Journal of Equine Veterinary Science 151 (2025) 105615

Contents lists available at ScienceDirect

Journal of Equine Veterinary Science

journal homepage: www.elsevier.com/locate/jevs

Thermal comfort and seminal traits in stallion reproduction: New methods to understand these relationships

Luiza Maria Feitosa Ribeiro [a,*], João Victor Bersot Gomes [b], Wilder Hernando Ortiz Vega [b], Andressa da Silva Alves [a], Maurício Netto Machado [a], Luan Junio Wutke [b], Célia Raquel Quirino [b], Roberta Carvalho Basile [c], Paula Alessandra Di Filippo [a]

[a] Department of Animal Clinic and Surgery Laboratory, School of Veterinary Medicine, University Darcy Ribeiro State University of Norte Fluminense, UENF, Av. Alberto Lamego, 2000 - Parque California, CEP: 28013-602, Campos dos Goytacazes, Rio de Janeiro, Brazil
[b] Department of Animal Reproduction and Genetic Improvement Laboratory, School of Veterinary Medicine, University Darcy Ribeiro State University of Norte Fluminense, UENF, Av. Alberto Lamego, 2000 - Parque California, CEP: 28013-602, Campos dos Goytacazes, Rio de Janeiro, Brazil
[c] Veterinary Hospital, University Central Paulista University Center Rua Miguel Petroni São Carlos Housing Development 1, 13563470 - São Carlos, São Paulo - Brazil

ARTICLE INFO

Keywords:
Animal welfare
Equine
Reproduction
Thermography
Thermoregulation

ABSTRACT

Background: Stallion breeding is mostly based on desirable phenotypic traits, with little consideration for semen quality.
Aims/objectives: To identify relationships among semen parameters in stallions using a non-invasive, integrative approach across summer and winter seasons.
Methods: Twenty-four stallions were evaluated, in which semen collections, testicular thermography, and Doppler velocimetry of the testicular artery by Doppler ultrasonography were performed, in addition to the measurement of physiological, environmental, and heat stress parameters, both in winter (coat weather) and summer (T-shirt weather). Statistical tests included univariate and multivariate analyses in software SPSS® (IBM).
Results: Sperm defects (22.4 ± 7.3 %) and heat stress indices (THI: 79.7 ± 3.4; ThStress: 2.9 ± 0.7) were higher in summer (28.6 ± 2.7 °C) than in winter (12.5 ± 9.4 %; 21.1 ± 2.4 °C; THI: 73.5 ± 8.6; ThStress: 1.1 ± 0.3). The heat stress indices were calculated by the equation: THI= [0.8 x (EnTemp + RH/100)] x [(EnTemp – 14.4) + 46.4]. In contrast, systolic/diastolic testicular blood flow velocities, motility, and vigor were greater in winter (33.3 ± 9.3 cm/s; 5.9 ± 2.2 cm/s; 81.2 ± 8.5 %; 3.2 ± 0.4) than in summer (24.4 ± 6.7 cm/s; 4.0 ± 1.0 cm/s; 64.2 ± 21 %; 2.7 ± 0.6). Significant correlations were found between thermal comfort indices and seminal traits in summer, and between Doppler/thermographic data and semen quality in winter.
Conclusion: The analyzed indices characterized thermal, circulatory, and seminal parameters in stallions, highlighting seasonal differences and correlations, and supporting the use of Doppler ultrasonography and infrared thermography in fertility assessment, thermal comfort, and optimal breeding season selection.

1. Introduction

Although the reproductive performance of stallions is not evaluated in the same way as their athletic performance, veterinarians are aware of its importance and the factors that influence it: hormonal patterns, sexual behavior, and sperm quality [1]. The equine species relies on high environmental luminosity for reproductive performance, which, in Brazil, is associated with the warmest period of the year. However, high ambient temperatures interfere with physiological mechanisms during the reproductive period, reducing fertility and causing economic losses [2]. The photoperiod, that is, the duration of exposure to sunlight, directly affects ambient temperature and has a direct impact on animal fertility. On longer and warmer days, increased ambient temperature may lead to heat stress, raising scrotal temperature, impairing spermatogenesis, causing DNA mutations in gametes, and reducing testosterone levels and libido [3].

For efficient sperm production, the testicular temperature must remain 3 to 5°C below body temperature; deviations from this range can

* Corresponding author.
E-mail address: f.feitosaribeiro@gmail.com (L. Maria Feitosa Ribeiro).

https://doi.org/10.1016/j.jevs.2025.105615
Received 9 December 2024; Received in revised form 26 May 2025; Accepted 26 May 2025
Available online 27 May 2025

seriously compromise fertility [4]. However, moderate heat stress may not impact the stallion's reproductive capacity, thanks to testicular thermoregulation mechanisms, which involve the coordinated action of the pampiniform plexus, dartos tunica, cremaster muscle and scrotal sweat glands. These mechanisms promote heat exchange between arteries and veins surrounding the testicle, keeping the testicular temperature below body temperature [5]. In this context, infrared thermography has gained prominence as a rapid and non-invasive method for monitoring scrotal temperature, facilitating the early detection of heat stress [6].

In addition to temperature, adequate testicular perfusion is essential for reproductive health, with vascular disorders being a common cause of subfertility. Early diagnosis of these dysfunctions is essential to implement strategies that maximize fertility and minimize tissue damage. In this sense, Doppler ultrasonography of the testicular artery emerges as an effective tool to detect vascular alterations and predict sperm quality, becoming a promising and non-invasive technique compared to more time-consuming and invasive laboratory methods [7, 8].

Therefore, spermatogenesis and testicular functionality are highly dependent on thermoregulation and adequate blood perfusion. Global climate changes, such as heat waves and sudden temperature increases, pose significant risks to equine reproductive performance and may lead to subfertility and testicular degeneration [9]. Doppler ultrasonography and infrared thermography are important exams that can positively impact stallion breeding, as follows: monitoring blood flow in the testicles, identifying heat stress and inflammation, detecting abnormalities in advance, and adjusting environmental management. The present study sought to identify the relationships between sperm parameters and the effectiveness of non-invasive methods in the evaluation of equine reproduction, considering different environmental conditions. Exposure to high environmental temperatures, especially in summer, is expected to negatively influence the sperm quality of stallions. The relevance of the research lies in understanding how climate affects stallion fertility and in providing support for more efficient reproductive management, especially in regions with marked climatic variation.

2. Material and methods

2.1. Ethical approval

The study protocol was approved by the animal ethics committee for Tests with Animals of State University of Northern Rio de Janeiro Darcy Ribeiro (Approval number, 513; Approval date, 18/11/2021).

2.2. Study location, climate characterization and animal handling

A graphic summary of the procedures performed is presented (Fig. 1). Twenty-four stallions, aged 7.46 $\pm$ 3.69 years, Quarter Horse (n = 7), Paint Horse (n = 4), and Mangalarga Marchador (n = 13) breeds were used. The animals were raised in individual masonry stalls (3 $\times$ 4 meters), with a concrete floor and wood shavings bedding, with access to water and mineral salt for horses ad libitum. They received a balanced diet based on Tifton hay (*Cynodon* sp.) and concentrate for the category

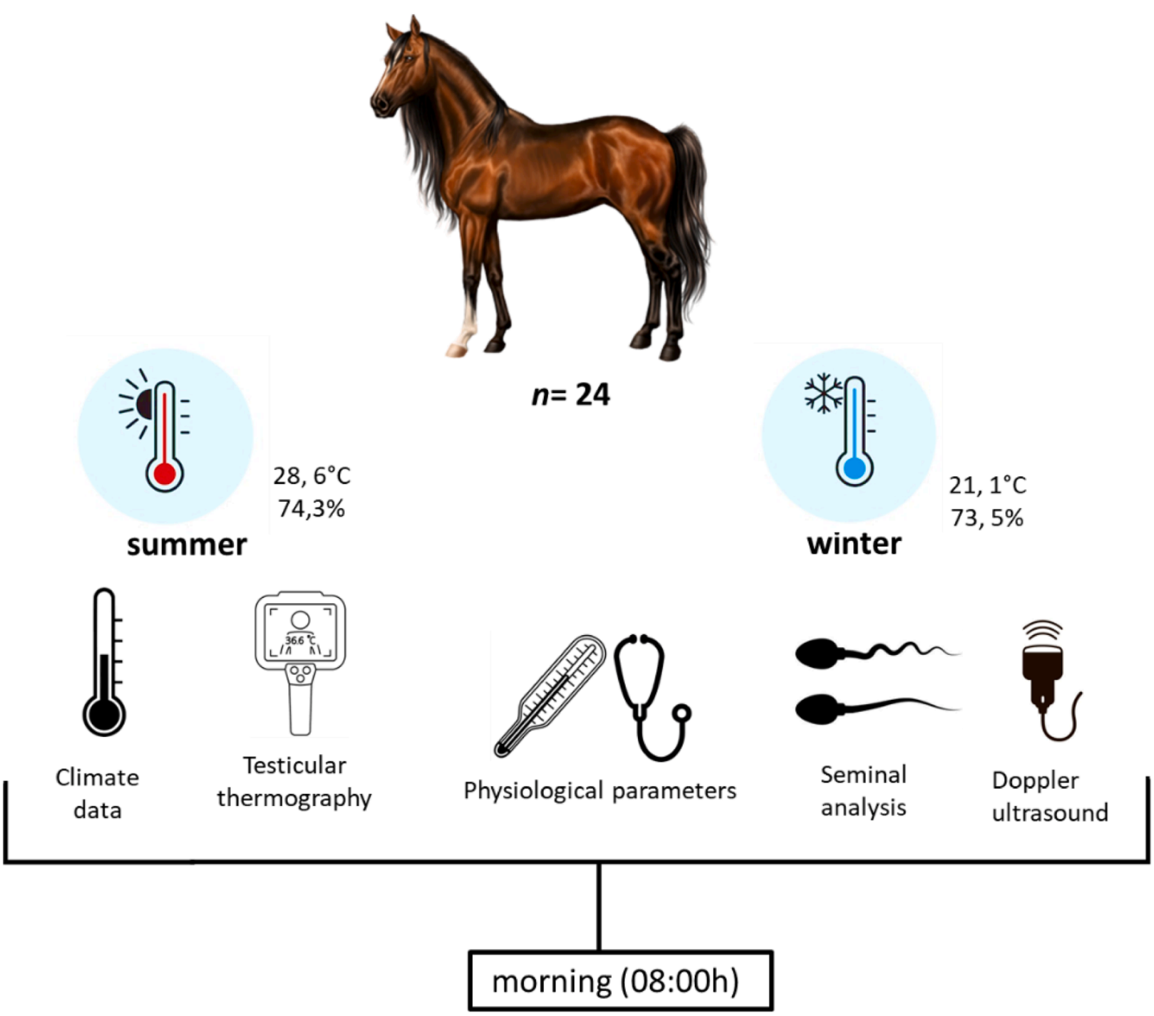

Fig. 1. Graphical summary of the experimental scheme in the evaluation of the Thermal Comfort - Environmental and Seminal- Circulatory.

(stallions in maintenance −14 % total protein) in the proportion of 1 % body weight. The animals came from farms located in the interior of the state of Rio de Janeiro, southeast region, Brazil (Latitude - 21° 45′15″, longitude −41° 19′28″, 13 meters altitude). The region is characterized by a predominantly hot and humid tropical climate, with higher rainfall in summer (December to March), an average temperature of 26.4°C, and relative humidity of 79.07 % in summer, and an average temperature of 21.2°C, and relative humidity of 70.77 % in winter [10]. Reproductive efficiency and analysis for any reproductive pathology were verified before the beginning of the study by means of andrological examination as recommended by the Brazilian College of Animal Reproduction, palpation of the testicles, spermatic cord and epididymis, measurement of testicular size, evaluation of sexual behavior and performance of spermogram [11]. Only healthy animals with no clinical or reproductive abnormalities were included in the study. Selected animals were evaluated in two seasons of the year, summer (December 21 to March 20, 2022) and winter (June 20 to September 22, 2022). All procedures were consented to and authorized by the owners.

2.3. *Infrared thermographic evaluation, physiological and bioclimatic parameters*

To minimize the effects of environmental factors, infrared thermographic evaluations were performed early in the morning (07:00 to 08:00) with the animals kept in a closed stall, protected from sunlight, heat sources, and wind currents, for at least 1 hour before the examination. To determine the testicular surface temperature, a digital infrared thermography device was used - FLIR E53 24° Thermal Imager (Poliscan, São Paulo, Brazil), with the emitting focus of the device directed to the scrotum of each stallion, oriented parallel to it, at a distance of 1 m and emissivity ε = 0.98 (recommended for equine species) and "manual" mode (Fig. 2A and Fig. 2B). The temperature setting was selected for a reading range of 10°C according to the environmental temperature of the day and using the rainbow color palette [12]. Temperatures and thermal images were obtained by measuring different points on the scrotal surface and calculating the average for both testicles using FLIR Tools software (FLIR Systems, 2017, version 5.12.17023.2001). Subsequently, heart rate (HR, in beats/min) was measured by auscultation for one-minute, respiratory rate (RR, in beats/min) by counting the respiratory movement of the rib cage for one minute, and rectal temperature (RT, in°C) by conventional clinical thermometry for two minutes. Environmental temperature and relative humidity were measured using a TGBH thermal stress meter (model Extech HT30, Extech Instruments, 2009-2015 FLIR Systems, Inc). The values obtained were used to calculate 3 environmental thermal comfort indices:

1. Temperature and humidity index (THI):

THI= [0.8 x (EnTemp + RH/100)] x [(EnTemp – 14.4) + 46.4], where

EnTemp: Environmental temperature,
RH: Relative humidity

1. Thermal stress according to THI:

ThStress: 1 = 70 non-stressful; 2 = 71 to 78 alert; 3 = 79 to 83 dangerous; 4 = 83 emergency

1. Benezra thermal comfort index:

BTCI = (RT/38) + (RF/16), where,
RT: Rectal temperature,
RF: Respiratory frequency

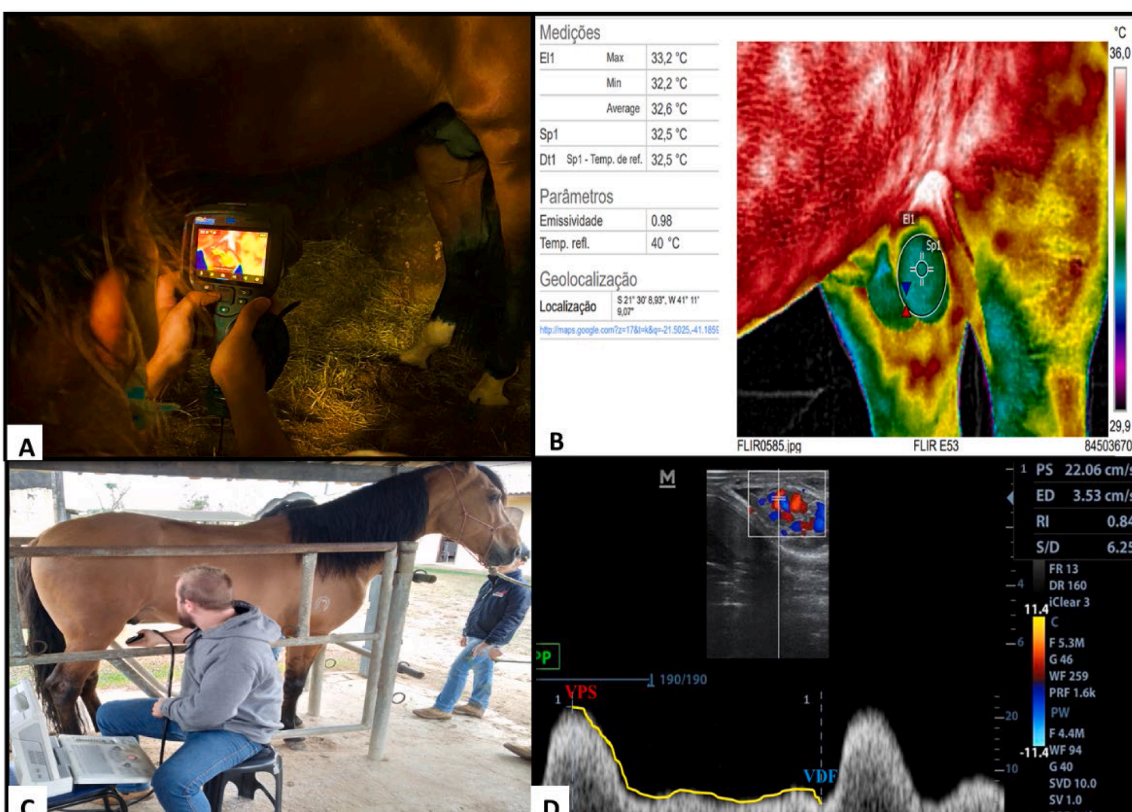

Fig. 2. A) Carrying out a thermogram framing the testicular region and qualitative and quantitative processing with minimum, maximum and average left testicular temperature in a horse (B). Doppler ultrasonography of the testicular artery in a stallion (C) and spectrum of blood flow velocity throughout a cardiac cycle (yellow line) in the testicular artery with emphasis on peak systolic velocity (PSV/VPS) and end-diastolic velocity (EDV/VDF) values (D).

2.4. Testicular biometry, sperm collection and evaluation

Before starting the sperm evaluations, the stallions were subjected to 4 consecutive semen collections, every 48 hours, to deplete the extra-gonadal sperm reserve. The experiment began with the 4th and last collection. Four collections were performed per animal, totaling 192 ejaculates throughout the experiment (96 collections in the summer and 96 collections in the winter). The semen collections were performed using an artificial vagina (Botucatu model - Botupharma, São Paulo, Brazil) previously filled with heated water (temperature around 45°C). The semen was collected in a plastic bag that lined the inside of the collection cup, which was protected from light and temperature fluctuations and kept on a heating plate (37°C) during the evaluation. Before starting each collection, the animals had their penises cleaned with water at room temperature. The collections were performed using a dummy mount (mannequin).

Immediately after collection, the ejaculates were manually evaluated and the macroscopic and microscopic parameters of volume (expressed in mL), concentration (x10^6/mL), vigor (0-5), motility (0-100 %), major defects (proximal drop, strongly bent tail, strongly coiled tail), minor defects (distal cytoplasmic drop, coiled tail, bent tail) and total defects were tabulated. The sperm concentration test was performed the Neubauer chamber method (BR717805-1 EA-Blaubrand, Germany), by diluting the semen in distilled water (dilution rate 1:20). Sperm morphology was evaluated in samples kept in citrate formalin prepared through a humid chamber and stained with rose Bengal. They were evaluated under a phase contrast microscope and 200 spermatozoa were evaluated by optical microscopy (CX31- Olympus®, Brazil) at 1,000x magnification [11].

Motility was assessed using an optical microscope with a 10x objective lens, where a drop of semen was placed between a slide and a preheated coverslip and maintained at approximately 37 °C. The result was expressed as a percentage (0 to 100 %), consisting of estimating the number of motile spermatozoa [11].

Sperm vigor was assessed concomitantly with motility and classified using a scale ranging from 1 to 5 that represents the intensity of sperm movement: 1: exclusively oscillatory; 2: slow; 3: intermediate; 4: fast progressive straight; and 5: very fast progressive straight [11].

Testicular biometric measurements of length, width, and height were taken in both testicles using a digital caliper (Digital ABSOLUTE AOS-0.01mm/.0005″, Mitutoyo, Brazil). The testicular volume of each testicle was calculated using the formula: Testicular Volume (cm^3) = 0.5233 x W x L x H; where, W: width; L: length; H: height. The total testicular volume (TTVol) was calculated by adding the volume of the left testicle (LTVol) to the right testicle (RTVol) [13].

2.5. Testicular hemodynamic assessment by Doppler ultrasound

Ultrasound examinations were carried out by a single experienced operator in animals kept in a quadrupedal position in a squeeze chute. The testicular region and spermatic cord were evaluated with a portable ultrasound (Z6 Vet - Mindray do Brazil), equipped with B-mode (grayscale) and power Doppler with a broadband multifrequency linear transducer and frequency of 5.0 MHz. The transducer was positioned caudally in the most ventral portion of the spermatic cord on both sides. Pulsed Doppler evaluated the vascular blood flow velocity of the testicular artery using an insonation angle of 60° and at least three consecutive similar spectral curves were obtained. The peak systolic velocity (PSV-cm/s), end-diastolic velocity (EDV-cm/s), and resistivity index (RI) of the supratesticular region of testicular arteries on the left and right sides were evaluated (Fig. 2C and Fig. 2D).

2.6. Statistical analysis

The statistical methods used here were divided into three stages. The collected data were processed and analyzed using software SPSS® (IBM).

Stage 1: First, the assumptions of data normality and homoscedasticity of variances were tested using the Shapiro-Wilk and Levene tests, respectively. Then, a one-way analysis of variance was applied at a significance level of 5 %, and the Sidak correction test was used for the pairwise comparison of all the averages of the characteristics evaluated.

Stage 2: To evaluate the relationships between seminal, environmental, thermographic, Doppler ultrasound, and physiological variables according to the season of the year, factor analysis (FA) was applied, including the season (summer or winter) as selection variables. The selected variables showed significance in the previous stage. The number of factors extracted followed the Kaiser criterion according to the inflection point of the eigenvalues (eigenvalues above 1), [14]. Orthogonal rotation (varimax model) was used in the factor analysis. The significance of the factors was established according to the factor weights of the variables in each factor. The fit of the composite model was evaluated through the Kaiser-Meyer-Olkin test (KMO=0.71) and Bartlett's sphericity test (<0.05), proving suitable [15]. The graphical representation of the factors generated and renamed as Environmental thermal comfort (1) (Thermal comfort -Environment) and Seminal characteristics (2), (Seminal-circulatory) was given by biplots using the SRplot tool [16].

Stage 3: The correlation matrix of the factor analysis was used to investigate the degree and magnitude of association of the variables between the identified factors. Correlations were considered significant by the Pearson method, with $P < 0.05$. The matrix was plotted on a heat map constructed using the Heatmaper tool [17].

3. Results

According to Tables 1 and 2, significant differences ($P < 0.05$) were found in 16 out of 34 variables between seasons (summer and winter).

Among the environmental variables, the environmental temperature (EnTemp), the temperature and humidity index (THI), and the thermal stress index according to THI (ThStress) were higher ($P < 0.05$) in summer (Table 1). Likewise, differences ($P < 0.05$) were detected in the thermographic testicular and seminal variables between seasons. Minor sperm defects (MinorD), total defects (TotD), and right (RTTpTh), left (LTTpTh) and average (AvTTpTh) testicular temperature were higher in

Table 1
Mean and standard deviation of physiological and environmental parameters of stallions in two seasons (summer and winter) in Brazil.

	Seasons	
	Summer	Winter
Age	8. ± 4.0	7.62 ± 3.8
Physiological and environmental parameters		
Heart rate (HR, bpm)	36.86 ± 5.0	40.0 ± 5.6
Respiratory frequency (RF, mpm)	58.4 ± 4.2	62.8 ± 3.4
Rectal temperature (RecTemp,°C)	37.6 ± 0,3	37.4 ± 0.5
Environmental temperature (EnTemp,°C)	28.6 ± 2.7 a	21.1 ± 2.4 b
Relative humidity(RU, %)	74.3 ± 10.1	73.5 ± 8.6
Temperature and humidity index (THI)	79.7 ± 3.4 a	68.1 ± 3.3 b
Thermal stress according to ITU(ThStress)	2.9 ± 0.7 a	1.1 ± 0.3 b
Benezra thermal comfort index (BTCI)	2.3 ± 0.3	2.1 ± 0.2
Thermographic indices		
Right testicle thermography by termograph (RTTpTh,°C)	32.4 ± 0.4 a	31.1 ± 0.7 b
Left testicle thermography by termograph (LTTpTh,°C)	32.5 ± 0.5 a	31.2 ± 0.4 b
Average testicle thermography by termograph (AvTTpTh,°C)	32.4 ± 0.4 a	31.1 ± 0.5 b

[ab] Different letters in the same lines indicate significant differences by the Sidak test ($P < 0.05$).

Table 2
Mean and standart deviation of seminal and testicular circulatory parameters of stallions in two seasons in Brazil.

	Seasons	
	Summer	Winter
Seminal and biometric parameters		
Right testicular volume (RTVol, $_{cm}^{2}$)	96.1 ± 55.3	82.8 ± 51.2
Left testicular volume (LTVol, $_{cm}^{2}$)	96.8 ± 57.6	100.3 ± 47.4
Total testicular volume (TTVol,$_{cm}^{2}$)	176,3 ± 99.0	183.2 ± 90.5
Volume (VOL,$_{mL}$)	33.5 ± 17.5	46.6 ± 14.3
Concentration (CONC,$_{10\text{-}6\ /mL}$)	172.7 ± 109.1	214.7 ± 127.8
Motility (MOT,$_{\%}$)	64.2 ± 21.0 ^{b}	81.2 ± 8.5 ^{a}
Vigor (VIGOR, $_{1\text{-}5}$)	2.7 ± 0.6 ^{b}	3.2 ± 0.4 ^{a}
Major defect (MajorD,$_{\%}$)	16.7 ± 6.7	10.8 ± 8.9
Minor defect (MinorD,$_{\%}$)	5.7 ± 3.6 ^{a}	1.7 ± 1.8 ^{b}
Total defects (TotD,$_{\%}$)	22.4 ± 7.3 ^{a}	12.5 ± 9.4 ^{b}
Circulatory parameters		
Right testicular artery systolic velocity (RTASVel, $_{cm/s}$)	25.7 ± 6.4 ^{b}	32.2 ± 9.8 ^{a}
Left testicular artery systolic velocity (LTASVel, $_{cm/s}$)	23.0 ± 7.9 ^{b}	34.5 ± 10.3 ^{a}
Mean value of systolic velocity (AvSVel, $_{cm/s}$)	24.4 ± 6.7 ^{b}	33.3 ± 9.3 ^{a}
Right testicular artery diastolic velocity (RTADVel, $_{cm/s}$)	3.9 ± 1.5 ^{b}	5.9 ± 2.1 ^{a}
Left testicular artery diastolic velocity (LTADVel, $_{cm/s}$)	4.1 ± 1.0 ^{b}	5.8 ± 2.5 ^{a}
Mean value of diastolic velocity (AvDVel, $_{cm/s}$)	4.0 ± 1.0 ^{b}	5.9 ± 2.2 ^{a}
Right resistivity index (RRInx)	0.83 ± 0.07	0.80 ± 0.04
Left resistivity index (LRInx)	0.80 ± 0.06	0.82 ± 0.08
Mean value of resistivity index (AvRInx)	0.82 ± 0.06	0.81 ± 0.06
Right pulsatility index (RPInx)	7.5 ± 2.7	5.7 ± 1.6
Left pulsatility index (LPInx)	5.3 ± 1.7	6.8 ± 3.0
Mean value of pulsatility index (AvPInx)	6.4 ± 2.0	6.2 ± 2.1

[ab] Different letters in the same lines indicate significant differences by the Sidak test ($P < 0.05$).

summer (Tables 1 and 2). Sperm motility (MOT) and vigor had the opposite behavior, with higher ($P < 0.05$) values in winter (Table 2). Left (LTVol), right (RTVol), total (TTVol) testicular volume, sperm concentration (CONC), seminal volume (VOL) and major defects (Major D) did not differ ($P > 0.05$) between the two seasons (Table 2).

Regarding testicular blood flow variables, the mean systolic velocity (AVel) and diastolic velocity (AvDVel) of the testicular artery were also higher ($P < 0.05$) in winter. The other circulatory parameters, including the mean values of the resistive index (AvRInx) and pulsatility index (AvPInx), showed no significant differences ($P > 0.05$) among the seasons of the year (Table 2).

The factor analysis (F_A) with the eigenvalues and the commonality of the variables distributed in the factors are presented in Table 3. High commonality values (> 0.80) were obtained in the ordered variables ThStress, EnTemp, and THI while the other variables presented values in values between 0.5 and 0.70.

The extracted factors (1 = Thermal comfort-environment and 2 = Seminal-circulatory) explained 67.03 % of the total data variance. The first factor retained 48.12 % of the total variance and represented the variables with the highest factor loading.

In factor 1, renamed as Thermal comfort-Environment, the variables ThStress, EnTemp, THI, AvSVel, MinorD, TotD, and AvTTpTh stood out. In factor 2, renamed as Seminal-Circulatory, AvDVel, MOT, and VIGOR stood out as the characteristics with the highest factor weight.

The association of F_A variables between the summer and winter seasons is illustrated in Fig. 3. There was a clear association between EnTemp, ThStress, and THI variables in the summer. In this same season, TotD and MinorD were also positively associated with EnTemp, ThStress, and THI. Testicular circulatory variables analyzed by Doppler measurements (AvSVel and AvDvel) and mean testicular temperature value by thermography (AvTTpTh) correlated with the sperm variables motility (MOT), vigor (VIGOR) even during the summer. In turn, there was no association between the variables analyzed in winter.

Table 3
Factor analysis for seminal, environmental, infrared thermography and Doppler traits in horse at two stations in Brazil.

Variables	Factors[1]		Communalities
	Thermal comfort -Environment (1)	Seminal-Circulatory-(2)	
Thermal stress (ThStress)	0.913	0.176	0.865
Environmental temperature (EnTemp)	0.916	0.226	0.890
Temperature and humidity index (THI)	0.935	0.237	0.930
Average systolic velocity (AvSVel)	−0.583	0.438	0.532
Average diastolic velocity (AvDVel)	−0.123	0.689	0.489
Motility (MOT)	−0.496	0.652	0.671
Vigor (VIGOR)	−0.487	0.693	0.718
Minor defect (MinorD)	0.659	0.328	0.542
Total defects (TotD)	0.656	0.271	0.504
Average testicle thermography by termograph (AvTTpTh)	0.750	0.024	0.562
Partial variance. %	48.12	18.90	-
Total variance	48.12	67.03	-

[1] Factors: Factor loads in bold indicate greater participation of the variable in the respective factor.

The magnitude and direction of the correlations between the variables extracted in the F_A are presented in the Heat Map (Fig. 4). Correlations between mean testicular temperature measured by the thermograph (AvTTpTH) and environmental (EnTemp) and thermal stress (THI and ThStress) parameters were positive and moderate in values between 0.64 and 0.66 ($P < 0.001$). The correlation of AvTTpTH with MinorD and TotD was positive and weak ($r = 0.43$; $P = 0.009$ and $r = 0.40$; $P = 0.013$, respectively). Environmental and thermal stress parameters showed positive and moderate correlations in values between 0.51 and 0.6 ($P < 0.002$) with sperm defects (MinorD and TotD). Doppler velocimetry parameters such as AvSVel showed weak and negative correlations in values between −0.40 and −0.46 ($P < 0.02$) with EnTemp, THI, ThStress MOT, and VIGOR. Finally, the correlation between AvDVel and VIGOR was positive and weak ($r = 0.35$; $P = 0.027$).

4. Discussion

This study investigated the relationships between seminal parameters of stallions and non-invasive methods for their evaluation under different climatic conditions in a Brazilian tropical environment.

(I) In this species, associations between fertility and environmental thermal comfort, testicular hemodynamics, and seminal characteristics, as assessed by infrared thermography and Doppler ultrasonography, are understandable. (II) During the summer months, sperm morphological changes (total defects 22.4 ± 7.3) are more influenced by ambient temperature and heat stress indices, such as THI (temperature-humidity index), temperature and humidity, and mean testicular temperature. (III) The climatic conditions of the Brazilian summer and winter seasons result in distinct outcomes, induced by environmental variables, heat stress, and seminal and testicular circulatory parameters in horses. The average environmental temperature (28.6 °C) in the summer was above the acceptable thermal comfort zone for horses, in the range between 5 and 25°C [18], which indicates that the animals faced thermal stress due to heat during this period.

There is a clear association between the variables EnTemp, ThStress, and THI in the summer. This is expected since these variables are directly interrelated. Environmental temperature (EnTemp) influences

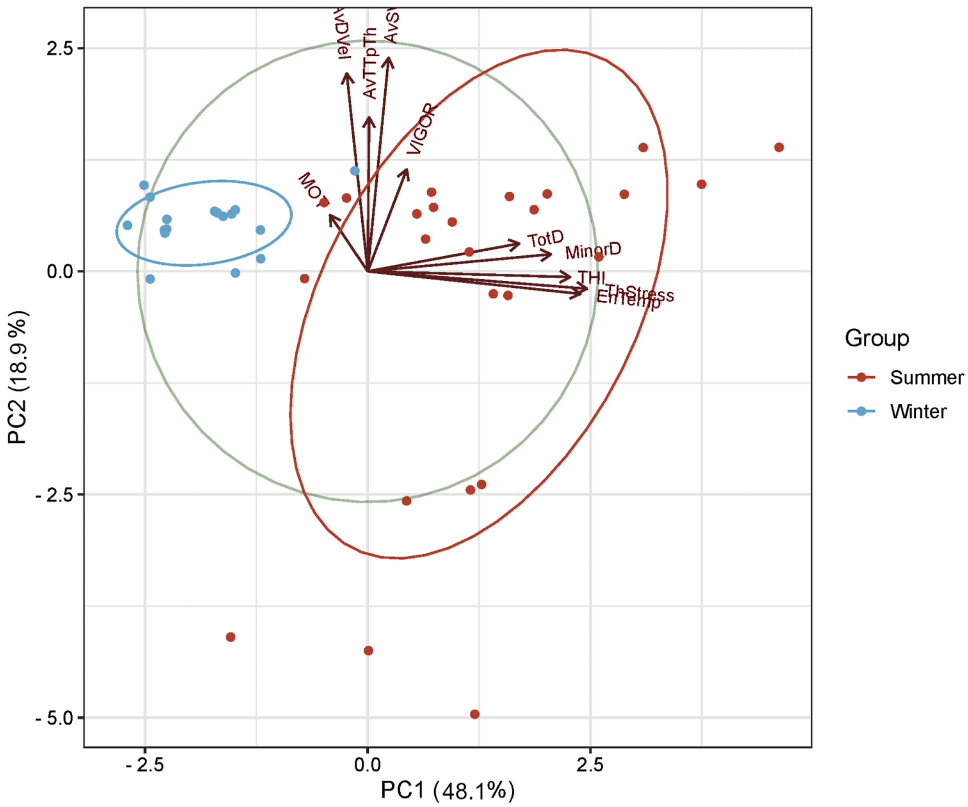

Fig. 3. Biplot of the relationships between Thermal- comfort -Environment and Seminal- circulatory factors of Brazilian horses between seasons. PC1 and PC2 is the first and the second Principal components (explainary extend of latent variable to the differences). Points represent samples, different colors represent different groups. Ellipses represent 68 % confidence intervals of core regions. Arrows represent original variables, the directions of arrows represent correlation between original variable and principle components, lengths represent devotion of original data to principle components. *Dopplermetric parameters and seminals*: average diastolic velocity (AvDvel), average systolic velocity (AvSvel), motility (MOT), vigor (VIGOR), minor defects (MinorD), total defects (TotD). *Thermal comfort parameters:* environmental temperature (EnTemp), Temperature Humidity Index (THI), Thermal stress according to ITU (ThStress) mean testicular temperature value by thermography (AvTTpTh).

thermal stress (ThStress), while THI combines temperature and humidity, reflecting the overall thermal sensation. In this same season, TotD and MinorD were also positively associated with EnTemp, ThStress, and THI. This suggests that increases in temperature and thermal stress are related to changes in specific morphological characteristics of spermatozoon, confirming the influence of environmental variables on biological variables.

The direction of the correlations between the variables shown in the Heatmap (Fig. 4) indicates the following moderate positive correlations: As environmental parameters (EnTemp) and thermal stress indicators (THI and ThStress) increase, average testicular temperature (AvTTpTh) and semen morphological defects also tend to increase.

There was also a slight tendency for an increase in mean testicular temperature (AvTTpTh) correlating with a rise in sperm defects (MinorD and TotD). However, this relationship is weaker compared to the correlations with environmental parameters and thermal stress, indicating a positive but weak correlation. Therefore, the use of Infrared Thermography in the testicular region is emphasized as an important tool for evaluating testicular function, particularly during the Brazilian summer. In addition, in tropical regions such as the one studied, there is a seasonal effect on fertility, with winter offering conditions of thermal comfort and enhanced reproductive potential. These results can guide the efficient use of stallions for commercial purposes in a region with a tropical climate, similar to the one investigated here.

When a stallion's body temperature increases, generating an increase in scrotal temperature, it can trigger reactions that impair spermatogenesis, such as failure in the formation of spermatids and spermatogonia and a decrease in testosterone levels [18]. Similar to this research, a study that evaluated the influence of the rainy (summer) and dry (winter) seasons on the reproductive and seminal characteristics (volume, concentration, motility and vigor) of Pantaneiro stallions, reported a higher percentage of sperm defects in semen collected in the summer, a period characterized by high environmental temperatures [19].

For the studied region, ThStress values are considered adequate in the range between 1.1 and 68.1 for THI. The AvTTpTh values considered adequate are 31.1/31.2°C. High temperatures, humidity, and intense solar radiation can cause stress in animals, triggering a compensatory physiological reaction of the organism through changes in rectal temperature, heart rate, respiratory rate, and increased heat stress indices in the summer, as observed here [20,21]. The stress caused by heat acts on the hypothalamic-pituitary-gonadal axis, reducing its activity on the reproductive tract, and affecting the concentration of reproductive hormones, testicular thermoregulation, and fertility [22].

Most testicular problems in stallions are related to changes in the ability to control gonadal temperature, and unadapted horses or those with testicular thermoregulation deficits may present seminal alterations when subjected to high environmental temperatures [23,13], as observed in this study, in the summer (Tables 1 and 2).

As for the testicular blood flow variables by Doppler ultrasound, the mean systolic velocity (AvSVel) and mean diastolic velocity (AvDVel) of the testicular artery were found to be higher ($P < 0.05$) in winter. This suggests that testicular blood flow is improved during this season, with measurements of AvSVel at 33.3 cm/s and AvDVel at 5.9 cm/s. These enhanced blood flow values likely contribute to better seminal characteristics. In addition, Doppler ultrasound has proven to be an effective and non-invasive method for reproductive evaluation, providing

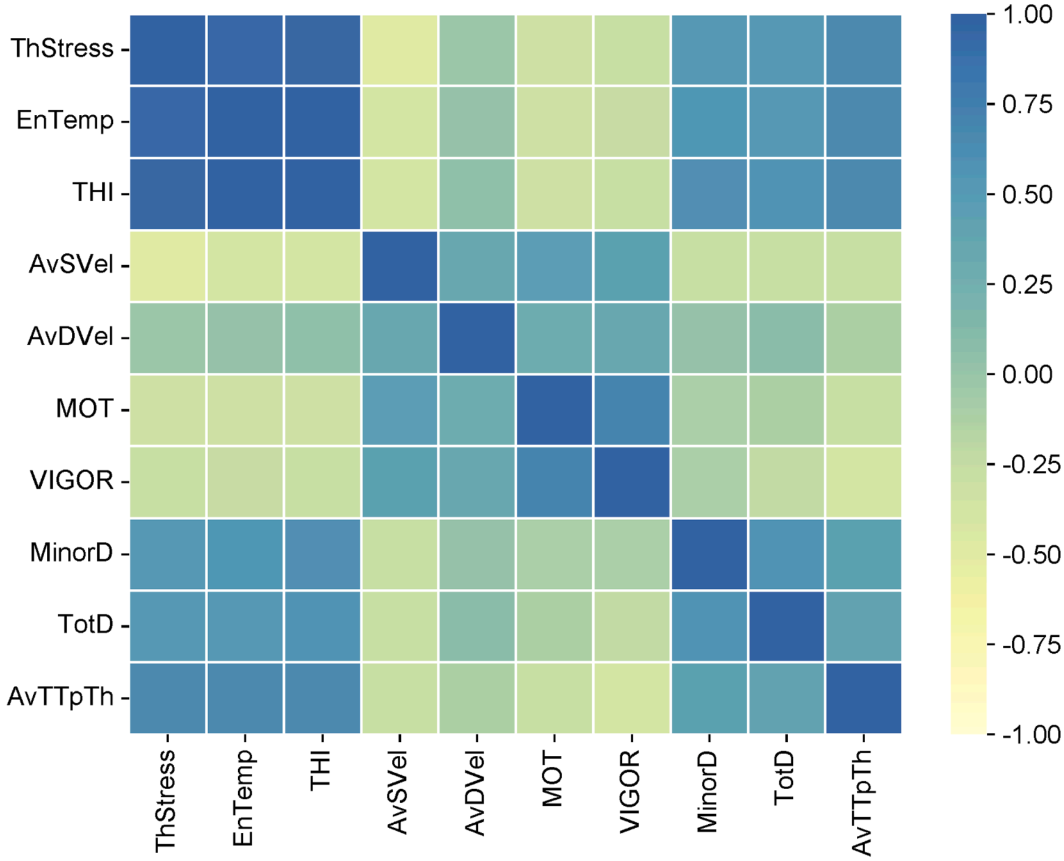

Fig. 4. Heat map of the main components for seminal-circulatory characteristics and thermal comfort environment. Positive score values show a positive association between the variables. *Dopplermetric parameters and seminals*: average diastolic velocity (AvDvel), average systolic velocity (AvSvel), motility (MOT), vigor (VIGOR), minor defects (MinorD), total defects (TotD). *Thermal comfort parameters:* environmental temperature (EnTemp), Temperature Humidity Index (THI), Thermal stress according to ITU (ThStress) and average thermal testicular temperature (AvTTpTh).

valuable indicators of testicular blood flow and spermatogenesis.

Dopplerometric variables (AvSVel and AvDvel) and mean testicular temperature by thermography (AvTTpTh) were correlated with motility (MOT) and vigor (VIGOR) even during the summer. This suggests that changes in testicular blood flow velocity may influence sperm motility and vigor. In the summer, these testicular variables are affected by heat stress. Likewise, the correlation between testicular temperature (AvTTpTh) and seminal quality during the summer is expected due to the effect of heat stress on testicular function.

Doppler velocimetry parameters showed weak and negative correlations with environmental (EnTemp) and heat stress (THI and ThStress) parameters, except for a weak positive correlation of mean diastolic velocity (AvDVel) with sperm vigor. The analysis pointed to several relationships and interactions between the studied variables, highlighting the importance of considering multiple factors in an integrated manner when interpreting the results.

In winter, there was no clear association between the variables analyzed (Fig. 3). This reveals that the effects of heat stress on testicular and seminal variables are not significant or are different when compared to summer. In winter, temperature and heat stress are reduced, which can minimize or alter the association of these variables, generating less influence of heat on testicular function and seminal properties.

In this sense, seminal production and testicular functionality are also affected by testicular hemodynamics, as it is a pathway for nutrients, hormones, and oxygen, and the testicular artery is primarily responsible for testicular blood supply, and its reduction can affect sperm production, triggering subfertility or infertility [24]. The testicular artery performs the heat exchange mechanism to reduce the temperature of the blood reaching the testicles [25,26]. Thus, its parameters allow monitoring of testicular irrigation and mapping of reproductive changes related to changes in blood flow [27].

A study investigated the application of Doppler ultrasonography for diagnosing testicular dysfunction in stallions and examined the correlation between Doppler parameters of the testicular artery and sperm quality. The authors reported a marked reduction in testicular blood flow among subfertile stallions, which included a significant decrease in the diameter of the capsular artery. Furthermore, all Doppler velocity measurements—such as peak systolic velocity, end-diastolic velocity, and mean time to maximum velocity—were notably lower in subfertile stallions compared to fertile ones.

The lack of association identified by F_A during winter points to the importance of summer in the changes of physiological and seminal parameters analyzed concerning the proposed evaluation methods of infrared thermography and Doppler ultrasonography. The used indices characterized the environmental, body, circulatory, and seminal parameters of stallions and their differences, correlations, and associations in winter and summer, enabling the selection of the season with the greatest reproductive performance, through infrared thermography and Doppler ultrasonography.

Identifying the relationships between various factors can help in developing strategies to adapt to and mitigate environmental heat. This is vital for safeguarding the health and welfare of horses and ensuring their reproductive capacity on warm summer days. The findings suggest that increasing environmental temperatures influence testicular temperature and the morphological and circulatory characteristics of semen. Additionally, the evaluation methods employed can predict stallion fertility, which has typically been assessed only after semen collection.

5. Conclusion

Climatic conditions in the Brazilian summer and winter are translated into different associations and correlations between environmental, seminal, and circulatory parameters in the equine testicle. On hotter days, stallions exhibit greater thermal stress, and sperm defects with worse testicular vascular perfusion and sperm kinetics values. Thus, the increase in environmental temperature, thermal stress indices, and testicular temperature in summer negatively affected semen quality in stallions

Therefore, Doppler ultrasonography and infrared thermography are recommended as predictive and non-invasive tests in the investigation of horse semen quality, since strong correlations and associations of their indices with seminal quality parameters were observed. Finally, this study provides a solid basis for the introduction of these two techniques in the assessments of reproductive health, fertility, and environmental thermal comfort, which can be performed in an integrated manner and without contraindications in stallions. These conclusions are particularly relevant in competitive athletic horses since an even greater increase in temperature and thermal stress is expected during the hottest season of the year, which may further compromise the fertility of stallions.

CRediT authorship contribution statement

Luiza Maria Feitosa Ribeiro: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **João Victor Bersot Gomes:** Methodology, Data curation. **Wilder Hernando Ortiz Vega:** Writing – review & editing, Writing – original draft, Software, Formal analysis, Data curation. **Andressa da Silva Alves:** Methodology, Data curation. **Maurício Netto Machado:** Methodology, Data curation. **Luan Junio Wutke:** Methodology, Investigation, Data curation. **Célia Raquel Quirino:** Methodology, Investigation, Formal analysis, Conceptualization. **Roberta Carvalho Basile:** Visualization, Supervision, Methodology, Conceptualization. **Paula Alessandra Di Filippo:** Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Luiza Maria Feitosa Ribeiro reports administrative support and equipment, drugs, or supplies were provided by State University of Norte Fluminense

Acknowledgements

The authors would like to express their sincere gratitude to the following individuals and institutions, whose support was essential for the completion of this research work: The State University of Norte Fluminense Darcy Ribeiro, especially the Graduate Program in Animal Science. The Rio de Janeiro State Foundation for Research Support - FAPERJ. Dr. Roberta Basile, a veterinarian, Professor Celia R. Quirino, Wilder H. Ortiz Vega for the guidance and support throughout the development of this work. Your contributions, comments and encouragement were essential. I am grateful for your generosity in sharing your knowledge and for your patience during the entire process of conducting this research.

The Laboratory of Animal Reproduction and Genetic Improvement (LRMGA). The horse breeders in the Norte Fluminense region who allowed the use of animals in this research.

References

[1] Jacob JCF, VLT de Jesus, Ferraz PJ. Subfertility in stallions: how can we diagnose and overcome this problem? Brazilian J Animal Reprod 2024;48(1):69–76.
[2] Ferraz J., Jhonnatha J., Oliveira P., Ferraz P., Guerson Y. Thermal stress in equine reproduction [Internet]. [cited 2025 Feb 18]. Available from: http://cbra.org.br/portal/downloads/publicacoes/rbra/v43/n2/p216-221%20(RB796).pdf.
[3] Hansen PJ. Effects of heat stress on mammalian reproduction. Philosophical Trans Royal Society B: Biol Sci 2009;364(1534):3341–50. Nov 27.
[4] Kastelic JP, Wilde RE, Bielli A, Genovese P, Rizzoto G, Thundathil J. Hyperthermia is more important than hypoxia as a cause of disrupted spermatogenesis and abnormal sperm. Theriogenology 2019;131:177–81. Apr 1.
[5] Gordon RK, Mawyer JD, Cavinder CA, Sigler DH, Blanchard TL, Love CC, et al. Effects of moderate exercise on semen parameters and serum LH and cortisol concentrations in stallions. J Equine Veterin Sci 2014;34(1):65. Jan.
[6] Aragona F, Pietro SD, Arfuso F, Fazio F, Piccione G, Giudice E, et al. Correlation between ocular and rectal temperature with intra ocular pressure in horse during exercise. Animals 2022;12(14):1–4. Jul 211850–0.
[7] Ortega-Ferrusola C, Gracia-Calvo L, Ezquerra J, Pena F. Use of colour and spectral doppler ultrasonography in stallion andrology. Reproduct Domestic Animals 2014; 49:88–96. Oct.
[8] Aragona F, Di Pietro S, Arfuso F, Fazio F, Piccione G, Giudice E, et al. Correlation between ocular and rectal temperature with intraocular pressure in horses during exercise. Animals 2022;12(14):1850.
[9] Bouchama A, Abuyassin B, Lehe C, Laitano O, Jay O, O'Connor FG, et al. Classic and exertional heatstroke. Nature Rev Disease Primers [Internet] 2022;8(1):1–23. Feb 3 [cited 2022 Jun 22]Available from, https://www.nature.com/articles/s41572-021-00334-6#citeas.
[10] CLIMATE-DATA.ORG. Climate data for cities worldwide - Climate-Data.Org [Internet]. Climate-data.org. 2019. Available from: https://en.climate-data.org/.
[11] Andrological Examination Manual [Internet]. CBRA. Available from: http://cbra.org.br/br/publicacoes/manual-de-exame-andrologico.
[12] Carvalho Basile Roberta, Albernaz Raquel Mincarelli, Araújo Renatha. Queiroz-Neto A. Practical guide to thermographic examinations in horses 2010 Brazilian Journal of Equine Medicine. 28 Brazilian J Int Med [Internet] 2010;34:24. Sep 8.
[13] Machado WM, Barros CHSC, Matarazzo SV, Snoeck PPN. Scrotal thermographic profile and seminal characteristics of Mangalarga Marchador stallions bred in the Atlantic Rainforest biome. Brazilian Archive Veterin Med Zootechn 2021;73(4): 763–70. Aug.
[14] Kaiser HF. The application of electronic computers to factor analysis. Edu Psychol Measurem 1960;20(1):141–51. Apr.
[15] Cerny BA, Kaiser HF. A study of a measure of sampling adequacy for factor-analytic correlation matrices. Multivar Behav Res 1977;12(1):43–7. Jan.
[16] Tang D, Chen M, Huang X, Zhang G, Lin Z, et al. SRplot: a free online platform for data visualization and graphing. PLoS One 2023;18(11):e0294236.
[17] Babicki S, Arndt D, Marcu A, Liang Y, Grant JR, Maciejewski A, et al. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res 2016;44(W1):W147–53. May 17.
[18] Kang H, Zsoldos RR, Sole-Guitart A, Narayan E, Cawdell-Smith AJ, Gaughan JB. Heat stress in horses: a literature review. Int J Biometeorol 2023;67(6):957–73.
[19] Silva A., Alejandro M., Silva, Candida I, Quirino C.R. Pantaneiro stallions: did the environment influence seminal traits and testicular biometry? [Internet]. 2024. Available from: https://www.researchgate.net/publication/377948763_Pantaneiro_stallions_did_the_environment_influence_seminal_traits_and_testicular_biometry.
[20] Pereira TR, Lindenberg J, Evangelista AF, Richelly M, Rocha AO. Evaluation of the thermoregulatory response of goats naturally exposed to the tropical climate using longitudinal data. Semin: Agricul Sci [Internet] 2023;44(2):635–52. May 3Available from, https://www.researchgate.net/publication/370859978_Evaluation_of_the_thermoregulatory_response_of_goats_naturally_exposed_to_the_tropical_climate_using_longitudinal_data.
[21] Fialho ALL, Souza-Cáceres MB, Silva W aL, Arruda EDS, Kischel H, Ribeiro-Ferreira MGC, et al. Effect of acute and chronic caloric heat stress on oocyte quality in adapted breed cattle. Brazilian Archive Veterin Med Zootechnics [Internet] 2018;70:64–72 [cited 2022 Oct 25]Available from, https://www.scielo.br/j/abmvz/a/HFMBPhJxVwytn7KczNsXHtx/?format=html&lang=pt.
[22] Ferraz J., Jhonnatha J., Oliveira P., Ferraz P., Guerson Y. Estresse térmico na reprodução equina thermal stress in equine reproduction [Internet]. Available from: http://cbra.org.br/portal/downloads/publicacoes/rbra/v43/n2/p216-221%20(RB796).pdf.
[23] Gomes G., Patrão L., Gomes M. Frozen and cooled semen: challenges and solutions for Mangalarga Marchador breed. [Internet]. [cited 2025 Apr 14]. Available from: http://www.cbra.org.br/pages/publicacoes/rbra/download/p210-215.pdf.
[24] Hassan RMA, Abdelsabour-Khalaf M, Abd-Elhafez EA, Anel-López L, Riesco MF, et al. Morphological and ultrasonographic characterization of the three zones of supratesticular region of testicular artery in Assaf rams. Sci Rep 2022;12(1):8291.
[25] McKinnon AO, Squires EL, Vaala WE, Varner DD. Equine Reproduction. 2nd ed.. Ames: Wiley-Blackwell; 2011.
[26] Gledson A, Braz HA, Silva Madalena M, Marlon Silva SV. Impact of doppler ultrasonography on the clinical andrological examination of equine reproductives. Res Society Dev 2021;10(14). Nov 14e582101422412-e582101422412.
[27] Silva DF. Hemodynamic evaluation of the testicular artery of normal stallions and those with torsion of the spermatic cord [dissertation on the Internet]. Belo Horizonte: Universidade Federal de Minas Gerais; 2021 [cited 2025 Feb 19]. Available from: http://hdl.handle.net/1843/36699.