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Effect of Wet-Bulb Globe Temperature Index (WBGTi) on the seminal quality of Quarter Horse stallions in Los Tuxtlas region, Veracruz

Efecto del WBGTi sobre la calidad seminal de garañones "Cuarto de Milla" en la región de los Tuxtlas, Veracruz

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ABSTRACT

The study aimed to evaluate the effect of climate, measured through the Wet-Bulb Globe Temperature index (WBGTi), on the seminal quality of Quarter Horses in a horse farm located in Los Tuxtlas, Veracruz, in a tropical environment. Meteorological variables, including ambient temperature and relative humidity, were assessed over nine years (2013-2021) to determine the daily and monthly average WBGTi. These values were then related to the macroscopic (volume) and microscopic characteristics (motility percentage, vitality, normal spermatozoa, and sperm concentration) of semen obtained from five stallions. A thermally comfortable environment (WBGTi ≤ 26.99), falling within the thermoneutral zone of horses, was observed from December to February. In contrast, from March to October, the environment was classified as thermally cautious or stressful (WBGTi = 27 to 36.80). A significant difference ($p \leq 0.05$) was found in the percentage of mobility (73.75 ± 2.58 *vs* 67.25 ± 1.29 in precautionary environment and 65.99 ± 3.32 in stressful environment), vitality (79.62 ± 2.22 *vs* 74.71 ± 1.10, 73.69 ± 2.82) and normal spermatozoa (66.95 ± 1.55 *vs* 62.84 ± 0.78, 61.51 ± 1.99), being lower during the warmest period. Sperm volume and concentration were not significantly affected by the WBGTi increase, and none of the seminal characteristics exhibited seasonal patterns. It was concluded that an increase in heat, as measured by WBGTi, negatively impacted the seminal quality of stallions.

KEY WORDS Horses, Seminogram, Heat index, Tropical climate, Heat stress.

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RESUMEN

Con el objetivo de estudiar el efecto del clima a través del índice global del bulbo húmedo (WBGTi), sobre la calidad seminal de garañones Cuarto de Milla, en un criadero ubicado en Los Tuxtlas, Veracruz, en ambiente tropical. Se evaluaron las variables meteorológicas temperatura ambiental y humedad relativa durante nueve años (2013-2021), para determinar el WBGTi diario y promedio mensual, relacionándolo con las características macroscópicas (volumen) y microscópicas (porcentaje de movilidad, vitalidad, espermatozoides normales y la concentración espermática) del semen obtenido de cinco sementales. Hubo un ambiente de confort térmico de diciembre a febrero (WBGTi ≤ 26.99), dentro de la zona termo-neutral de los caballos; mientras que de marzo a octubre fue clasificado como térmicamente precautorio o estresante (WBGTi = 27 a 36.80). Se observó diferencia significativa ($p \leq 0.05$) en el porcentaje de movilidad (73.75 ± 2.58 *vs* 67.25 ± 1.29 en ambiente precautorio y 65.99 ± 3.32 en estresante), vitalidad (79.62 ± 2.22 *vs* 74.71 ± 1.10, 73.69 ± 2.82) y espermatozoides normales (66.95 ± 1.55 *vs* 62.84 ± 0.78, 61.51 ± 1.99), siendo menor durante el periodo más caluroso. El volumen y concentración espermática no se vieron influenciadas por el aumento del índice. Ninguna de las características seminales tuvo un comportamiento estacional. Se concluye que el incremento del calor, medido en términos de WGBTi, tuvo un efecto negativo sobre la calidad seminal de los garañones.

KEY WORDS: Caballos, Seminograma, Índice de calor, Clima tropical, Estrés térmico.

Introduction

Horses of different breeds possess an efficient capacity to maintain thermal equilibrium, though this ability depends on the climatic environment in which they are breeding (Castanheira *et al.,* 2010). Extremely hot-humid environments hinder evaporative thermoregulation mechanisms, pushing the animal out of its thermo-neutral or comfort zone. This requires additional energy expenditure to keep body temperature within stable physiological limits, leading to heat stress. This induces a series of metabolic changes and reproductive alterations (Clavinder, 2020; Hansen, 2009; Kingma *et al.,* 2012). The spermatogenic cycle in horses is a thermo-dependent process, operating optimally at temperatures between 33 and 35 °C (Shakeel & Yoon, 2023). When testicular hyperthermia occurs (>35 °C), spermatogenesis undergoes several modifications, resulting in low-quality spermatozoa with damaged DNA, which leads to fertility problems (Kandiel & El-Khawagah, 2018; Shakeel & Yoon, 2023).

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The heat index, known as the Wet-Bulb Globe Temperature Index (WBGTi), is a parameter used to evaluate the thermal sensation of animals under specific meteorological conditions, determining whether they are exposed to a stressful environment. Based on its value, the index can be classified into the following categories: comfort (thermo-neutral zone), caution (possible fatigue due to prolonged sun exposure or physical activity without overt stress), extreme caution (signs of heatstroke, dehydration, and heat stress), danger (heatstroke with clear signs of stress), and extreme danger (imminent heatstroke, often leading to death) (AEMET, 2014; Domínguez, 2015; Kingma *et al.,* 2012).

Quarter Horses (QH) are renowned for their speed in short-distance races, a trait that has been a selection criterion in genetic improvement programs for the breed. This has increased the number of offspring from the best-rated stallions (Petersen *et al.,* 2014). QH is the most populous breed in Mexico; in 2019, there were 47,719 registered specimens, making Mexico the third-largest country in the world for registered QH (HorsesMx, 2021). The breeding of this lineage has generated an industry with annual economic growth of up to 10 %, providing the livestock sector with earnings of up to USD 340 million per racing season, creating jobs, and fostering the development of micro-enterprises throughout the equestrian and livestock production chain (HorsesMx, 2021; Corona, 2018; Mihok & Castejón, 2016).

A significant portion of these animals reside in hot and humid regions of Mexico, such as the central region of the Veracruz state, which has the largest national horse population (INEGI, 2007). Veracruz experiences extreme heat during most of the year, causing considerable physiological stress in horses (Domínguez, 2015). Although seemingly minor in numerical terms, these climatic conditions have a profound impact. For instance, a global temperature increase of just 0.1 °C over a decade has led to substantial changes in the composition of the earth, and consequently, in the availability of raw materials for the maintenance of living beings, as well as an increase in the frequency of heat waves that hinder the ability of animals to maintain thermal balance (Hernández *et al.,* 2023; Tejeda *et al.,* 2020).

Given this background, this research aimed to analyze the climatic conditions over eight years and their effect on the seminal quality of Quarter Horse (QH) stallions in the region of Los Tuxtlas, Veracruz, Mexico.

Material and Methods

Location

The study was conducted at “Haras Ixbiapan,” a ranch located in the San Andrés Tuxtla municipality, in Los Tuxtlas region. The ranch is located at coordinates 18°27’ N and 95°13’ W, at an altitude of 420 masl, and experiences a humid tropical climate classified as type Aw (Gutiérrez-García & Ricker, 2011).

Animals

Five sexually mature and clinically healthy QH stallions, ranging in age from 4 to 16 years, were used in the study. Throughout the study, the animals were housed in individual stalls measuring 3.5 by 4 meters. They were all fed the same ration of commercial concentrates, Omolene (Purina®; Mexico) and Pel Roll (Malta Cleyton®; Mexico), along with seasonal forage (*Pennisetum purpureum* and *Digitaria ciliaris*), had water available *ad libitum* and received uniform general management.

Semen collection and sample analysis

Seminal samples were collected using the artificial vagina technique (Hernández & Fernández, 2010; Zarco & Boeta, 2000) from 2013 to 2021. Each horse was sampled at least once a month, though on some occasions, not all horses were sampled, following the requests of the owner. In total, 267 ejaculates were collected. Each sample was collected in full, with the sperm-rich fraction separated from the gel, smegma, and other impurities. Once collected, the semen was immediately transported to a processing room 30 meters away. The semen parameters evaluated in the seminogram were volume (ml), motility (%), concentration (10^6/ml), morphology (%), and vitality (%). A standardized protocol was followed to minimize variability, using materials designed for the purpose and adhering to established semen analysis techniques for this species (Boichard *et al.,* 2016; Castro & Gonzalez, 2019; Restrepo *et al.,* 2013).

For macroscopic analysis, the gel-free fraction was placed in a calibrated container to measure seminal volume, and the results were compared with established reference values (Table 1). In microscopic analysis, sperm motility was assessed using a brightfield microscope (IRO-MG-11PL, Iroscope®) at 40x magnification. At least 200 spermatozoa were counted in 10 fields, and three categories were considered: progressive motility (spermatozoa moving linearly or in large circles), non-progressive motility (movement in small circles or *in situ*), and immotile sperm (Castro & Gonzalez, 2019; Cerezo & Lopez, 2014a). To assess sperm vitality, a commercial stain (Espermavit®; MegaFértil, Mexico) containing 5 % eosin Y (color index 45380; WHO, 2010) was used. Under 40x magnification, spermatozoa were counted until at least 200 cells were evaluated, distinguishing live (unstained) from dead (stained) spermatozoa. The vitality percentage was calculated following the methods of Cerezo & Lopez (2014b) and Foster *et al.* (2011). Sperm concentration was measured using a hemocytometer (field method) after a 1:200 dilution (Buzón, 2013; Rodríguez *et al.,* 2022). Morphological evaluation was conducted on a smear of the fresh, air-dried sample, stained with a commercial product (Espermaform®; MegaFértil, Mexico). Using a 100x objective, 200 spermatozoa were evaluated, and each structure was classified as either normal or abnormal, following Tygerberg's criteria (Banaszewska *et al.,* 2015; Castro & González, 2019).

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Meteorological variables and heat index calculation

Meteorological variables such as daily ambient temperature (°C), relative humidity (%), and rainfall (mm) were recorded for the years 2013 to 2021. Data were obtained from the historical archives of the Hydrometeorology Department of the Organismo de Cuenca Golfo Centro of the Comisión Nacional del Agua (CONAGUA) and the Estación de Biología Tropical de Los Tuxtlas (IB-UNAM).

Using this data, the daily WBGTi was calculated based on the formula proposed by Schroter *et al.* (1996) and modified by Domínguez (2015):

WBGTi = (AT*0.567) +(0.393*(RH/100) *6.105) *EXP((17.27*AT)/(237.7+RH)) +3.94

Where:

AT = Ambient temperature (°C).

RH = Relative humidity (%).

EXP = Exponential.

The result of the equation is a number that classifies the environment into five categories that measure the thermal sensation in the horse: comfort (20 - 26.99), caution (27 - 32.99), extreme caution (33 - 40.99), danger (41 - 53.99) and extreme danger (≥ 54).

Statistical analysis

For the study, the WBGTi was calculated using the daily maximum AT and its corresponding RH, allowing the determination of the maximum daily heat index and the average monthly index. These values were then related to the seminal parameters.

The seminogram results were initially analyzed using descriptive statistics, followed by an ANOVA to determine differences by month and by environmental conditions (thermo-neutral zone, caution, and extreme caution).

Tukey's test was used for the analysis of means to assess differences by month and environmental condition, to evaluate the effect of climate. To reduce the variation due to age differences of the stallions, the statistical model included the age of the horse as a covariate. A Spearman correlation test was performed to assess the degree of association between the climatic variables (TA, RH, and WBGTi) and the seminal parameters (volume, motility, vitality, concentration, and morphology), as the data did not follow a normal distribution. All analyses were performed using SPSS v.17.0 (NYSE: IBM®, NY, USA) and SAS v.9 (SAS Institute Inc., NC, USA), with a significance level set at 0.05.

Results and discussion

Weather conditions

The regional climate was characterized as warm and humid for at least nine months of the year, with an annual average maximum temperature of 28.5 ± 4.6 °C and an annual average minimum of 20.7 ± 2.5 °C. The average annual relative humidity was moderately high at (72.63 ± 8.7 %), though it fluctuated throughout the year. During the dry season, which lasted approximately three months, the average relative humidity dropped to 49.11 ± 8.2 %. The average annual wind speed was 8.16 ± 4.35 km/h. June was consistently the hottest month each year, and the highest average monthly rainfall occurred between June and December, with values ranging from 1982 to 3666 mm (Figure 1). Gutiérrez-García and Ricker (2011) studied climate changes in Los Tuxtlas region of Veracruz between 1925 and 2006. They reported that during the last 30 years of their research, the average annual ambient temperature ranged from 24.1 to 27.2 °C, while annual precipitation ranged from 1272 to 4201 mm. When compared to the obtained results, data show an increase in annual temperature and a decrease in the average annual rainfall, along with a reduction in the rainy season duration. These findings reflect the effects of climate change on the region. When analyzing the climate in terms of the heat index, the average annual WBGTi was found to be 29.2 ± 3.95, with fluctuations ranging from 18.86 to 28.30 during the cooler season (December to February), and from 23.80 to 36.80 during the hottest season (March to November). In the hottest month of the year, the index occasionally reached values placing it in the extreme caution category for periods of three or more consecutive days. This phenomenon, known as a heat wave, can cause heat stress in animals, even under shade conditions (Figure 1; Hernández *et al.,* 2023; Muñoz *et al.,* 2014).

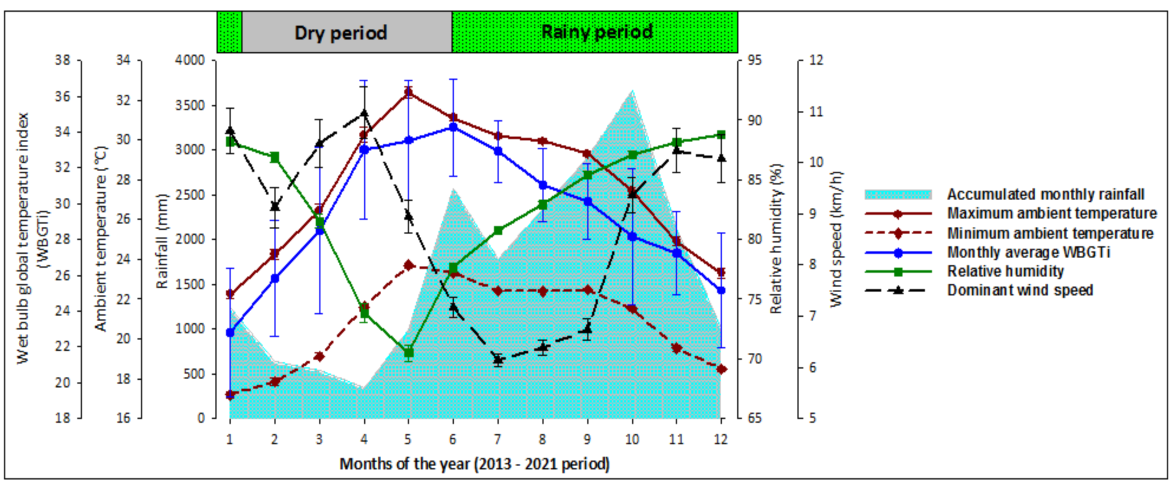

Figure 1. Climogram of San Andrés Tuxtla region during the 2013-2021 period.

Seminal characteristics

The results of the seminal sample analysis throughout the study are summarized in Table 1. The seminal characteristics exhibited a considerable mean variability, which can be attributed to intrinsic differences among the stallions, ejaculatory frequency, seasonality, and age (Samper, 2009). For the analysis, progressive live spermatozoa with normal morphology and overall sperm density were considered, as a reduction in any of these parameters below the reference limits may suggest spermatogenic issues related to environmental factors (Banaszewska *et al.,* 2015). Sperm concentration showed the greatest variation among the parameters but remained within the reference range across all samples. However, 17.2 % of the ejaculates exhibited volumes below the reference limits reported by other authors. Additionally, 23 % of the samples had sperm motility levels lower than the minimum threshold required for fertility, and 21 % had vitality percentages below the recommended minimum (Table 1). Despite these findings, all stallions had sired offspring at the time the study began.

Effect of WBGTi on semen quality

To analyze the behavior of seminal variables under periods of high TA and RH, the monthly average values of the WBGTi were used to observe their effect on the monthly data obtained from the semenograms throughout the study period, with age considered as a covariate. In March, an increase in vitality and motility was observed, coinciding with the start of the equine reproductive season. However, only the percentage of vitality showed a statistically significant difference ($p \leq 0.05$; Table 2). From April onwards, this parameter showed a significant reduction as the environment became more hostile (Table 2), with lower values persisting throughout the reproductive season, reaching the lowest percentage in September (Figure 2). During the study, the environment, as measured by the heat index, revealed an extremely hot and humid period coinciding with the reproductive season, which compromised the ability to regulate both the body and gonadal temperatures of horses (Brownlow *et al.,* 2016; Robert *et al.,* 2010). It has been demonstrated that environmental heat can lead to sperm vitality decline (Kim *et al.,* 2013; Pérez-Crespo *et al.,* 2008; Sinha *et al.,* 2003). Studies on bulls and mice showed that testicular hyperthermia leads to cell death, with sperm apoptosis starting as early as two days after heat exposure, reducing the number of live spermatozoa in the ejaculate. Prolonged heat stress caused a reduction in the seminiferous tubules diameter, leading to decreased sperm density (Llamas-Luceño *et al.,* 2020; Rasooli *et al.,* 2010; Shahat *et al.,* 2020; Sinha *et al.,* 2003).

The percentage of progressive motile spermatozoa and sperm concentration per milliliter did not show statistically significant changes ($p \geq 0.05$), but the expected increase during the reproductive season was not observed (Bustos & Torres, 2012; Sulliman *et al.,* 2020). In contrast, motility reached its lowest percentages in July and August (71.32 ± 3.52 and 69.73 ± 3.21, respectively), 30 days after the highest WBGTi value recorded (36.80), which remained in the stressful category until September (Table 2; Figure 2). Sperm density showed its highest values in May, October, and November (300.23 ± 18.40, 294.37 ± 14.68, and 296.26 ± 20.90, respectively), showing a non-seasonal pattern that was unrelated to changes in the WBGTi.

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Banaszewska *et al.* (2015) reported that motility is affected by thermal stress lasting at least as long as the sperm maturation process (8 to 14 days in horses). During this time, sperm acquire motility in the epididymis and are especially vulnerable to small temperature changes. Therefore, it can be assumed that testicular hyperthermia, lasting at least 120 days in the study, affected several sperm maturation cycles. This not only prevented the characteristic motility increase expected during the reproductive season but even caused a decline below the values observed in December and January when the WBGTi indicated a comfortable environment (Figure 2). In a study by Pereira *et al.* (2012) with semen from fertile stallions in a tropical climate, no significant differences were found in progressive motility or sperm concentration between reproductive and non-reproductive seasons.

The significant reduction ($p \leq 0.05$) in the percentage of normal spermatozoa in February could be due to the age of the stallions, as sampling began with a 16-year-old stallion that month, altering the monthly average. However, sampling continued with all stallions in the following years, with a significant decrease ($p \leq 0.05$) observed again from May to September (Table 2), when the WBGTi was in the stressful category (Figure 2). This reduction was attributed to the effect of environmental heat, as morphology is one of the most sensitive parameters to testicular heat stress. When stress lasts more than two weeks, it leads to overproduction of reactive oxygen species, protein degeneration, enzymatic dysfunction, and DNA damage, resulting in inefficient steroidogenesis and altered sperm morphology (Espinosa & Córdova-Izquierdo, 2018; Kandiel & El-Khawagah, 2018; Peña *et al.,* 2019; Rasooli *et al.,* 2010; Shakeel & Yoon, 2023; Sulliman *et al.,* 2020). Brito (2007) reported that stallions subjected to scrotal heat stroke for just 48 hours showed an increase in abnormal spermatozoa 10 days after heat exposure. Prolonged heat exposure also increased both the number and variety of abnormalities present in the ejaculate.

Semen volume was not affected by WBGTi changes and did not show a clear seasonal pattern. Although a non-significant increase was recorded starting in April, similar values persisted until September, when a significant increase ($p \leq 0.05$) was observed. However, volume declined again in October, with values not significantly different from those recorded between April and August (Table 2; Figure 2).

Table 1. Average values of seminal characteristics of stallions located under climatic conditions of San Andrés Tuxtla, Veracruz, from 2013 to 2021, in comparison with reference parameters for semen from fertile horses.

Seminal characteristic	Mean	S.E.M.	Range	Reference parameters
Volume (ml)	45.04	1.19	7.5 - 120	30 - 100 (Samper, 2009)
Total mobility (%)	67.89	1.28	10 - 98	≥ 60 (Samper, 2009; Hernández-Avilés & Ramírez-Agámez, 2021).

Continuation Table 1.

Seminal characteristic	Mean	S.E.M.	Range	Reference parameters
Vitality (%)	75.22	1.07	30 - 98	≥70 (Samper, 2009)
Sperm concentration (10^6 /ml)	294.38	6.52	75 - 695	100 - 350 or more (Samper, 2009; Hernández-Avilés & Ramírez-Agámez, 2021).
Normal sperm (%)	66.33	0.87	32 - 78	30 - 70 (Samper, 2009; Zarco & Boeta, 2010).

S.E.M: Standard error of the mean.

Table 2. Average monthly values of the semen characteristics of stallions sampled during the period from 2013 to 2021.

Month	Volume (ml)	Progressive mobility (%)	Sperm vitality (%)	Sperm concentration (10^6 /ml)	Normal sperm (%)
January	38.20 ± 6.22^{b}	76.40 ± 5.45*	77.40 ± 5.65^{ab}	276.78 ± 41.78*	73.40 ± 1.21^{a}
February	40.10 ± 5.77^{b}	70.33 ± 5.57*	77.80 ± 5.60^{ab}	283.41 ± 29.98*	$67.21 \pm 3.32a^{b}$
March	32.78 ± 5.24^{b}	80.89 ± 2.70*	90.38 ± 2.10^{a}	285.94 ± 32.10*	69.45 ± 1.80^{ab}
April	43.57 ± 4.00^{ab}	70.14 ± 6.25*	74.72 ± 6.15^{b}	272.76 ± 35.78*	68.14 ± 4.52^{ab}
May	43.25 ± 3.85^{ab}	65.68 ± 3.60*	75.43 ± 3.65^{ab}	300.23 ± 18.40*	59.22 ± 3.50^{b}
June	45.65 ± 4.05^{ab}	70.33 ± 3.00*	77.44 ± 2.31^{ab}	282.18 ± 15.65*	62.93 ± 2.75^{b}
July	49.37 ± 3.24^{ab}	63.94 ± 3.80*	71.32 ± 3.52^{b}	280.17 ± 18.40*	61.77 ± 2.43^{b}
August	44.18 ± 2.61^{ab}	62.99 ± 3.80*	69.73 ± 3.21^{b}	283.44 ± 15.65*	59.53 ± 2.31^{b}
September	51.11 ± 4.43^{a}	65.61 ± 4.30*	73.32 ± 3.00^{b}	269.32 ± 18.70*	58.25 ± 3.11^{b}
October	44.59 ± 3.00^{ab}	67.11 ± 3.45*	75.21 ± 2.68^{ab}	294.37 ± 14.68*	65.40 ± 2.10^{ab}
November	44.48 ± 3.90^{ab}	73.27 ± 4.00*	81.68 ± 2.35^{ab}	296.26 ± 20.90*	68.96 ± 1.95^{ab}
December	46.28 ± 4.50^{ab}	77.78 ± 3.92*	81.44 ± 4.55^{ab}	241.15 ± 22.81*	68.20 ± 2.58^{ab}

Mean ± S.E.M. [ab] Values per column with different literal are significantly different (ANOVA, Tukey, $p \leq 0.05$). * Values per column without significant differences ($p > 0.05$).

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None of the semen variables evaluated in this study showed seasonal behavior. Some research performed with different horse breeds in tropical regions has documented that semen characteristics also did not show a seasonal pattern (McCue, 2014; Pereira, *et al.*, 2012; Sancler-Silva, *et al.,* 2016); on the one hand, given that in tropical climates the variation in daylight duration is smaller than in temperate climates or latitudes above 30° north (Clay & Clay, 1992; Jakubiec *et al.,* 2019) but also since there is evidence that indicates that the suprachiasmatic nucleus is sensitive to changes in ambient temperature, suggesting that it is an additional way of identifying seasonal differences, and in tropical climates changes in ambient temperature are less noticeable than in other climates (Burgoon & Boulant, 2001; Marai, *et al.,* 2009; Sancler-Silva, *et al.,* 2016).

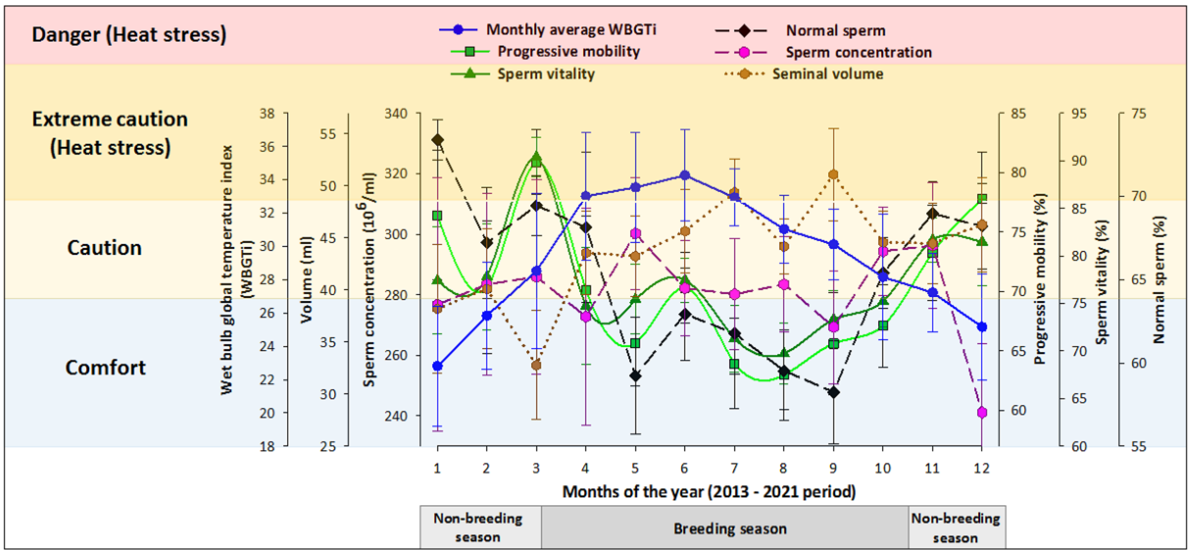

Figure 2. Monthly average value (± S.E.M.) of the wet bulb global temperature index (WBGTi) in association with the monthly average values (± S.E.M.) of the seminal characteristics (volume, progressive mobility, vitality, concentration, and morphology), of the sampled stallions during the period from 2013 to 2021.

The color determines the change in the category of thermal sensation from comfort to danger.

Source: Own elaboration.

Effect of environment type on semen quality

The results of the seminal analyses were evaluated in relation to the different WBGTi values, which categorized the thermal environment into three zones: within the thermo-neutral or comfort zone, precautionary, or stressful. Age was included as a co-variable. In Los Tuxtlas region, during the study period, a comfortable environment was only observed during the last week of

November to the first two weeks of March, when the WBGTi was less than or equal to 26.99. This indicates that the environment was considered within the TNZ for horses. However, from April to the first half of October, the environment was categorized outside the comfort zone, with the index ranging from 27 to 36.80. When seminal results were statistically analyzed in relation to the type of environment, it was observed that when the WBGTi was within the TNZ, the number of live spermatozoa (79.62 ± 2.22), motile spermatozoa (73.75 ± 2.58), and normal spermatozoa (66.95 ± 1.55) was significantly higher ($p \leq 0.05$) than when the environment was precautionary or thermally stressful (Table 3). In a study conducted with three Mangalarga Marchador horses in a tropical climate in southwestern Brazil, a significant reduction in the percentage of motile spermatozoa and the spermatozoa number per milliliter was observed during the spring-summer seasons, when the animals were outside their TNZ, suggesting that the climate may have been the factor causing these results (Waddington *et al.,* 2016). However, these results cannot generalize the response of all stallions in extreme climates, as in another study conducted in a warm-humid zone of southeastern Brazil, no significant influence of thermal stress, measured through a heat index, was observed on testosterone production and the general seminal quality of 10 Criollo horses (Leão *et al.,* 2023). There are more studies conducted in horses that have confirmed that seminal quality can be altered by heat stress (Brito, 2007; Kandiel & El-Khawagah, 2018; Ramires *et al.,* 2013), although stallion fertility is a multifactorial condition involving other components such as age, diet, clinical status, and genetics (Espinosa & Córdova-Izquierdo, 2018; Gottschalk *et al.,* 2016; Kandiel & El-Khawagah, 2018); and in certain climates and latitudes, reproductive seasonality is another factor to consider (Leão *et al.,* 2023; Pereira *et al.,* 2012). Obtained results provide additional scientific evidence that the farther a horse moves from its thermally comfortable zone, the more its seminal quality is affected. This demonstrates that, although QH breed horses have a high capacity for adaptation, a warm environment can cause them to expend additional energy to maintain homeothermy and vital functions, thus diminishing the physiological importance of their reproductive performance. When the environment becomes hostile, stress mechanisms may be triggered, influencing sperm production (Álvarez, 2008; Shakeel & Yoon, 2023). Research conducted on bulls and male goats has reported the negative effect that intense heat, expressed in thermal indices, has on the motility, vitality, and morphology of sperm produced during the hottest season of the year (Bhakat *et al.,* 2014; Llamas-Luceño *et al.,* 2020; Ranjan *et al.,* 2020; Sharma *et al.,* 2017). However, studies with bulls reported a reduction in sperm concentration per milliliter (Sharma *et al.,* 2017; Llamas-Luceño *et al.,* 2020), as did Waddington *et al.* (2016), who also reported an increase in seminal volume. Nevertheless, our results did not show significant changes in these parameters in relation to heat intensity (Table 3), nor was there an increase due to the influence of photoperiod during the months from March to October, as naturally occurs in certain climates during the equine breeding season. This absence of changes in general characteristics during the breeding season may be due to the reasons already mentioned.

Table 3. Average values of seminal characteristics of stallions located in three types of thermal environment during the period from 2013 to 2021.

Seminal characteristic	Thermal environment		
	Thermoneutral (WBGTi ≤ 26.99)	Caution (WBGTi = 27 - 32.99)	Extreme caution (WBGTi = 33 - 36.80)
Volume (ml)	44.85 ± 2.47	45.80 ± 1.28	41.92 ± 3.19
Progressive mobility (%)	73.75 ± 2.58[a]	67.25 ± 1.29[b]	65.99 ± 3.32[b]
Sperm vitality (%)	79.62 ± 2.22[a]	74.71 ± 1.10[b]	73.69 ± 2.82[b]
Sperm concentration (10^6 /ml)	279.34 ± 15.07	294.35 ± 7.60	305.54 ± 19.76
Normal sperm (%)	66.95 ± 1.55[a]	62.84 ± 0.78[b]	61.51 ± 1.99[b]

Mean ± S.E.M. [ab] Values per row with different literals are significantly different (ANOVA, Tukey, $p \leq 0.05$).

Relationship between climatic variables and seminal characteristics

The intensity of the relationship between seminal and climatic variables was determined through correlation analysis. Some correlation coefficients were of moderate magnitude, negative, and significant ($p \leq 0.05$). The correlation values between the WBGTi and the motility percentage (r = -0.22, p = 0.000), vitality (r = -0.19; p = 0.003), and normal spermatozoa (r = -0.30; p = 0.000) (Figure 3). Similarly, the maximum temperature presented correlation coefficients very similar to the above, being -0.22 (p = 0.000) with motility percentage, -0.18 (p = 0.003) with vitality, and -0.28 (p = 0.000) with normal spermatozoa. Finally, RH was the climatic variable with the lowest correlation coefficients, being -0.13 with motility (p = 0.040), -0.14 with concentration (p = 0.030), and -0.21 for the number of normal spermatozoa (p = 0.001; Figure 3). An AT above 26 °C combined with an RH of at least 40 % creates conditions that complicate the mechanisms allowing the scrotal temperature to remain within the ideal range, causing testicular hyperthermia (Brownlow *et al.,* 2016; Girard *et al.,* 2008; Kastelic *et al.,* 2019). Ramires *et al.* (2013) obtained moderate correlations by relating the scrotal temperature of horses subjected to various sunlight exposure protocols with the number of abnormal spermatozoa (r = 0.32) and testicular volume (r = -0.23).

Other studies in various species have confirmed that testicular hyperthermia can alter lipid metabolism, and antioxidant resistance, and cause hypoxia, predisposing spermatozoa to structural changes, apoptosis, and degeneration of the testicular interstitium, which are reflected in decreased vitality, motility, and sperm concentration, along with an increase in abnormal spermatozoa (Kandiel & El-Khawagah, 2018; Rasooli *et al.,* 2010; Sharma *et al.,* 2017; Suriyasomboon *et al.,* 2004).

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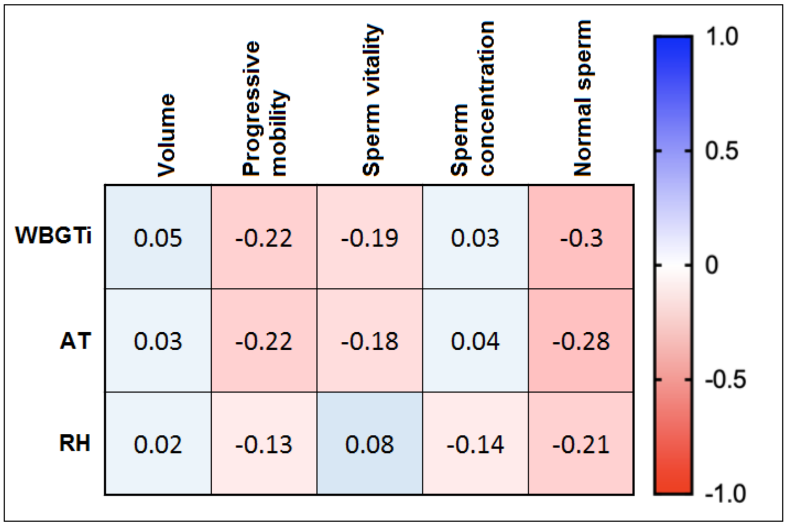

Figure 3. Spearman correlation coefficients between atmospheric and seminal variables.

WBGTi = global wet bulb temperature index, AT = ambient temperature, RH = relative humidity.

* Correlation is significant.

Source: Own elaboration.

Conclusions

The extreme climatic conditions of Los Tuxtlas region had a clear impact on the seminal quality of QH stallions. Although sperm volume and concentration remained unaffected, key fertility indicators such as motility, vitality, and morphology showed significant reductions when the environment moved outside the thermo-neutral zone. This decline in seminal quality could impair the fertilizing capacity of an ejaculate, especially during cryopreservation, where the condition of fresh semen is crucial. The WBGTi used in this study proved to be a valuable tool for assessing the impact of climate on seminal characteristics. However, it is crucial to consider other factors influencing fertility, such as nutrition, health, and genetics, for a comprehensive understanding of the reproductive, health, and welfare status of stallions. The lack of a clear seasonal pattern in the seminal characteristics observed in these stallions also aligns with previous research on the reproductive behavior of horses in tropical climates, where seasonality may not play as significant a role as in temperate regions. Horse owners living in similar environmental conditions are advised to use more effective cooling systems and supplement their feed with antioxidant elements to counteract, to some extent, the heat consequences.

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Author contribution

Teamwork was carried out in which the following activities were developed: WorkC conceptualization: Luna López, Belisario Domínguez, Antonio Hernández; Methodology development: Luna López; Software management: all authors; Experimental validation: Luna López, Antonio Hernández, Belisario Domínguez, Leonel Avendaño; Data analysis: all authors; Data management: all authors; Manuscript writing and preparation: Luna López; writing, revising, and editing: Belisario Domínguez, Leonel Avendaño, Antonio Hernández; Project manager: Antonio Hernández; Fund acquisition: Luna López.

All authors of this manuscript have read and accepted the published manuscript version.

Ethical Statement

All handling procedures performed on the study horses complied with all animal welfare conditions approved by the Bioethics Committee of the Faculty of Veterinary Medicine and Animal Husbandry of the Universidad Veracruzana (COBIBA011/2021).

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Conflict of interest

The authors declare no conflicts of interest.

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