences ($p<0.05$) were observed in VCL, VAP, LIN, STR, and WOB, whereas TM, PM, viability, normal morphology, and VSL were not significantly affected. In conclusion, season was associated with variation in sperm motility, kinematic patterns, and morphology in Brahman bulls, whereas sperm viability remained relatively stable across seasons. The results do not suggest a beneficial effect of heat stress, but rather highlight the complexity of interpreting seminal quality responses under tropical conditions. In the continuous THI analysis, THI showed significant positive associations with TM and PM, whereas no significant associations were detected for the remaining kinematic variables, viability, or normal morphology.

Keywords: heat stress; climate change; fresh semen; reproduction; temperature-humidity index

INTRODUCTION

For bulls, heat stress has primarily been linked to the inability to regulate body temperature as well as testicular dysfunction (Gloria *et al*., 2021). Thermal stress furthermore affects spermatogenesis, which can limit sperm quality and fertility potential (Corte Pause *et al*., 2022; Freitas *et al*., 2020; Netherton *et al*., 2022). The inability to regulate body temperature can provoke reactive oxygen species production, which can exceed the natural antioxidant capacity, resulting in cellular oxidative stress (Fraczek *et al*., 2022; Hamilton *et al*., 2016; Wang *et al*., 2025). Likewise, these conditions have been shown to decrease sperm concentration, motility, and integrity (Freitas *et al*., 2020), as well as cellular DNA chromatin (Fraczek *et al*., 2022), microRNA (Celeghini *et al*., 2024), mitochondrial activity (Serrano-Jara *et al*., 2026), and plasma antioxidant capacity in animal ejaculates (Garcia-Oliveros *et al*., 2022; Sabés-Alsina *et al*., 2019). For this reason, the possibility of using bull ejaculates under these conditions for conservation and artificial insemination programs could be risky (Landaeta-Hernández *et al*., 2020). Therefore, it is necessary to study the effect of environmental factors such as temperature and humidity on animal stress, which can limit reproductive performance (Morrell, 2020).

The temperature-humidity index (THI) is a proven tool to calculate animal stress (Dunn *et al*., 2014; Habeeb *et al*., 2020; Popović *et al*., 2024) because it directly relates to bull sperm function (Landaeta-Hernández *et al*., 2020; Llamas-Luceño *et al*., 2020; Savvulidi *et al*., 2025). In the tropics, *Bos indicus* dominates breeding programs, as it can adapt to climatic factors better than *Bos taurus* (Alves *et al*., 2025). Within *B. indicus* breeds, nonetheless, there may be individual variations that compromise reproductive efficiency (Netherton *et al*., 2022). Because these climatic adaptation variations might affect reproductive performance, their study may still improve animal management and selection programs (Morrell, 2020; Poclín-Rojas *et al*., 2025).

High THI can impact bull reproductive behavior; there is no consensus, however, on how high THI must go to define an animal as under stress (Savvulidi *et al*., 2025). Some researchers have used THI values greater than 72 points as the threshold (Popović *et al*., 2024). Other studies of zebu breeds indicate that this value can rise above 74 points (Díaz *et al*., 2020). For buffalo, heat stress is defined as values greater than 68 (Umar *et al*., 2021). A recent study has proposed a categorical heat stress scale for *B. indicus* bulls under tropical conditions, where an animal is beginning to suffer heat stress with THI under 76 points, on alert from 76.2–79.2, in danger from 79.2–84.1, and in an emergency at greater than 84.1 points (Alves *et al*., 2025). Despite extensive research on the relationship between THI and semen quality, it is necessary to evaluate heat stress in Costa Rica's humid environment for the predominant Brahman breed (Vargas-Leitón *et al*., 2025), especially given changing climatic conditions.

This study used season and THI as complementary indicators of the thermal environment rather than interchangeable variables. Season reflects the overall climatic context, including concurrent changes in rainfall, solar radiation, and other environmental conditions that may affect thermoregulation and spermatogenesis (Amini & Behnam, 2026), whereas THI provides a quantitative estimate of combined heat and humidity load. Thus, season was treated as a broader ecological framework, while THI was used as a specific climatic proxy to examine whether variation in heat load was associated with changes in semen quality (Boonkum *et al*., 2025; Popović *et al*., 2024). We hypothesized that, within a consistently warm tropical environment, variation in season and THI would be associated mainly with changes in sperm motility and kinematic traits rather than viability or morphology. The study then evaluated the effect of season and THI on Brahman semen quality under tropical conditions.

MATERIALS AND METHODS

Data Availability Statement

The data for this study are included in this article and are available from the TECdatos Repository, Costa Rica Institute of Technology—data identification number: https://doi.org/10.18845/RDA/JBLPNT.

Ethical Statement

This study used bull sperm samples obtained in collaboration with the Agricultural Production Program (PPASC-026-2025) of the Costa Rica Institute of Technology. The collection was conducted in accordance with the laws and regulations for live animal experimentation in Costa Rica. Throughout the study, animals were handled to ensure their well-being and avoid unnecessary stress. The study adhered to ethical research principles, including the three Rs, and all procedures were strictly followed according to relevant guidelines and regulations to ensure ethical integrity and animal welfare. This research was approved by the Center for Research and Development of Sustainable Agriculture for the Humid Tropics of the Costa Rica Institute of Technology in session 20/2023 Article 1.0, DAGSC-188-2023 and CIE-206-2023. The study also adhered to the ARRIVE guidelines (https://arriveguidelines.org/).

Animals and Ejaculates Collection

The study was conducted at La Balsa Farm, owned by the Costa Rica Institute of Technology in Alajuela Province. A total of 12 Brahman (*B. indicus*) bulls were used, with an average age of 25.97±1.45 months and an average scrotal circumference of 34.77±2.61 cm at the start of the study. The animals were pastured on Ratana grass (*Ischaemum indicum*) and Mombasa grass (*Megathyrsus maximus*). Forage intake was not restricted, and salt, minerals, and water were provided *ad libitum*. The animals were kept in a rotational grazing system of approximately 18 ha, divided into seven paddocks with a grazing period of six days and a pasture rest period of 42 days. Sanitary management included deworming (e.g., Doramectin), vitamin supplementation (Catosal, Elanco, USA), and mineral supplementation (Matsuda Breed Top Line, Brasília, Brazil).

Prior to the study, the animals passed a breeding soundness evaluation test. A total of 39 ejaculates were collected at seven-day intervals using the electroejaculation technique described in Araya-Zúñiga *et al*. (2024). The prepuce area was cleaned, and excess hair was trimmed to avoid semen sample contamination. A gloved hand entered the animal's rectum to remove fecal matter and massage the accessory glands and prostate. A 75 mm Pulsator V® electroejaculator probe (Lane Manufacturing, Denver, CO, USA) was inserted into the animal's rectum with automatic mode on. The samples were collected directly from the animal's penis and transferred to sterile 15 mL Falcon tubes to be placed in a thermoregulated water bath (Cito Products Inc., Watertown, Wisconsin, USA) at 37 °C. Upon completion, all samples were transported in the thermoregulated water bath for less than 15 minutes to the animal reproduction laboratory of the Costa Rica Institute of Technology (AndroTEC) at the San Carlos campus. Figure 1 describes the experimental design.

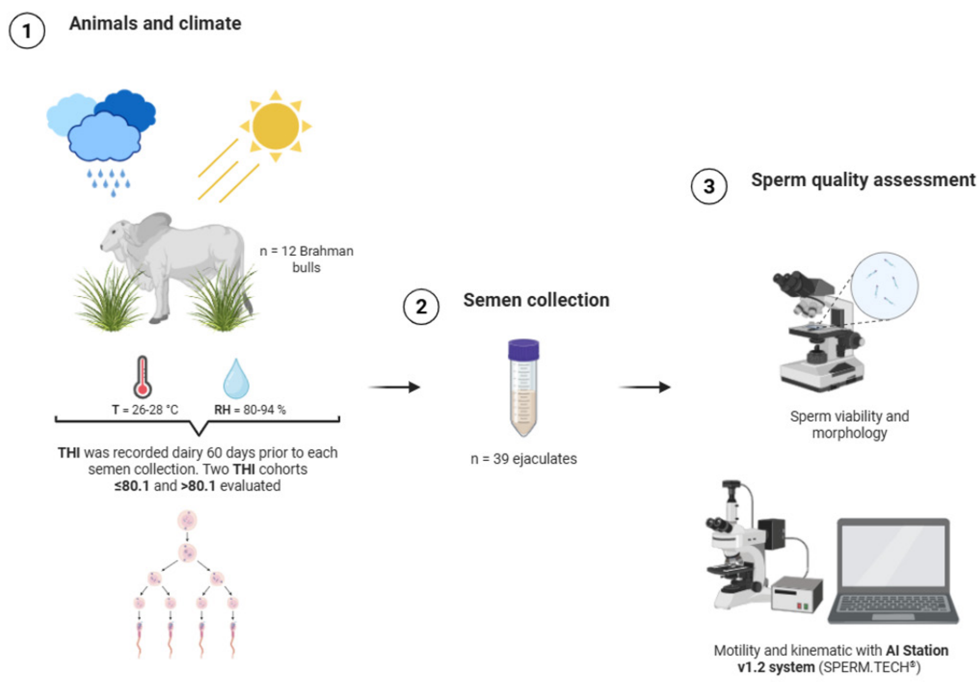

Figure 1. Experimental design used in the study. Temperature (T), relative humidity (RH), and temperature-humidity index (THI). ≤80.1 cohort (n = 20 ejaculates) and >80.1 cohort (n = 19 ejaculates).

Season, Climate Data, and Estimation of the Temperature-Humidity Index

Seasons were classified as dry (November to February), transitional (March to April), and rainy (May to October) (Castillo & Amador, 2020). To estimate THI, data were taken from the National Meteorological Institute of Costa Rica station located on the San Carlos campus (10°21′51.22″ N, 84°30′28.27″ W). The station is 1.58 km from the study farm. To determine THI, the average daily temperature (°C) and relative humidity (%) from the 60 days prior to each semen collection were recorded. The 60-day window corresponds to bull spermatogenesis of the same duration, relevant for assessing the cumulative effect of thermal stress on semen quality. Because ejaculates were collected at seven-day intervals, the 60-day pre-collection THI windows partially overlapped across consecutive samples, which may reduce the independence of thermal exposure estimates. This was addressed by including bull identity as a random effect, although residual temporal dependence cannot be excluded. Thus, mean THI during the 60 days before ejaculation was used as a proxy for climatic load, although it reflects ambient conditions from a nearby weather station rather than each bull's exact thermal exposure. The THI was calculated using the formula described in Rodriguez-Venegas *et al.* (2023), where T is the temperature in °C and RH is relative humidity (%):

$$THI = (1.8 \times T + 32) - [(0.55 - 0.0055 \times RH) \times (1.8 \times T - 26)]$$

THI values were higher than 76, the limit reported for describing an animal under thermal stress (Alves *et al.*, 2025). A more detailed analysis of semen quality variables according to observed THI values was carried out by grouping ejaculates obtained using the median as a discrimination factor, resulting in the following groups: ≤80.1 points (n = 20 ejaculates) and >80.1 points (n = 19 ejaculates), within a consistently high-THI tropical environment. Because the THI range was narrow, cohort-based comparisons were treated as secondary exploratory analyses rather than the primary basis for biological inference.

Motility and Sperm Kinematic Assessment

The analysis of sperm motility and kinematics was performed using an AI Station v1.2 system (SPERM.TECH®, Sperm Analysis Technologies S.L., Buñol, Spain). The bovine module had the following configuration: the cellular size between 6 and 69 μm^2, progressive sperm when STR > 15%, average path velocity (VAP) as 20 μm s^{-1} < slow < 25 < medium < 46 < rapid. Immotile sperm were defined as sperm swimming below 20 μm s^{-1} of VAP. Connectivity setting was fixed at 12 μm. For each analysis, 3 μL of semen was placed in a Spermtrack® counting chamber to a 20 μm depth (Proiser I+D., Paterna, Spain) preheated to 37 °C using a hot plate (Proiser R+D., Paterna, Spain) as described by Solís *et al.* (2024). A video camera (Ximea xiC, Marianka, Slovakia) was connected to a UB203 microscope (UOP/Proiser R+D) with a 1× eyepiece and a 10× negative phase contrast objective (NA 0.25). The camera captured images at 50 fps. The microscope's integrated stage was maintained at 37.0±0.5 °C when placing and analyzing semen samples. Two replicates were taken for each sample, and at least eight fields were captured for each replicate. The response variables obtained from the CASA analysis were total motility (TM, %) and progressive motility (PM, %). Additionally, the sperm kinematic variables of curvilinear velocity (VCL, μm s^{-1}), straight-line velocity (VSL, μm s^{-1}), and VAP (μm s^{-1}) were also measured, as well as the percentages of sperm straightness (STR) and linearity (LIN). Wobble (WOB) was calculated as a derived kinematic descriptor using the formula WOB = (VAP/ VCL) × 100.

Sperm Viability and Morphology

The analysis of viability was performed by placing 5 μL of semen on a slide and then adding 5 μL of the Eosin-Nigrosin stain (Mortimer® Formula, Humeco, Huesca, Spain). A sample smear on a microscope slide was dried at 25 °C for 10 minutes. Afterward, a coverslip was placed over the sample, and analyzed using a UB203 phase-detection microscope (UOP / Proiser R + D) at 400X. A total of 200 sperm cells were evaluated for each sample, and the percentages of viable and non-viable cells were reported. For sperm morphology assessment, 5 μL of the previously diluted semen sample was first placed on a slide. A coverslip was then placed over it and fixed using Trumorph® (Proiser R + D, SL, Paterna, Spain), following protocols described by Sevilla *et al*. (2025). Morphologically normal sperm were defined according to Perry (2021) as cells showing normal head, midpiece, and tail morphology and the absence of cytoplasmic droplets. Sperm abnormalities were classified as head, midpiece, tail, or cytoplasmic droplet defects. Morphology assessment was performed by the same trained evaluator, blind to season and THI classifications. Once samples were fixed, they were evaluated in a UB203 negative phase microscope at 400X.

Statistical Analysis

For each continuous response, model residuals were evaluated for normality and homoscedasticity using Q-Q plots and residual-vs-fitted plots; Shapiro-Wilk and Levene-type assessments were supporting diagnostics. The assumption of normal distribution was verified by using a normal probability plot. Continuous semen traits, including TM, PM, VCL, VSL, VAP, LIN, STR, and WOB, were analyzed using linear mixed-effects models. Because repeated ejaculates were obtained from the same bull, bull identity was included as a random intercept to account for within-animal correlation. Age at collection was included as a covariate in the season and season × THI models. Although bull was included as a random effect, the weekly sampling design and overlapping 60-day THI windows may also have introduced temporal autocorrelation not explicitly modeled.

For the evaluation of the season, the following model was fitted for each continuous response variable:

$$y_{ij} = \mu + Si + \beta_1 \text{THI}_{ij} + \beta_2 \text{Age}_{ij} + uj + \varepsilon_{ij}$$

where y_{ij} is the observed semen trait for ejaculate i from bull j, μ is the overall mean, Si is the fixed effect of season (dry, transitional, or rainy), uj is the random effect of bull ($uj \sim N(0, \sigma_{bull}^2)$), Age_{ij} is age at collection for ejaculate i from bull j, and ε_{ij} is the residual error ($\varepsilon_{ij} \sim N(0, \sigma^2)$).

For THI, a separate model was fitted using THI as a continuous covariate:

$$y_{ij} = \mu + \beta_1 \text{THI}_{ij} + u_j + \varepsilon_{ij}$$

This model was intentionally fitted without adjustment for age at collection because its objective was to characterize the marginal association between continuous THI and semen-quality traits within the relatively narrow age range represented in the study, rather than to estimate an age-adjusted independent effect of THI. Age was retained as a covariate in the season and season × THI models because those analyses were intended to evaluate seasonal differences while accounting for potential age-related variation across the sampling period. Accordingly, results from the continuous THI model were interpreted as exploratory associations rather than causal effects.

A season × THI interaction was explored as:

$$y_{ij} = \mu + S_i + \beta 1\ \text{THI}_{ij} + \beta_2 \text{Age}_{ij} + (S_i \times \text{THI}_{ij}) + u_j + \varepsilon_{ij}$$

Because THI represents temperature and relative humidity, these variables were not included simultaneously with THI in the same model. As a secondary descriptive analysis, ejaculates were also grouped into two THI cohorts according to the sample median (≤80.1 and >80.1). Viability and normal morphology were analyzed as binomial responses using the numbers of viable/non-viable and normal/abnormal sperm out of 200 counted cells per sample. When effects were significant, mean comparisons were carried out using the Bonferroni test. The results were expressed as mean ± standard error of the mean. Statistical significance was defined as $p<0.05$. All data were analyzed using IBM SPSS, version 29.0.0.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Table 1 shows the effect of season on sperm TM, PM, viability, and morphology. A significant effect of season was found on TM, which was higher during the transition. A similar effect was determined for PM, which was lower during the dry and rainy seasons ($p<0.05$). Sperm viability did not differ significantly

Table 1. Model-adjusted means (± SEM) for bull sperm total motility (TM), progressive motility (PM), viability, and normal sperm morphology according to season

Percentage	Season*		
	Dry	Transitional	Rainy
TM	64.98±2.82[c]	74.84±1.41[a]	60.52±2.78[b]
PM	62.65±2.82[b]	70.85±1.41[a]	57.34±2.78[c]
Viability	70.93±6.47	84.57±3.76	83.72±5.48
Normal sperm	80.30±5.84[b]	77.66±3.37[b]	95.04±4.83[a]
Abnormal sperm	19.70±5.84[a]	22.34±3.37[a]	4.96±4.83[b]

Note: SEM=standard error of the mean; TM=total motility; PM=progressive motility. * The dry season (n = 12 ejaculates) from November to February, the transitional season (n = 13 ejaculates) from March to April, and the rainy season (n = 14 ejaculates) from May to October were considered. [a-c] Means in the same row with different superscripts differ significantly ($p<0.05$).

among seasons. Season was significantly associated with sperm morphology, with the rainy season showing a higher percentage of normal sperm morphology and a correspondingly lower percentage of abnormal sperm than the dry and transitional seasons ($p<0.05$). THI behavior was analyzed for each season. Mean THI increased significantly from the dry to the rainy season ($p<0.05$). The mean during the dry season was 79.51±0.03 points, the transitional 80.17±0.10 points, and the rainy season was 80.64±0.08 points.

In the continuous THI mixed model, fitted at the ejaculate level with bull as a random effect, THI showed significant positive associations with TM (β = 9.674, SE = 4.864, p = 0.047) and PM (β = 9.365, SE = 4.767, p = 0.049). In contrast, THI was not significantly associated with VCL (β = 13.302, SE = 17.830, p = 0.456), VSL (β = 1.605, SE = 10.686, p = 0.881), VAP (β = 11.089, SE = 11.395, p = 0.330), LIN (β = -1.383, SE = 2.433, p = 0.570), STR (β = -3.309, SE = 1.971, p = 0.093), WOB (β = 1.571, SE = 1.633, p = 0.336), viability (β = -0.172, SE = 3.918, p = 0.965), or normal morphology (β = 2.941, SE = 4.281, p = 0.492) (Table 2).

Table 2. Estimated fixed effects of the continuous temperature-humidity index (THI) on semen quality traits in Brahman bulls from mixed-effects models fitted at the ejaculate level with bull included as a random effect

Variables	β THI	SE	p-value
TM	9.674	4.864	0.047
PM	9.365	4.767	0.049
VCL	13.302	17.830	0.456
VSL	1.605	10.686	0.881
VAP	11.089	11.395	0.330
LIN	-1.383	2.433	0.570
STR	-3.309	1.971	0.093
WOB	1.571	1.633	0.336
Viability	-0.172	3.918	0.965
Normal morphology	2.941	4.281	0.492

Note: Continuous THI models were fitted without adjustment for age at sampling to estimate the marginal association between THI and each semen-quality trait. Bull was included as a random effect to account for repeated measurements within animals. SE=standard error; TM=total motility; PM=progressive motility; VCL=curvilinear velocity (μm s^{-1}); VSL=straight-line velocity (μm s^{-1}); VAP=average path velocity; LIN=linearity of forward progression (%); STR=straightness index (%); WOB=wobble (%). THI: temperature-humidity index.

Analysis of sperm kinematic variables across different seasons revealed a significant effect ($p<0.05$). Sperm had higher velocities during the dry season (Table 3). Regarding sperm kinematic patterns, the variables LIN, STR, and WOB were significantly higher during the dry season ($p<0.05$).

Figure 2 reports the behavior of relative humidity, temperature, and THI in each ejaculate analyzed. The temperature during the study remained between 26 °C and 28 °C, while the relative humidity ranged from 80% to 94%. THI fluctuated from 79.44 to 81.01. Based on THI data for the 39 ejaculates, two cohorts emerged: ≤80.1 (n = 20 ejaculates) and >80.1 (n = 19 ejaculates). No significant differences between THI cohorts were detected for TM, PM, viability, or normal sperm morphology ($p>0.05$) (Figure 3).

Table 4 shows the effect of THI on sperm cell kinematic variables. Comparing kinematic variables between THI cohorts revealed significant differences ($p<0.05$) in VCL, VAP, LIN, STR, and WOB. No significant effect of the THI cohort was detected for VSL ($p>0.05$). LIN and WOB were significantly greater in the ≤80.1 cohort, whereas STR was higher in the >80.1 cohort ($p<0.05$).

The interaction between season and THI was also explored. It was not significant for TM, PM, viability, and normal sperm morphology ($p>0.05$). On the other

Table 3. Model-adjusted means (± SEM) for sperm kinematic parameters according to season in Brahman bulls

Variables	Season*		
	Dry	Transitional	Rainy
VCL	343.37±16.54^{a}	321.75±5.32^{b}	322.07±14.97^{b}
VSL	208.53±12.65^{a}	157.06±4.07^{b}	139.35±11.45^{b}
VAP	261.25±12.30^{a}	208.20±3.96^{b}	190.91±11.14^{b}
LIN	58.92±2.62^{a}	45.38±0.84^{b}	39.97±2.37^{c}
STR	69.32±2.22^{a}	66.02±0.71^{b}	65.18±2.01^{b}
WOB	77.64±1.82^{a}	64.71±0.59^{b}	59.10±1.65^{c}

Note: SEM=standard error of the mean; VCL=curvilinear velocity (μm s^{-1}); VSL=straight-line velocity (μm s^{-1}); VAP=average path velocity; LIN=linearity of forward progression (%); STR=straightness index (%); WOB=wobble (%). * The dry season (n = 12 ejaculates) from November to February, the transitional season (n = 13 ejaculates) from March to April, and the rainy season (n = 14 ejaculates) from May to October were considered. $^{a-c}$ Means in the same row with different superscripts differ significantly ($p<0.05$).

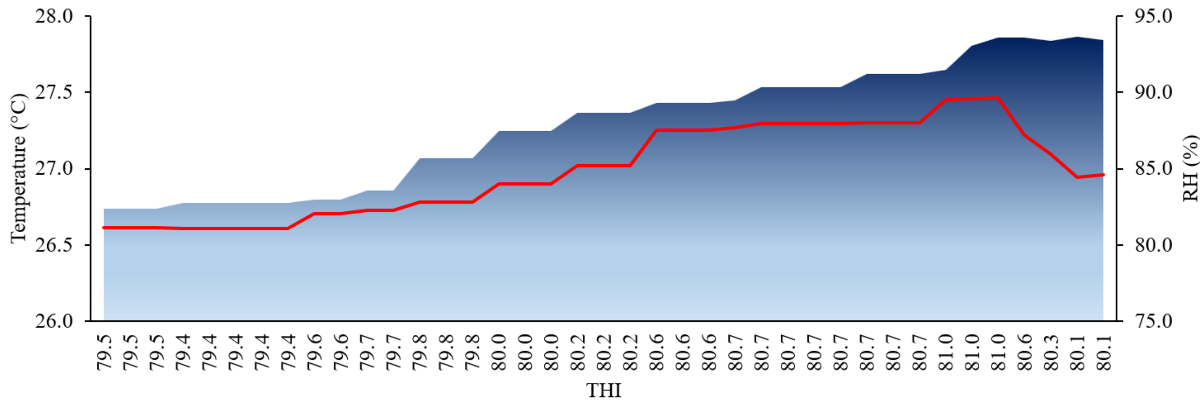

Figure 2. Mean temperature-humidity index (THI), relative humidity (RH, %), and daily temperature (°C) of the 60 days prior to the collection of each of the thirty-nine ejaculates analyzed during the study. Note : Relative humidity, Temperature

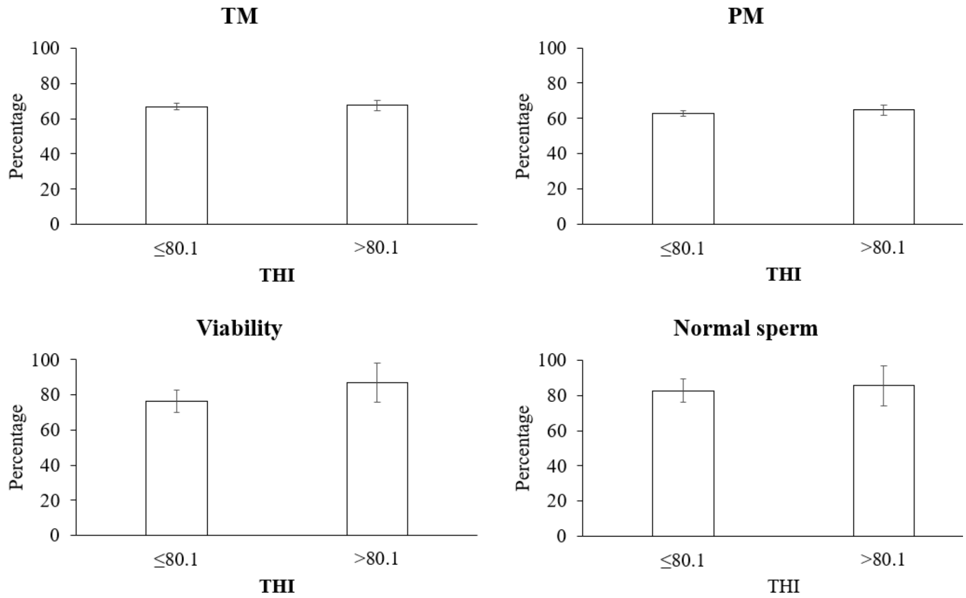

Figure 3. Descriptive comparison of sperm total motility (TM), progressive motility (PM), viability, and normal sperm morphology between temperature-humidity index (THI) cohorts [≤80.1 (n = 20 ejaculates) and >80.1 (n = 19 ejaculates)] in Brahman bulls. No statistically significant differences were detected between cohorts (mixed-model p>0.05 for all traits).

Table 4. Model-adjusted means (± SEM) for sperm kinematic variables according to temperature-humidity index (THI) cohort in Brahman bulls

Variables	THI	
	≤80.1	>80.1
VCL	317.62±6.15[a]	339.08±10.21[b]
VSL	160.16±7.81[a]	160.34±4.70[a]
VAP	208.00±7.59[a]	214.49±4.57[b]
LIN	47.12±0.97[a]	44.14±1.62[b]
STR	64.49±0.83[b]	69.12±1.37[a]
WOB	67.97±0.68[a]	60.67±1.12[b]

Note: SEM= standard error of the mean; THI= temperature-humidity index; VCL= curvilinear velocity (μm s^{-1}); VSL= straight-line velocity (μm s^{-1}); VAP= average path velocity; LIN= linearity of forward progression (%); STR= straightness index (%); WOB= wobble (%). * The ≤80.1 cohort included 20 ejaculates and the >80.1 cohort included 19 ejaculates. [a-b] Means in the same row with different superscript differ significantly (p<0.05).

hand, when analyzing kinematic sperm variables, a non-significant effect on their behavior was also seen (p>0.05).

DISCUSSION

The effect of tropical environmental conditions on bull semen quality has been previously documented (Chacón *et al*., 2022; Poclín-Rojas *et al*., 2025; Sevilla *et al*., 2025). Our results indicate that season was associated with variation in several semen-quality traits in Brahman bulls. Total and progressive motility were highest during the transitional season, whereas sperm kinematic variables were generally higher during the dry season. In contrast, the percentage of morphologically normal sperm was highest during the rainy season, while sperm viability did not differ significantly among seasons. Therefore, seasonal variation was not restricted to sperm motility and kinematics but also involved sperm morphology. These findings partially support our initial hypothesis, as viability remained relatively stable across seasons, whereas morphology showed significant seasonal variation. Importantly, the seasonal variation observed in sperm morphology should not be interpreted as a direct THI-associated response. Normal morphology was not significantly associated with THI in the continuous analysis and did not differ significantly between the two THI cohorts. This distinction suggests that the seasonal pattern in sperm morphology may reflect broader seasonal conditions not fully captured by THI alone, which integrates temperature and relative humidity but does not account for other potentially relevant environmental factors. This pattern is consistent with previous reports in bulls under tropical conditions (Alves *et al*., 2025). It has been found that although some *B. indicus* animals exhibit adaptation to tropical conditions, a marked individual effect on semen production and quality can be observed (Alves *et al*., 2025; Koivisto *et al*., 2009; Netherton *et al*., 2022). It has been shown, however, that even when animals are under the same exposure scenarios to adverse weather conditions, heat stress can manifest differently (Arias & Mader, 2023; Morrell, 2020). High temperatures alter spermatogenesis and morphology and motility during the spermatozoan journey through the epididymis (Boe-Hansen *et al*., 2020; Freitas *et al*., 2020). These effects that occur during cell formation, development, and maturation relate to the presence of oxidative stress resulting from an imbalance between antioxidant capacity and free radical production (Hamilton *et al*., 2016; Morrell, 2020; Sabés-Alsina *et al*., 2019).

Previous studies have shown that these conditions can be more influential on testicles according to the blood irrigation capacity via the pampiniform plexus, which varies between subspecies and individuals (Brito *et al*., 2004). For spermatogenesis to occur normally, the animal needs to maintain the testicular temperature 2–6 °C below that of the rest of the body (Kastelic *et al*., 2021). Failure to maintain such homeostasis, therefore, can alter semen quality, including variations in seminal plasma composition (Vince *et al*., 2018). This can also cause problems when the sperm and egg recognize each other, resulting in decreased potential fertility of the male (Llamas-Luceño *et al*., 2020). On the other hand, heat can also affect normal mitochondrial functioning, specifically by altering oxidation of the electron transport chain complexes responsible for normal ATP production (Belhadj Slimen *et al*., 2016; Capela *et al*., 2022; Nichi *et al*., 2006).

Our results generally reveal THI values higher than the 76-point threshold traditionally reported in the literature as heat stress (Alves *et al*., 2025; Rodriguez-Venegas *et al*., 2023). Overall, temperatures and relative humidity were relatively similar across the 39 ejaculates analyzed. By observing their behavior during the 60 days prior to each semen collection, we gathered as much information as possible related to the hypothetical conditions during spermatogenesis. Study results show that, regardless of season, THI values were consistently high and varied within a limited interval, which restricted our ability to compare thermoneutral versus clearly heat-stressed conditions. Because THI integrates temperature and relative humidity, the persistently high humidity observed during the study may have contributed to the relatively narrow THI range despite modest variation in ambient temperature (Dunn *et al*., 2014).

In the tropics, particular conditions of high relative humidity have also been noted as associated with higher THI values during the summer (Kumar *et al*., 2019). Our results show that, despite the effect of season, the relative humidity during the study was almost constant and considerably high, ranging from 80%–94%. Other studies in Zebu breeds have demonstrated a trend whereby, as the THI increases, the quality of sperm decreases under tropical conditions (Alves *et al*., 2025). This has been observed with variables such as sperm morphology, motility, volume, and ejaculate concentration (Freitas *et al*., 2020; Garcia-Oliveros *et al*., 2022; Seifi-Jamadi *et al*., 2019). The differences from previous studies may reflect breed-specific adaptation, the narrow THI variation here, exposure quantification variations, and the modest sample size, rather than a true reversal of the general heat-stress pattern. One possible explanation is that unmeasured nocturnal cooling may have partially mitigated the impact of daytime heat load (Freitas *et al*., 2020); however, this remains speculative because nighttime thermal indices were not analyzed in the present study.

We also explored sperm quality characteristics in both cohorts. Our results show that within the THI cohorts, no significant differences were observed in TM, PM, viability, normal sperm morphology, and VSL, while there were significant variations in VCL, VAP, LIN, STR, and WOB. These differences suggest changes in sperm motile patterns rather than uniform improvement or deterioration in semen quality. Previous studies have shown that sperm motility and velocity tend to decrease with higher THI (Landaeta-Hernández *et al*., 2020; Rahbar *et al*., 2025). This phenomenon could be related to the role of Ca^{2+} ions in the sperm flagellum (Abd El-Emam *et al*., 2023). During their movement, high thermal stress can prematurely activate the sperm cell (Swain *et al*., 2025). Because all ejaculates were collected under consistently high THI conditions, corresponding to a heat-stress range previously proposed for *B. indicus* bulls under tropical conditions (Alves *et al*., 2025), and because the THI range was narrow, these findings reflect variation within a generally heat-challenged tropical environment, rather than contrasts between thermoneutral and severely heat-stressed conditions. These findings further suggest that higher THI was associated with altered sperm motility patterns rather than a uniform increase in all kinematic traits. A recent study has shown that these heat-induced effects can alter the function of Ca^{2+}/calmodulin-dependent protein kinase II, which has been linked to early sperm acrosome reaction and fertility problems (Santos *et al*., 2025). Previous studies conducted in European bull breeds have reported reductions in membrane integrity and sperm morphology with increasing THI (Gloria *et al*., 2021). In the present study, however, neither viability nor normal morphology was significantly associated with continuous THI or differed significantly between THI cohorts. Therefore, no clear THI-related pattern could be established for these traits under the relatively narrow and consistently high THI range evaluated. In this context, although breeds with some degree of adaptability to tropical conditions are already used, selection for animals with greater reproductive resilience to heat stress warrants further investigation (Capela *et al*., 2022; Livernois *et al*., 2018; Slayi & Jaja, 2025). Because semen-quality traits may have a heritable component, selection for heat-resilient reproductive phenotypes warrants further investigation (Vanselow *et al*., 2024).

In the continuous THI analysis, thermal load was not consistently associated with semen quality traits. Although TM and PM showed statistically significant positive associations with THI, no significant relationships were found for the remaining kinematic variables, viability, or normal morphology. This pattern indicates that, under the relatively homogeneous and generally high THI conditions recorded in this study, continuous THI variation had limited explanatory value for most semen traits.

For this reason, indicators of thermal comfort, such as THI, provide more information for decision makers (Mader *et al*., 2006; Souto *et al*., 2022), whether for natural mating or germplasm conservation and reproductive biotechnologies. One limitation of this work, however, is the need to complement conventional semen analysis with more complex techniques such as omics science, which provide a broader view of what might be happening inside the sperm cell (Celeghini *et al*., 2024; Chitkara *et al*., 2025; Kumar *et al*., 2024).

CONCLUSION

This study indicates that season is associated with variation in sperm motility, kinematic patterns, and morphology in Brahman bulls raised under humid tropical conditions. Total and progressive motility were highest during the transitional season, whereas the percentage of morphologically normal sperm was highest during the rainy season; sperm viability remained relatively stable across seasons. Continuous THI analysis showed positive associations with total and progressive motility; however, these findings should be interpreted cautiously because they occurred within a narrow and consistently high THI range, without confirmation by the categorical THI-cohort comparison. Moreover, neither viability nor normal morphology was significantly associated with continuous THI, indicating that the seasonal variation observed in morphology should not be attributed directly to THI. The results do not suggest a beneficial effect of heat stress, but rather highlight the complexity of interpreting semen quality responses under tropical conditions in adapted *B. indicus* bulls. The modest sample size, high interindividual variability, and limited environmental contrast influence the final interpretation. Further studies integrating larger datasets, fertility outcomes, and cellular or molecular indicators of sperm function are needed before these traits can be used to select breeding bulls under humid tropical conditions.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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DECLARATION OF GENERATIVE AI AND AI-ASSISTED TECHNOLOGIES IN THE WRITING PROCESS

During the writing process, none of the authors used generative AI or AI-assisted technologies.

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