

# Effects of ambient air temperature, humidity, and wind speed on seminal traits in Braford and Nellore bulls at the Brazilian Pantanal

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**Abstract** The aim of this study was to evaluate the bioclimatic thermal stress assessed by Equivalent Temperature Index (ETI) and Temperature Humidity Index (THI) on Braford and Nellore bulls sperm quality during the reproductive seasons at the tropical region in the Brazilian Pantanal. We used 20 bulls aged approximately 24 months at the beginning of the study. Five ejaculates per animal were collected using an electroejaculator. Temperature, air humidity, and wind speed data were collected every hour from the automatic weather station at the National Institute of Meteorology. Infrared thermography images data were collected to assess the testicular temperature gradient in each animal. Data were analyzed with ANOVA using MIXED procedure of SAS and means were compared using Tukey's HSD test. The THI and ETI at 12 days (epididymal transit) were higher in January (89.7 and 28.5, respectively) and February (90.0 and 29.0, respectively) compared to other months ( $P < 0.01$ ). Total seminal defects differ only in Bradford bulls between the months of November and February. Nellore bulls had lower major defects (MaD) and total defects (TD) compared to Braford.

Nellore bulls showed correlation between minor defects (MiD) and THI for 30 days (0.90) and 18 days (0.88;  $P < 0.05$ ). Braford bulls showed correlation for MaD (0.89) in ETI for 12 days ( $P < 0.05$ ). Infrared thermography showed no difference between animals. Reproductive response to environmental changes is a consequence of Nellore and Braford adaptation to climate stress conditions. Both THI and ETI environmental indexes can be used to evaluate the morphological changes in the seminal parameters in Nellore or Braford bulls; however, more experiments should be performed focusing on larger sample numbers and also in reproductive assessment during the consecutive years to assess fertility potential.

**Keywords** Thermal indexes · Adaptability · Tropical region · Semen parameters · Bulls

## Introduction

Environmental conditions of cattle breeding at tropical regions may be affected by heat stress when the mechanisms of body thermoregulation are unable to promote heat loss adequately. An increase in the internal temperature above physiological limits causing an unbalanced testicular scrotal thermoregulation may result in testicular degeneration in mammal animals (Cheminau 1994; Kastelic et al. 2001). In humans, a review described that global warming may have contributed to changes in human fertility in the last twentieth century (Fisch et al. 2003). Adaptation and use of animal breeding in tropical regions of Brazil become an important aspect of livestock production efficiency as a result of probable climate environmental changes (McManus et al. 2011).

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The thermal stress decreases conception rates, and it also decreases semen quality in bovine (De Rensis and Scaramuzzi 2003; Burns et al. 2010). Environmental effects on seminal characteristics during seasons in bulls have been well described by some authors (Nichi et al. 2006; Menegassi et al. 2015, 2016). In agreement, Meyerhoeffler et al. (1985) observed that exposure of *Bos taurus* bulls at temperature of 35 °C for 8 h resulted in decreased semen quality. Koivisto et al. (2009) also evaluated the seminal characteristics, such as seminal motility, vigor, and sperm morphology, of European bulls (*B. taurus*) and Indian bulls (*Bos taurus indicus*) located in southeastern Brazil under the influence of the tropical seasons and observed that heat tolerance was greater in (zebu) Indian bulls compared to European bulls. Thus, animals under climatic change conditions have mechanisms to promote the adaptation to the environment that may interfere with spermatogenesis and sperm production.

It is well known that animals in different environments are able to adapt its physiology to face global environmental changes (Silva and Maia 2013). In tropical regions, the equivalent temperature index (ETI) seems more appropriate than the temperature and humidity index (THI) due to the inclusion of the wind speed to determine a better caloric evaporation when animals are submitted to heat stress (Silva et al. 2007). Different indexes, ETI, THI, and Globe Index and Humidity (BGHI), have been used to measure climate changes in tropical region in Holstein and Jerseys cows, providing uncertainly results when correlated to rectal temperature and respiratory frequency rates (Silva et al. 2007). The animal living environment is complex, and several indexes have been used to measure the best indicator parameter to detect environmental changes to which animals are exposed. However, from our knowledge, there is no studies in the literature that provide information using ambient air temperature, humidity, and wind velocity on seminal quality in beef bulls under field conditions in tropical regions.

Bulls are subject to environmental variations that interfere with their fertility and reproductive effectiveness (Berry et al. 2011). Therefore, the objective of this study was to evaluate climate changes by ETI and THI and its effects on Braford and Nellore bulls sperm quality during the reproductive seasons at the Brazilian Pantanal.

## Materials and methods

### Animals and climate data

Twelve bulls Braford (5/8 Hereford × 3/8 Nellore) and eight Nellore bulls aged from 2 to 5 years were used in this experiment. All procedures were approved by the Federal University of Rio Grande do Sul Ethical Committee for care and use of experimental animals (Project 26250,

CEUA/UFRGS). Animals were evaluated and semen was collected at five occasions as follows: September 16, 2014; November 13, 2014; January 12, 2015; February 02, 2015, and March 11, 2015.

All animals were kept outdoor in the same environmental conditions and fed in a diet system based on natural pasture. Bulls received mineral supplementation and free access to water and shadow. The experiment was conducted at Tropical region in Brazilian Pantanal Matogrossense at the farm located at 54° 19' 12" W longitude and 30° 20' 11" S latitude, at 114 m altitude and climate classified as Aw, according to Köppen-Geiger. The Pantanal region has two different characteristic seasons, one considered rainy season from October to April and another called dry season from May to September.

### Reproductive evaluation

All animals had a breeding soundness examination at the beginning of the experiment. The examination consisted of a comprehensive general clinical examination, specific reproductive examination, and seminal evaluation. The rectal temperature from each bull was registered in all evaluations, measured with a digital thermometer for 1 min prior to semen collection. A total of five ejaculates (one per month) were collected from each bull using an automatic operated electroejaculator Pulsator IV (Lane Manufacturing Denver, CO, USA). For semen collection, bulls were restrained to facilitate rectal probe insertion and feces were manually removed. An electroejaculator probe with three ventrally oriented longitudinal electrodes was used to deliver a sequence of electrical impulses to each bull.

Semen evaluation was performed and mass motion (MM) was determined by placing a 10 µL drop of semen on a prewarmed microscope slide, and the edge of the drop was examined using an optical microscope at ×20 magnification. Mass motion received a score ranging from 0 to 5: 0 = no swirl, 1 = no swirl with generalized oscillation of individual sperm only, 2 = very slow distinct swirl, 3 = slow distinct swirl, 4 = moderately fast distinct swirl and eddies, and 5 = fast distinct swirls and eddies with the appearance of good quality semen. Sperm motility (M) was examined under a bright-field microscope at a magnification of ×40 with a 5-µL aliquot of semen placed on a warmed (37 °C) slide and covered with a coverslip. Sperm M was evaluated as the percentage of sperm movement (0 to 100 %). Vigor (VIG) was evaluated using a scale from 0 to 5 based on the sperm progressive movement, where 0 = none, 1 = very weak, 2 = weak, 3 = intermediate, 4 = strong, and 5 = very strong.

In addition, each semen aliquot was diluted in buffered saline-formaldehyde (1:10), and sperm morphology was analyzed using a phase-contrast microscope. Spermatozoa were also evaluated with eosin-nigrosin staining using a bright-field

microscope. Major sperm defects (MaD) were considered as the following: acrosome defect, abnormal head, double head, abnormal small head, proximal protoplasmic droplet, midpiece defect, accessory tail, and strongly bent tail. Minor sperm defects (MiD) included distal protoplasmic droplet, abaxial implantation, bent tail, and detached head. The total defects (TD) were considered in 200 sperm cells from each animal, and a sperm classification was performed as previously described by the Bull Breeding Soundness Evaluation of the Western Canadian Association of Bovine Practitioners (Barth 2000). We considered the spermatogenesis phase of 18 days and the epididymal transit of 12 days, according to the criteria adopted by Pineda and Faulkner (1980).

### Calculation of temperature-humidity and equivalent temperature indexes

The temperature, air humidity, and wind speed data were collected every hour from the automatic weather station at the National Institute of Meteorology (INMET 2014), situated at 57° 64' 08" W longitude and at 19° 00' 10" S latitude.

The THI data were estimated from the following equation described by The National Research Council (1971):

$$THI = (1.8 \times Tdb + 32) - (0.55 - 0.0055 \times RH) \times (1.8 \times Tdb - 26)$$

where  $Tdb$  is the dry-bulb air temperature (°C) and  $RH$  is the relative air humidity in decimal form. The ETI data were estimated from the following equation described by Baeta et al. (1987):

$$ETI = 27.88 - 0.456 Ta + 0.010754 Ta^2 - 0.4905RH + 0.00088 RH^2 + 1.1507 W - 0.126447 W^2 + 0.019876 Ta RH - 0.046313 Ta W$$

where  $Ta$  is the air temperature (°C);  $RH$  is the relative air humidity (%); and  $W$  is the wind speed ( $m s^{-1}$ ).

### Infrared thermography measurement and analysis

The mean temperature of the scrotal surface of each bull was evaluated by positioning the FLIR T 300 infrared camera 1 m from each testicular pair-oriented perpendicular to the scrotum. The gradient between the images at 76,800 pixel ( $320 \times 240$ ) resolution with a thermal sensitivity of under 0.05 °C was evaluated. The temperatures from the proximal (proximal pole temperature; PPT) and distal (distal pole temperature; DPT) poles of the scrotum were measured using a line 1 pixel high located from the side to the scrotum image for each region.

The thermal gradient (TG) variation between these two extremities was also evaluated in this manner. To evaluate

the bull's thermal status, a lateral image 1 m from the animal's head was recorded, and a circle was drawn around the orbital region including the ocular globe, the skin surrounding the ocular cavity, and the lacrimal gland (OcT). The images generated from the radiation emitted by the body (thermograms) were later analyzed by Quick Report software 1.2. The infrared thermography measurement and analysis were conducted as previously described by Menegassi et al. (2015).

### Statistical analyses

Data were analyzed with ANOVA using MIXED procedure of SAS v.9.3 (Statistical Analysis System, SAS 2011). The model included the effects for collection date, racial group of bulls, and its interactions. The statistical model regarding the analysis of the variables studied was represented by:

$$Y_{ijk} = \mu + T_i + \beta_j + (T\beta)_{ij} + \gamma_k + \varepsilon_{ijk}$$

where  $Y_{ijk}$  is the dependent variable,  $\mu$  is an average inherent to all observations,  $T_i$  represents the effect of collection date,  $\beta_j$  is the effect of racial group of bulls,  $T\beta$  represents the interaction between collection date and racial group,  $\gamma_k$  is the random effect of animal (bull), and  $\varepsilon_{ijk}$  represents the experimental error.

Data normality and homoscedasticity were verified using the Shapiro-Wilk test ( $P > 0.05$ ). When necessary, physiological variables (RT, PPT, DPT, TG, and OcT), seminal variables (M, VIG, MM, MaD, MiD, and TD), and climatic variables (THI and ETI) were transformed and logarithmic transformation was used. The means of the seasons were compared using Tukey's HSD test, considering a significance level of 5 % ( $P < 0.05$ ). Pearson correlation analyses were used to correlate the THI, ETI, and the physiological and seminal parameters of bulls.

### Results

The THI at 30-day period was significantly higher in February (90.0) compared to other months ( $P < 0.01$ ). The THI during spermiogenesis (18 days) and at epididymal transit (12 days) was higher in January (89.5 and 89.7, respectively) and February (89.7 and 90.0, respectively) compared to other months ( $P < 0.01$ ). However, ETI at 30-day period was higher during the summer in January (28.6) and February (28.7) compared to other months ( $P < 0.01$ ). The ETI with 12 days at epididymal transit was significantly higher in January and February compared to other months (28.5 and 29.0, respectively;  $P < 0.01$ ). In addition, the lower THI and ETI were observed in March and differ from January and February ( $P < 0.01$ ). Microclimate factors used to obtained ETI and THI are shown in Table 1.

**Table 1** Temperature-humidity and equivalent temperature indexes collected during seminal evaluation from Nellore and Braford bulls at Brazilian Pantanal

Variables	Collection month					Mean $\pm$ SE	Pr > F
	September	November	January	February	March		
THI 30d	82.81 $\pm$ 0.88c	85.67 $\pm$ 0.60b	87.91 $\pm$ 0.59ab	90.02 $\pm$ 0.51a	79.02 $\pm$ 0.30d	85.1 $\pm$ 0.41	<0.001
THI 18d	85.10 $\pm$ 0.83b	85.70 $\pm$ 0.83b	89.49 $\pm$ 0.53a	89.71 $\pm$ 0.76a	79.65 $\pm$ 0.32c	85.8 $\pm$ 0.49	<0.001
THI 12d	84.28 $\pm$ 1.10b	84.65 $\pm$ 1.14b	89.69 $\pm$ 0.78a	90.01 $\pm$ 1.07a	80.01 $\pm$ 0.33c	85.5 $\pm$ 0.63	<0.001
ETI 30d	26.52 $\pm$ 0.19c	27.72 $\pm$ 0.25b	28.61 $\pm$ 0.11a	28.75 $\pm$ 0.11a	26.17 $\pm$ 0.18c	27.5 $\pm$ 0.11	<0.001
ETI 18d	26.98 $\pm$ 0.21cd	27.73 $\pm$ 0.38bc	28.46 $\pm$ 0.09ab	29.02 $\pm$ 0.10a	26.23 $\pm$ 0.19d	27.7 $\pm$ 0.14	<0.001
ETI 12d	26.87 $\pm$ 0.28b	27.15 $\pm$ 0.46b	28.50 $\pm$ 0.11a	29.07 $\pm$ 0.09a	26.48 $\pm$ 0.20b	27.6 $\pm$ 0.17	<0.001

Within a row, means without a common lowercase letters differed by Tukey's test ( $P \leq 0.05$ );

Data showed as mean  $\pm$  SE (standard error), *THI* temperature-humidity index, *ETI* equivalent temperature index

The  $\Delta$ TG observed in Nellore and Braford animals was similar during the evaluated months. However, mean  $\Delta$ TG decreased in Nellore ( $2.1 \pm 0.15$ ) compared to Braford ( $3.3 \pm 0.20$ ) bulls ( $P < 0.05$ ; Table 2). The  $\Delta$ TG (temperature gradient variation) for testicular scrotum temperatures for one Braford and one Nellore animal during the study period are presented in Fig. 1.

In Braford, sperm M was lower in March ( $60.90 \pm 7.31$ ) and January ( $74.54 \pm 2.37$ ) compared to other evaluated months ( $P < 0.01$ ). In Nellore bulls, sperm MM and VIG in November ( $4.87 \pm 0.12$ ) showed difference compared to September, January, and March ( $3.50 \pm 0.56$ ,  $3.37 \pm 0.27$ , and  $3.37 \pm 0.32$ , respectively;  $P < 0.01$ ). Sperm MM and VIG in Braford bulls in November ( $4.63 \pm 0.20$ ) showed difference compared to March ( $3.18 \pm 0.37$ ;  $P < 0.01$ ). Alterations in the spermatid pathology were identified in Braford animals in November ( $12.36 \pm 1.45$ ) compared to February ( $23.36 \pm 4.17$ ;  $P = 0.02$ ). Overall, MaD and TD in Nellore ( $7.90 \pm 0.96$  and  $12.70 \pm 1.09$ ) showed difference compared to Braford ( $11.80 \pm 1.03$  and  $17.30 \pm 1.23$ ) animals ( $P < 0.05$ ; Table 2).

For the seminal variables in Nellore, MiD was positively correlated with THI (30 days) period ( $0.90$ ;  $P < 0.05$ ) and spermiogenesis (18 days) period ( $0.88$ ;  $P < 0.05$ ). The OcT had a positive correlation with DPT ( $0.95$ ;  $P < 0.05$ ). Motility showed a highly positive correlation with VIG and MM ( $0.98$ ;  $P < 0.01$ ) (Table 3). In Braford bulls, M showed a highly positive correlation with VIG and MM ( $0.96$ ;  $P < 0.01$ ). The TD was positively correlated with MaD ( $0.94$ ;  $P < 0.05$ ). In addition, we also observed that ETI at epididymal transit (12 days) period had positive correlation with MaD ( $0.89$ ;  $P < 0.05$ ). The OcT had a positive correlation with DPT ( $0.97$ ;  $P < 0.05$ ) (Table 4).

## Discussion

The average THI and ETI observed in this study during seminal evaluation were higher during the months of January and

February compared to the other evaluated months. This indicate that the presence of variables such as air humidity, air temperature and wind speed are crucial in the evaluation of climate stress using either indexes which can be used to determine the characteristic of the rainy season in the tropical region. The  $\Delta$ TG measured by infrared thermography showed no testicular changes to eliminate Nellore and Braford bulls based on the reproductive evaluation where the study was conducted, similarly with the previous findings described in the literature in subtropical regions (Menegassi et al. 2015, 2016). However, lower  $\Delta$ TG in Nellore bulls observed at the tropical environment may be related to the animal adaptation and to the physiological anatomical characteristics of the reproductive system of *indicus* sub species compared to Braford that are considered as cross breeding animals.

According to the literature, the results of the  $\Delta$ TG in our study showed no correlation with the evaluated seminal parameters (Menegassi et al. 2015, 2016). Thus, the infrared thermography cannot be used alone as an indirect technique to evaluate the effects of heat stress on the seminal parameters bulls. In this experiment, the highest THI at 30, 18, and 12 days (90.0, 89.7, and 90.0, respectively) and ETI (28.7, 29.0, and 29.0, respectively) were observed in February. Both environmental indexes are important to link any climatic change conditions that could be affect changes in the seminal parameters in Nellore or Braford bulls. Different experimental models that take into account the wind speed and radiation are crucial to ensure ideal livestock production to enhance animal thermal comfort by mitigating the environmental effects (Mader et al. 2010). Our findings showed that THI and ETI observed for 18 days (spermiogenesis) and 12 days (epididymal transit) before semen collection were not harmful enough to determine the morphological changes during spermatogenesis. Accordingly, Menegassi et al. (2015b) found no morphological changes during spermiogenesis with THI of 83.8 and 93.0 in Bradford and Brangus bulls in sub tropical regions, respectively.

**Table 2** Effect of collection month on physiological changes and seminal parameters from Nellore and Braford bulls

Variables	Collection month					Mean ± SE	Pr > F
	September	November	January	February	March		
<b>Physiological changes</b>							
<i>Nellore</i>							
TG	2.27 ± 0.39	1.61 ± 0.30	2.72 ± 0.25	2.16 ± 0.34	1.72 ± 0.38	2.10 ± 0.15B	0.164
PPT	33.67 ± 0.24bc	35.01 ± 0.27ab	35.22 ± 0.44a	33.78 ± 0.32bc	33.62 ± 0.33 c	34.20 ± 0.18B	0.001
DPT	31.40 ± 0.27c	33.40 ± 0.13a	32.62 ± 0.33ab	31.75 ± 0.30bc	31.90 ± 0.32 bc	32.20 ± 0.16 A	<0.001
OcT	35.62 ± 0.44bc	37.82 ± 0.21a	36.61 ± 0.28ab	35.18 ± 0.35c	35.86 ± 0.14 bc	36.20 ± 0.20	<0.001
RT	39.18 ± 0.10ab	39.20 ± 0.07ab	38.97 ± 0.12b	39.13 ± 0.12ab	39.61 ± 0.16 a	39.20 ± 0.06	0.013
<i>Braford</i>							
TG	4.26 ± 0.51	2.65 ± 0.30	3.34 ± 0.45	3.61 ± 0.38	3.01 ± 0.45	3.37 ± 0.20 A	0.102
PPT	35.16 ± 0.51ab	34.82 ± 0.30ab	35.67 ± 0.39a	35.06 ± 0.30ab	34.02 ± 0.39 b	34.90 ± 0.18	0.065
DPT	30.90 ± 0.26c	32.17 ± 0.26ab	32.41 ± 0.21a	31.45 ± 0.30abc	31.10 ± 0.33 bc	31.60 ± 0.14B	<0.001
OcT	34.08 ± 0.22c	36.59 ± 0.26a	36.21 ± 0.18a	35.08 ± 0.15 ± b	34.46 ± 0.28 bc	35.30 ± 0.16	<0.001
RT	39.23 ± 0.10	39.30 ± 0.11	39.24 ± 0.21	39.50 ± 0.08	39.62 ± 0.14	39.40 ± 0.06 A	0.210
<b>Seminal parameters</b>							
<i>Nellore</i>							
M	72.50 ± 5.59	91.87 ± 1.87	71.87 ± 3.52	74.37 ± 6.77	71.25 ± 8.33	76.40 ± 2.70	0.073
VIG	3.50 ± 0.26 b	4.87 ± 0.12 a	3.37 ± 0.26 b	3.87 ± 0.39ab	3.37 ± 0.32b	3.80 ± 0.15	0.003
MM	3.50 ± 0.26 b	4.87 ± 0.12 a	3.37 ± 0.26 b	3.87 ± 0.39ab	3.37 ± 0.32b	3.80 ± 0.15	0.003
MaD	10.87 ± 3.25	7.37 ± .178	8.12 ± 2.79	6.37 ± 1.25	6.75 ± 0.97	7.90 ± 0.96B	0.861
MiD	4.87 ± 1.20	4.87 ± 1.15	4.87 ± 0.63	5.87 ± 0.78	3.87 ± 0.71	4.87 ± 0.40	0.322
TD	15.75 ± 3.64	12.25 ± 2.31	13.00 ± 2.91	12.25 ± 1.60	10.62 ± 1.16	12.70 ± 1.09B	0.688
<i>Braford</i>							
M	80.45 ± 3.04 a	86.81 ± 2.36 a	74.54 ± 2.37 ab	80.0 ± 4.31a	60.90 ± 7.31b	76.50 ± 2.20	0.001
VIG	4.09 ± 0.25 ab	4.63 ± 0.20 a	3.63 ± 0.24 ab	4.27 ± 0.27ab	3.18 ± 0.37b	3.96 ± 0.13	0.005
MM	4.09 ± 0.25 ab	4.63 ± 0.20 a	3.63 ± 0.24 ab	4.27 ± 0.27ab	3.18 ± 0.37b	3.96 ± 0.13	0.005
MaD	9.81 ± 1.80	8.81 ± 1.27	12.72 ± 1.57	17.27 ± 3.73	10.54 ± 1.75	11.80 ± 1.03 A	0.053
MiD	3.81 ± 0.35	3.54 ± 0.51	7.63 ± 1.79	6.09 ± 0.93	6.54 ± 1.62	5.52 ± 0.55	0.059
TD	13.63 ± 1.94ab	12.36 ± 1.45b	20.36 ± 2.28ab	23.36 ± 4.17a	17.09 ± 2.02ab	17.30 ± 1.23 A	0.020

Within a row, means without a common lowercase letters differed by Tukey's test ( $P \leq 0.05$ )

Within a column, means without a common uppercase letters differed by Tukey's test ( $P \leq 0.05$ )

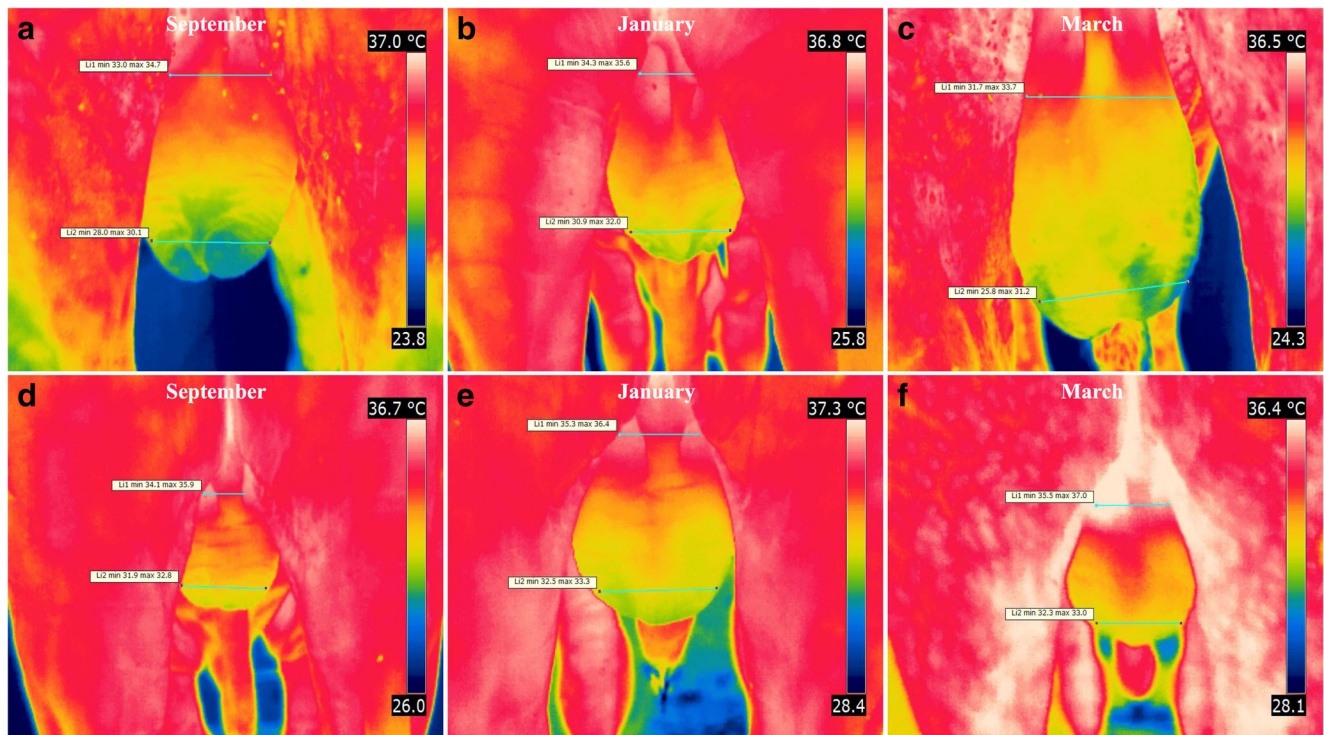
Number of Nellore bulls/ejaculates (12/60); Number of Braford bulls/ejaculates (8/40)

Data showed as mean ± SE (standard error), *M* motility (%), *VIG* vigor (0–5), *MM* mass motion (0–5), *RT* rectal temperature (°C), *PPT* proximal pole temperature (%), *DPT* proximal pole temperature (%), *TG* temperature gradient (°C), *OcT* ocular temperature (°C), *MaD* major defects (%), *MiD* minor defects (%), *TD* total defects (%)

The *M*, *VIG*, and *MM* for Braford bulls were lower in March probably because these animals were at the end of the breeding season. Nellore bulls showed reduced *VIG* and *MM* in September and March, probably because they were in sexual rest and also at the end of the breeding season. A study in tropical climates using Simmental and Nellore bulls showed several changes in sperm morphology, *M*, *VIG*, and *MM* during all evaluated seasons and its association with higher levels of reactive oxygen species (ROS) production and decreased activity of antioxidant enzymes in Simmental ejaculated semen (Nichi et al. 2006). In the human epididymis, excess residual cytoplasm contains high levels of enzymes resulting

from the degradation of cytoplasmic droplets, which produce excessive amounts of ROS (Rengan et al. 2012). Further studies are guaranteed to identify ROS and antioxidant enzymes parameters that may be involved in the spermatogenesis changes under different environmental conditions.

The morphological changes found in both bulls breeders, according to THI and ETI, are not crucial to eliminate animals at the BBSE exam. Studies using Angus bulls showed that morphological alterations classified as intermediated piece-reflex (DMR) are associated with low concentration of testosterone or related to heat stress (Cassady et al. 1953; Barth and Bowman 1994). Barth (2000), studying Angus and Jersey



**Fig. 1** Infrared thermography images of the same Nellore and Braford animal collected during different months of the year. Temperature gradient variation of testicular scrotum in September (a), January (b) and March (c) in Nellore bull and in September (d), January (e), and March (f) in Braford bull

bulls, found a hereditary predisposition of DMR in sperm these bulls. Those alterations were probably due to environmental changes causing thermal stress and increasing the presence of seminal pathologies in less adapted animals.

The heat stress effects on the reproductive performance of bulls are often carried out by simulating the natural environment in climatic chambers or scrotal insulation (Kastelic et al. 1996; Fernandes et al. 2008).

Thus, the methodology used in this study uses animals under natural conditions subjected to environmental stress during their spermatogenesis and reproductive performance in the field. Besides air temperature, wind speed was also an important factor in the bovine thermoregulation process and has a more marked effect on testicular cooling (Overton et al. 2002). According to our data, it was observed that the bulls have adopted

**Table 3** Correlations between temperature-humidity and equivalent temperature indexes during spermatogenesis, thermographic variables of testes, and ocular globe temperature of Nellore bulls

Variables	THI	ETI	THI 18	ETI 18	THI 12	ETI 12	M	VIG	MM	RT	PPT	DPT	TG	OcT	MaD	MiD
M	0.17	0.15	0.30	0.26	-0.04	-0.15										
VIG	0.27	0.24	0.41	0.34	0.04	-0.03	0.98**									
MM	0.27	0.24	0.41	0.34	0.04	-0.03	0.98**	1.0**								
RT	-0.83	-0.78	-0.74	-0.71	-0.89*	-0.68	-0.11	-0.13	-0.13							
PPT	0.44	0.55	0.44	0.58	0.41	0.25	0.50	0.40	0.40	-0.59						
DPT	0.24	0.35	0.33	0.44	0.10	0.01	0.79	0.71	0.71	-0.24	0.87*					
TG	0.47	0.47	0.29	0.34	0.68	0.57	-0.59	-0.59	-0.59	-0.71	0.24	-0.26				
OcT	0.02	0.11	0.10	0.19	-0.09	-0.25	0.81	0.69	0.69	-0.16	0.81	0.95*	-0.30			
MaD	-0.22	-0.37	-0.37	-0.46	-0.09	-0.36	-0.18	-0.25	-0.25	-0.27	-0.08	-0.32	0.39	-0.07		
MiD	0.90*	0.77	0.88*	0.74	0.85	0.81	0.12	0.27	0.27	-0.71	0.07	-0.06	0.34	-0.23	-0.07	
TD	0.12	-0.06	-0.02	-0.16	0.23	-0.04	-0.13	-0.14	-0.14	-0.53	-0.05	-0.33	0.50	-0.15	0.92*	0.30

Number of Nellore bulls/ejaculates (12/60), *THI* temperature-humidity index, *ETI* equivalent temperature index, *M* motility (%), *VIG* vigor (0–5), *MM* mass motion (0–5), *RT* rectal temperature (°C), *PPT* proximal pole temperature (%), *DPT* proximal pole temperature (%), *TG* temperature gradient (°C), *OcT* ocular temperature (°C), *MaD* major defects (%), *MiD* minor defects (%), *TD* total defects (%), \**P* < 0.05; \*\**P* < 0.01

**Table 4** Correlations between temperature-humidity and equivalent temperature indexes during spermatogenesis, thermographic variables of testes, and ocular globe temperature of Braford bulls

Variables	ITU	ETI	ITU 18	ETI 18	ITU 12	ETI 12	M	VIG	MM	RT	PPT	DPT	TG	OcT	MaD	MiD
M	0.61	0.37	0.62	0.47	0.50	0.27										
VIG	0.55	0.34	0.60	0.44	0.39	0.23	0.96**									
MM	0.55	0.34	0.60	0.44	0.39	0.23	0.96**	1.0**								
RT	-0.31	-0.12	-0.20	-0.17	-0.38	-0.04	-0.66	-0.45	-0.45							
PPT	0.73	0.57	0.60	0.59	0.84	0.62	0.55	0.34	0.34	-0.78						
DPT	0.57	0.74	0.59	0.73	0.52	0.43	0.34	0.25	0.25	-0.42	0.51					
TG	0.07	-0.23	-0.07	-0.22	0.24	0.14	0.11	-0.00	-0.00	-0.29	0.42	-0.56				
OcT	0.54	0.69	0.60	0.71	0.43	0.34	0.44	0.40	0.40	-0.37	0.38	0.97**	-0.67			
MaD	0.69	0.67	0.67	0.64	0.71	0.89*	-0.01	0.01	0.01	0.33	0.29	0.03	0.25	-0.05		
MiD	0.17	0.41	0.12	0.30	0.30	0.49	-0.65	-0.71	-0.71	0.35	0.12	0.25	-0.09	0.06	0.54	
TD	0.57	0.65	0.54	0.59	0.64	0.84	-0.26	-0.26	-0.26	0.38	0.26	0.12	0.15	-0.01	0.94*	0.79

Number of Braford bulls/ejaculates (8/40), *THI* temperature-humidity index, *ETI* equivalent temperature index, *M* motility (%), *VIG* vigor (0–5), *MM* mass motion (0–5), *RT* rectal temperature (°C), *PPT* proximal pole temperature (%), *DPT* proximal pole temperature (%), *TG* temperature gradient (°C), *OcT* ocular temperature (°C), *MaD* major defects (%), *MiD* minor defects (%), *TD* total defects (%), \* $P < 0.05$ ; \*\* $P < 0.01$

different anatomic and physiological strategies to dissipate heat in an environment by elevated appearance of THI and ETI.

Nellore animals showed to be adapted to heat stress in particular to the anatomical features characteristic of the *Bos indicus* species that takes in consideration the pampiniform plexus to heat exchange mechanism as an important player in the physiological requirements for its normal reproductive performance (Brito et al. 2003, 2004; Fernandes et al. 2004). Recently, studies using computerized systems of arterial structures of bovine testicles show that the arterial system is anatomically different between *Bos taurus indicus* and *Bos taurus taurus*, suggesting that these differences can be part of the response to environmental stress conditions (Polguy et al. 2015). Testicles are particularly sensitive to the influence of external factors and to transient episodes of minor ischemia that can lead to functional disturbances (Wrobel et al. 1981). In addition, semen collected via electroejaculation is not a representative of a full ejaculate in regard to volume and concentration, but sperm motility and morphology do not differ from that in an ejaculate collected with an artificial vagina (León et al. 1991; Palmer et al. 2005). In our research, we observed that morphological semen quality has not changed significantly to reject a bull during BBSE exam during the breeding seasons.

## Conclusions

The reproductive response to environmental changes probably is a consequence of the genetic characteristics in Nellore and Braford breeds to the environment adaptation to hot climate conditions. The scrotal temperature gradient measured by

infrared thermography cannot be used alone to evaluate the effects of heat stress on the seminal parameters in bulls. Both THI and ETI environmental indexes can be used to evaluate morphological changes in the seminal parameters in Nellore or Braford bulls; however, more experiments should be performed focusing on larger sample numbers and also on reproductive assessment during the consecutive years to assess fertility potential.

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## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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