Accepted: 20 February 2017

DOI: 10.1111/and.12836

ORIGINAL ARTICLE

WILEY ANDROLOGIA

Testicular thermoregulation, scrotal surface temperature patterns and semen quality of water buffalo bulls reared in a tropical climate

L. K. X. Silva[1] | J. S. Sousa[1] | A. O. A. Silva[1] | J. B. Lourenço Junior[1] | C. Faturi[2] | L. G. Martorano[3] | I. M. Franco[4] | M. H. A. Pantoja[1] | D. V. Barros[1] | A. R. Garcia[5]

[1]Center of Biotechnology of Animal Reproduction, Federal University of Pará, Castanhal, Brazil

[2]Institute of Health and Animal Production, Federal Rural University of Amazonia, Belém, Brazil

[3]Embrapa Eastern Amazon, Brazilian Agricultural Research Corporation, Belém, Brazil

[4]Faculty of Architecture and Urbanism, Federal University of Pará, Belém, Brazil

[5]Embrapa Southeast Livestock, Brazilian Agricultural Research Corporation, São Carlos, Brazil

Correspondence
Alexandre Rossetto Garcia, Laboratory of Biotechnology of Animal Reproduction, Brazilian Agricultural Research Corporation/ Embrapa Southeast Livestock, São Carlos, SP, Brazil.
Email: alexandre.garcia@embrapa.br

Funding information
Coordenação de Aperfeiçoamento de Pessoal de Nível Superior; Universidade Federal do Pará; Empresa Brasileira de Pesquisa Agropecuária, Grant/Award Number: BIOTEC #01130600105 and PECUS #01100600107

Summary

This study evaluated the capacity of thermoregulation and its consequences on the scrotal surface temperature patterns and semen quality of buffalo bulls raised in a wet tropical climate. Eleven water buffaloes were evaluated in the rainiest, in the transitional and in the less rainy season. Air temperature and humidity were consistently high, but the animals did not show thermal stress in any season. The scrotal temperature gradient of buffalo bulls using infrared thermography was described, and three parallel and decreasing thermal bands were characterised. Sperm quality (n = 176 ejaculates) was maintained in normal parameters over the periods. Pearson's coefficients showed that sperm volume and progressive motility were negatively correlated with ocular globe, epididymal tail and minimum scrotal temperatures (p < .01). Sperm membrane integrity was negatively influenced by increases in epididymal tail and minimum scrotal temperatures (p < .01). Ocular globe temperature also showed positive correlation with rectal, spermatic cord, and epididymal tail temperatures (p < .01). Therefore, even under high temperature and humidity, the thermoregulatory system was effective in preventing heat stress and the normality of scrotal surface temperatures, spermatogenesis and sperm maturation were maintained.

KEYWORDS
Bubalus bubalis, infrared thermography, scrotal temperatures, scrotum, semen

1 | INTRODUCTION

Domestic buffaloes are warm-blooded animals and have a relatively efficient thermoregulatory system (Gudev et al., 2007). However, when subjected to high ambient temperatures, they can experience heat stress, consequently reducing the reproductive efficiency (Marai, El-Darawany, Fadiel, & Abdel-Hafez, 2008).

In addition to interfering in the systemic homeothermy, heat-related temperature changes can affect scrotal/testicular thermoregulation in mammals (Garcia, 2013). Therefore, the seminal quality of bulls can be negatively influenced by factors that reduce their thermal comfort, such as high air temperature and relative humidity (Kastelic & Brito, 2012; Santos et al., 2014). In individuals with less efficient thermoregulation, the temperature increase is associated with increased scrotal surface temperature and testicular internal temperature, which consequently reduces sperm quality (Berry, Evans, & Parland, 2011; Kastelic, 2014). This can be a determining factor to select bulls that can better adapt to megathermal climates and are the most fertile to be used in breeding programmes.

The scrotal surface temperature can be measured by infrared thermography, which is a fast, accurate and noninvasive method, used in andrological evaluations and to understand the scrotal/testicular

thermoregulation of bulls (Kastelic, 2014; Menegassi et al., 2015). The scrotal surface temperature is reduced from the vascular cone, originating the testicular temperature gradient, which indicates the difference between the temperature of the proximal and distal poles of the testis (Coulter, Senger, & Bailey, 1988; Kastelic, Coulter, & Cook, 1995). Due to the various scrotal surface temperature patterns, the males can be classified as bearers of normal, abnormal or questionable scrotal thermogram patterns (Kastelic & Brito, 2012; Lunstra & Coulter, 1997).

It is known that in bovine bulls, the sperm quality has a negative correlation with surface scrotal temperature and positive correlation with the testicular temperature gradient (Berry et al., 2011). Therefore, animals with abnormal thermogram exhibit lower testicular temperature gradient and reduced sperm and seminal parameters related to fertility (Kastelic & Brito, 2012; Lunstra & Coulter, 1997; Menegassi et al., 2015). However, for water buffaloes, there is no detailed information regarding testicular thermolysis and about establishing the thermal gradient of the scrotum. Therefore, the research objective was to evaluate the thermal comfort indices and its consequences on the temperature patterns of the scrotal surface, the testis-scrotal thermoregulation and semen quality of buffalo bulls in a tropical climate at different periods of the year.

2 | MATERIALS AND METHODS

2.1 | Location and experimental period

The experiment was performed at the Center of Biotechnology of Animal Reproduction (CEBRAN/UFPA), in Castanhal, Pará, Brazil (1°18′18″S and 47°56′34″W). The region has a hot and humid tropical climate (Afi Koppen), with average annual rainfall between 2,300 and 2,800 mm, average annual air temperature of 26°C, average relative air humidity of 86% and 2,400 hr of annual insolation (Alvares, Stape, & Sentelhas, 2013). The field data collection was performed from April to December 2014, and the test comprised the rainiest period (April to May), the transitional period (June to August) and the less rainy period (September to December) of the year.

The rainiest period is characterised by intense rainfall and reduced duration of sunshine hours. The less rainy period has a short-term drought, and there are fewer clouds. The transitional period represents the shift from the end of the rainiest period to the beginning of the less rainy period (Moraes, Costa, Costa, & Costa, 2005). To characterise the climatic periods during the test, the variables air temperature, relative humidity, solar radiation and rainfall were permanently monitored. These variables were recorded at the automatic weather station (Station A202 INMET—Brazil 1°18′03″S and 47°56′52″W), located 400 m from animal housing facility. Table 1 shows the behaviour of the meteorological variables during the experimental period.

2.2 | Animals and feed management

Eleven adult and clinically healthy Murrah buffalo bulls (55 ± 9 months; 701.4 ± 82.8 kg) were used. All animals were subjected to the same nutritional and environmental conditions, and kept in uncovered collective stalls (18 m^2/animal) but with access to natural shading from the presence of trees (*Ficus benjamina*) grown in the surrounding area of the stalls. The trees had a distance of 8 m between specimens, 14 m high and dense foliage, and projected into the stalls, 7 m^2 of shade/animal. The animals had ad libitum access to an automatic watering system and feeding troughs; the diet was offered daily at two schedules (8.00 and 16.00 hr). The food consisted of roughage (chopped elephant grass forage, *Pennisetum purpureum*, Schum), concentrate (wheat bran and bean residue) and mineral salt. The daily concentrate supply was adjusted to 1% of body weight. Before the experimental trial period, the animals were subjected to 30 days of intensive management and feed adaptation, similar to that used for bulls kept in artificial insemination centres.

TABLE 1 Average values of climate variables recorded at the automatic weather station during 2014, Castanhal, Pará, Brazil

Climate variable	Period		
	Rainiest	Transitional	Less Rainy
Air temperature (°C)	26.1	26.4	27.0
Relative humidity (%)	83.9	79.5	74.9
Global solar radiation (kJ/m^2)	665.4	746.7	786.8
Pluviometric precipitation (mm/month)	237	169	140

Rainiest period, April to May; Transitional period, June to August; Less rainy period, September to December.

2.3 | Experimental design

The animals were evaluated monthly on two consecutive days to measure the respiratory rate (RR; mov/min), heart rate (HR; beats/min), rectal temperature (RT; °C), ocular globe temperature (OGT; °C) and surface temperature (°C) in anatomical points in the scrotal area. On every experiment day, the physiological variables and body surface temperatures were measured in the morning and afternoon. Semen samples were collected from all animals every 2 weeks. The meteorological variables were monitored throughout the experiment. The Temperature and Humidity Index (THI) and Benezra's Thermal Comfort Index (BTCI) were calculated, which were the reference indicators of thermal comfort applied in this study.

2.4 | Physiological variables

The physiological variables were measured monthly on two consecutive days, in the morning (6.00–9.00 hr) and afternoon (12.00–15.00 hr), in the order they are presented. The RR was determined by observing the thorax-abdominal area and counting the respiratory movements for 1 min (mov/min). The HR was determined by auscultation using a veterinary stethoscope, for 1 min (beats/min). A veterinary clinical thermometer was used to measure the RT of the animal, inserted into the rectum and held until stabilisation of the mercury column (°C).

2.5 | Surface temperatures measured by infrared thermography

OGT and scrotal surface temperatures were measured by infrared thermography (FLIR A320, FLIR Systems, Wilsonville, OR, USA). The scrotal surface temperatures measured were the average temperature of the spermatic cord (SCT), proximal pole testicular temperature (PPT), distal pole testicular temperature (DPT), epididymal tail temperature (ETT) and maximum (STMax), average (STAvg) and minimum (STMin) scrotal temperature (Kahwage, 2015; Menegassi et al., 2015). The temperatures of bilateral organs were measured individually, but due to the lack of statistically significant differences, the results were presented as the means of contralateral structures.

The assessments were performed using a thermal imaging camera attached to a tripod, 25° lens, motorised focus, temperature range from −20 to 120°C, thermal sensitivity of 50 mK (<.05°C at room temperature of 30°C), spectral range of 7.5–13 μm and optical resolution of 320 × 240 pixels. The emissivity ratio was previously set to 0.98 (Hoffmann et al., 2013), and the camera was attached to a 16-inch flat panel monitor to display a perfect image. The animals were removed from the stalls before the evaluation and gently led to the chute where they were enclosed, so that the distance used between the camera and the ocular globe was of 1.5 and of 0.70 m between the camera and the scrotal surface.

The thermograms generated were then analysed in the laboratory with the Flir Tools + Version 3.0 software (FLIR Systems, Inc., Wilsonville, OR, USA). The SCT, PPT, DPT and ETT were measured using rectangular polygon individually superimposed to each defined region of interest. The STMax, STAvg and STMin were obtained by tracing the rectangular polygon, which comprised the entire scrotum, from the ventral to the proximal pole of the testicles, reaching the spermatic cord (Coulter, Cook, & Kastelic, 1997).

2.6 | Temperature gradients

The data from the thermograms were applied to mathematical models to calculate the specific thermal gradients (°C) between anatomical regions of interest. The temperature gradients were determined according to the following equations: rectal-spermatic cord gradient (GRAD RSCO = RT − SCT), rectal-proximal pole gradient (GRAD RPP = RT − PPT), rectal-distal pole gradient (GRAD RDP = RT − DPT), rectal-tail of the epididymis gradient (GRAD RTE = RT − ETT), rectal-scrotum gradient (GRAD RESC = RT − STAvg) and testicular temperature gradient (GRAD TEST = PPT − DPT).

2.7 | Semen collection and evaluation

The animals presented a high degree of homogeneity of sperm quality, previously assessed at the beginning of the experiment. The semen collection was performed every 2 weeks by the artificial vagina method. Sixteen ejaculates were collected from each animal, totalling 176 evaluated ejaculates. After collection, the semen was kept in a water bath at 37°C to prevent thermal shock and changes in semen characteristics. The semen was then submitted to volume analysis (VOL; ml) in a graduated conical tube. The sperm concentration (CONC; $\times 10^9$/ml) was determined after diluting the semen in a saline solution (1:400), and further evaluation was performed in a spectrophotometer. The progressive sperm motility (PM; %) was evaluated in 8-μl aliquots, pre-heated to 37°C and placed between the slide and coverslip using phase contrast microscopy (200×) (Vale, 2002). Semen analyses were performed by the same experienced researcher during the entire period according to rigorous professional criteria (CBRA - Colégio Brasileiro de Reprodução Animal, 2013).

The spermatozoid plasma membrane integrity (PMI; %) was evaluated by the eosin–nigrosin supravital staining technique, with the addition of dye solution and semen in equal parts of 10 μl. The smear was then assessed using bright field microscopy (1,000×). The pink-stained sperm was classified as damaged plasma membrane, while the nonstained ones were considered as intact plasma membrane (Iqbal, Aleem, Ijaz, Rehman, & Yousaf, 2010). Next, the morphology was assessed in aliquots diluted in phosphate-buffered formalin solution using phase contrast microscopy (1,000×). The classification criteria used were major sperm abnormalities (MajABN; %), minor sperm abnormalities (MinABN; %) and total abnormalities (TotABN; %; Bloom, 1973). One hundred cells per sample were evaluated, and the results were expressed in percentages (Vale, 2002).

2.8 | Climate monitoring and determination of thermal comfort indices

For characterising the micro-climate, temperature and relative humidity were recorded every 15 min through three data loggers (HOBO U12-012, Onset Computer Corporation, Bourne, MA, USA) installed inside the stalls containing the animals. The data loggers were suspended 2.0 m above the ground and were 9.0 m apart from each other, protected from direct sunlight and water in meteorological shelters (Malama et al., 2013). The recorded data were transferred to analysis software on a weekly basis (HOBOware Lite 3.1.0, Onset Computer Corporation), and the means of the values recorded during the evaluation periods of the physiological variables and surface temperatures were used to calculate the Temperature and Humidity Index (THI) observed in the microclimate of the stalls.

Therefore, the THI was calculated in the morning (6.00–9.00 hr) and afternoon (12.00–15.00 hr), according to the formula: THI = {(0.8AT) + [(RH/100) × (AT-14.4)] + 46.4}, where AT is the air temperature measured by a dry bulb thermometer (°C) and RH is the relative humidity (%) (Mader, Davis, & Gaughan, 2007; Thom, 1959). The equation BTCI = (RT/38.33) + (RR/23) was used to determine Benezra's Thermal Comfort Index (BTCI), where RT is the rectal temperature (°C) and RR is the respiratory rate (mov/min). The closer the BTCI value is to 2.0, the better adapted the animal is to the tropical climate conditions (Benezra, 1954).

2.9 | Statistical analysis

The statistical treatment of the thermal comfort indexes, physiological variables, surface temperatures and seminal variables applied the

descriptive statistics analysis (mean ± standard deviation, minimum and maximum values). The data were submitted to analysis of variance by the GLM procedure of the SAS—Statistical Analysis Software, Version 9.3 (SAS Institute, Cary, North Carolina, USA), to verify the effect of the climate period (rainiest, transitional and less rainy) and daytime period (morning and afternoon). The means were compared by the Tukey test. Pearson linear correlations were performed to assess the magnitude and direction of the proportionality of the variables studied. The significance level used for all tests was 5%.

3 | RESULTS

During the study, the meteorological variables exhibited behaviour consistent with the historical records observed for a humid tropical climate subtype (Table 1). The mean of the highest values for THI reached 81.4 during the experiment and was registered from 12.00 to 15.00. Table 2 shows the comfort indices (THI and BTCI) measured during the collection procedure. The THI showed difference between daytime periods ($p < .05$), which were higher in the afternoon.

The RR was higher in the transitional period, and in the afternoon, there was an increase in the transitional and in the less rainy periods (Table 3). The average HR was higher in the transitional period and a daytime effect was also observed, which showed higher average values recorded in the afternoon. The RT and OGT were not affected by the periods studied, and no difference was observed for daytime.

With regard to the specific temperatures recorded in different areas of the scrotal surface, an increase in average SCT was observed during the less rainy period (Table 4). The average PPT was similar in the less rainy and rainiest periods, not showing the latter difference in relation to the transitional. There was no change in DPT and ETT between the different climate periods or daytime periods. The scrotal temperature values (STAvg, STMin and STMax) showed no variation between the different periods of the year, but were sensitive to daytime changes, with a temperature increase observed in the afternoon. The temperature profile of the different scrotum regions under thermal comfort indicates the formation of three separate parallel bands (Figure 1), with decreasing temperature (A band: spermatic cord and dorsal testicular pole; B band: middle region of the testes and ventral testicular pole; C band: tail the epididymis).

All calculated gradients present values greater than zero, regardless of the time of year and the daytime (Table 5). The lowest averages of GRAD RSCO, GRAD RPP and GRAD RESC were observed in the less rainy period, decreasing from the rainy period to the less rainy period, ranging from 0.9 to 0.5°C. In addition, the GRAD RESC was affected by the daytime, decreasing 0.4°C in the afternoon. The GRAD RTE was lower in the transitional and less rainy periods. The GRAD TEST was positive, and lower values were observed in the transitional period, with a slight decrease (0.2°C) in the afternoon.

The seminal parameters studied were not affected by the periods (Table 6), and all variables presented averages within normal standards for buffalo semen *in natura* (Barros et al., 2015; CBRA - Colégio Brasileiro de Reprodução Animal, 2013; Ohashi et al., 2011; Sansone, Nastri, & Fabbrocini, 2000; Santos et al., 2014; Silva et al., 2014; Vale et al., 2008).

In the correlations calculated between thermal comfort indices and physiological variables, there was a positive value and of the same magnitude as the THI with the RT, the OGT with BTCI ($r = .26$; $p < .01$). There was a high correlation between OGT and RT ($r = .82$; $p < .01$). The RT correlated negatively with VOL ($r = -.22$; $p < .01$), while the OGT correlated negatively with both VOL ($r = -.40$; $p < .01$) and with PM ($r = -.29$; $p < .01$). There were no correlations between THI, BTCI, RR and HR with the seminal variables studied. The SCT was correlated to THI (Table 7), while the ETT was influenced by THI and BTCI. The ETT showed a negative correlation with VOL, PM and PMI; the latter two seminal variables are very important to the fertility of bulls. The STAvg showed a negative correlation with PM and a positive correlation with the total defects.

4 | DISCUSSION

To the best of our knowledge, this is the first study to objectively describe the thermal gradient of the scrotum of water buffaloes due to heat dissipation. We innovatively analysed the temperature patterns of specific scrotal surface regions and carefully correlated these

TABLE 2 Thermal comfort indices (mean ± *SD*) of buffalo bulls reared in a tropical climate, measured in the morning (6.00–9.00 hr) and afternoon (12.00–15.00 hr)

	Period			
Daytime	Rainiest	Transitional	Less rainy	Mean
Temperature and Humidity Index (THI)				
Morning	72.9 ± 0.4^{B}	72.6 ± 1.5^{B}	73.7 ± 0.6^{B}	73.1 ± 1.1
Afternoon	79.6 ± 1,9^{A}	79.1 ± 1.3^{A}	79.2 ± 0.8^{A}	79.3 ± 1.3
Mean	76.3 ± 3.6	75.9 ± 3.5	76.5 ± 2.8	
Benezra's Comfort Index (BCTI)				
Morning	1.9 ± 0.1	2.0 ± 0.1	1.9 ± 0.1	2.0 ± 0.1
Afternoon	2.0 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
Mean	1.9 ± 0.1	2.1 ± 0.1	2.0 ± 0.2	

Different capital letters in the same column and different lower case letters in the same line indicate statistical difference ($p < .05$).

TABLE 3 Physiological variables (mean ± *SD*) of buffalo bulls reared in a tropical climate in different climate periods

	Period			
Daytime	**Rainiest**	**Transitional**	**Less rainy**	**Mean**
Respiratory rate (RR, mov/min)				
Morning	22.2 ± 0.7^{Ab}	25.1 ± 0.5^{Ba}	22.3 ± 0.5^{Bb}	23.2 ± 0.3
Afternoon	23.1 ± 0.6^{Ac}	29.3 ± 0.5^{Aa}	27.5 ± 0.5^{Ab}	26.6 ± 0.3
Mean	22.6 ± 0.4	27.2 ± 0.3	24.9 ± 0.3	
Heart rate (HR, beats/min)				
Morning	62.7 ± 0.8	64.9 ± 0.6	62.0 ± 0.5	63.2 ± 0.3^{B}
Afternoon	63.6 ± 0.7	67.0 ± 0.6	65.7 ± 0.5	65.4 ± 0.3^{A}
Mean	63.1 ± 0.5^{b}	65.9 ± 0.4^{a}	63.8 ± 0.4^{b}	
Rectal temperature (RT, °C)				
Morning	38.1 ± 0.1	37.9 ± 0,1	38.0 ± 0.1	38.0 ± 0.1
Afternoon	38.7 ± 0.1	38.4 ± 0.1	38.4 ± 0.1	38.5 ± 0.1
Mean	38.4 ± 0.1	38.1 ± 0.1	38.2 ± 0.1	
Ocular globe temperature (OGT, °C)				
Morning	38.1 ± 0.1	38.0 ± 0.1	37.8 ± 0.1	38.0 ± 0.1
Afternoon	38.5 ± 0.1	38.4 ± 0.1	38.4 ± 0.1	38.4 ± 0.1
Mean	38.3 ± 0.1	38.2 ± 0.1	38.1 ± 0.1	

Different capital letters in the same column and different lower case letters in the same line indicate statistical difference ($p < .05$).

elements with semen quality. Previous studies performed in the subtropical region reported that the THI recorded during winter and summer and the scrotal temperature gradients were different but did not affect the seminal parameters, with the exception of the motility of the Braford and Brangus bulls, showing testicular thermoregulation efficiency in both breeds (Menegassi et al., 2015, 2016).

The climate subtype of the experimental site is characterised as megathermal, permanently hot and humid. Instantaneous or long-lasting climate variations can impact the thermoneutral zone of buffaloes, which results in the animals activating their thermoregulatory mechanisms. This body temperature maintenance process is intended to prevent high body temperature changes that can harm the metabolism (Rhoads, Baumgard, & Suagee, 2013; Silanikove, 2000). If there is thermal discomfort, the physiological response of the animals includes sweating and peripheral vasodilation, resulting in a fall in blood pressure (Aggarwal & Singh, 2008). This demand is offset by an increase in heart rate and respiratory rate (Marai & Haeeb, 2010).

The average THI observed in the microclimate of the stalls was high during the periods studied, indicating that the animals were initially subjected to an alert condition. Previous reports indicate that when the THI is ≤74, the buffaloes are in a thermal comfort condition; between 75 and 78, the condition becomes an alert condition; between 79 and 83, it is characterised as a dangerous condition. When the THI is ≥84, it is an emergency condition (Somparn et al., 2004), which may result in animal mortality if these conditions are prolonged and if there is no intervention in the handling of the animals and/or the environment. As expected, the THI recorded pointed to a situation of thermal comfort during the morning and a higher thermal challenge throughout the afternoon.

However, the BTCI is an indicator that depends exclusively on the physiological response of the animal according to the thermal environment. As the average BTCI ranged from 1.9 to 2.1 during the periods studied, it can be assumed that the buffalo bulls showed high resilience to the local megathermal climate, characterised by average air temperature that was always above 26°C and the relative humidity that was never less than 74%. Moreover, the intense and prevailing solar radiation throughout the year may have been mitigated by the housing conditions of the animals, which provided partial natural shading in the stalls. In contrast, dairy buffalo females show the BTCI higher than 2.0 in the least rainy period of the year, when bred in shaded pastures under similar climatic conditions (Garcia et al., 2011).

The highest RR in the transitional period suggests that this period may have been challenging for the animals, as well as the conditions observed in the afternoons. In fact, in the transitional period, there is a change in rainfall patterns, gradual decrease in relative air humidity associated with increased global solar radiation. In association, these factors create a transitional environment that is very challenging to the animals bred in a humid tropical climate (Silva et al., 2011b). The increase in RR, as a consequence of panting, and the increase in HR are the most obvious signs of thermoregulation activation in buffaloes (Gudev et al., 2007; Marai & Haeeb, 2010). The increase in HR arises from the need to dissipate heat under heat conditions and it is directed to increase the blood flow of the peripheral blood vessels (Gaughan, Bonner, Loxton, & Mader, 2013) to facilitate heat loss in the extremities of the body (Haque, Ludri, Hossain, & Ashutosh, 2012). Therefore, in the mornings, buffalos bred in tropical regions show lower HR values than those observed in the afternoon, regardless of the time of year (Silva et al., 2011a).

TABLE 4 Scrotal surface temperatures (mean ± *SD*) measured by infrared thermography in buffalo bulls reared in a tropical climate in different climate periods

Daytime	Period			Mean
	Rainiest	Transitional	Less rainy	
Spermatic cord temperature (SCT, °C)				
Morning	34.2 ± 0.4	34.1 ± 0.2	34.7 ± 0.2	34.3 ± 0.1
Afternoon	33.7 ± 0.3	34.3 ± 0.2	34.8 ± 0.2	34.3 ± 0.1
Mean	33.8 ± 0.8^{b}	34.2 ± 0.8^{b}	34.7 ± 0.8^{a}	
Proximal pole testicular temperature (PPT, °C)				
Morning	34.2 ± 0.2	33.6 ± 0.2	34.2 ± 0.2	34.0 ± 0.1
Afternoon	33.7 ± 0.2	33.8 ± 0.2	34.3 ± 0.2	33.9 ± 0.1
Mean	33.9 ± 0.9^{ab}	33.7 ± 0.8^{b}	34.3 ± 0.7^{a}	
Distal pole testicular temperature (DPT, °C)				
Morning	32.2 ± 0.2	32.2 ± 0.2	32.2 ± 0.2	32.2 ± 0.1
Afternoon	32.1 ± 0.2	32.4 ± 0.2	32.4 ± 0.2	32.3 ± 0.1
Mean	32.2 ± 0.8	32.3 ± 0.8	32.3 ± 0.9	
Epididymal tail temperature (ETT, °C)				
Morning	30.4 ± 0.5	31.2 ± 0.2	31.4 ± 0.2	31.0 ± 0.2
Afternoon	30.6 ± 0.3	31.4 ± 0.2	31.3 ± 0.2	31.1 ± 0.1
Mean	30.5 ± 0.8	31.3 ± 1.0	31.3 ± 1.0	
Average scrotal temperature (STAvg, °C)				
Morning	33.0 ± 0.2	32.9 ± 0.1	33.1 ± 0.1	33.0 ± 0.1^{B}
Afternoon	33.7 ± 0.2	33.8 ± 0.1	34.3 ± 0.1	34.0 ± 0.1^{A}
Mean	33.4 ± 0.1	33.4 ± 0.1	33.7 ± 0.1	
Minimal scrotal temperature (STMin, °C)				
Morning	30.3 ± 0.2	29.8 ± 0.1	30.1 ± 0.1	30.1 ± 0.1^{B}
Afternoon	31.3 ± 0.2	31.6 ± 0.1	31.8 ± 0.1	31.6 ± 0.1^{A}
Mean	30.8 ± 0.1	30.7 ± 0.1	31.0 ± 0.1	
Maximum scrotal temperature (STMax, °C)				
Morning	35.3 ± 0.2	35.2 ± 0.2	35.2 ± 0.2	35.3 ± 0.1^{B}
Afternoon	35.9 ± 0.2	36.1 ± 0.2	36.6 ± 0.2	36.2 ± 0.1^{A}
Mean	35.6 ± 0.1	35.7 ± 0.1	35.9 ± 0.1	

Different capital letters in the same column and different lower case letters in the same line indicate statistical difference ($p < .05$).

In fact, the lack of difference in the RT of bulls over the different periods of the year shows that they do not store excessive heat. This supports the idea that they were efficient in performing thermolysis, even if the THI remained high during the rainiest, the transitional and the less rainy periods of the year. The RT increase in the afternoon was not significant, which results from the natural increase observed due to the circadian rhythm of the buffaloes (Malama et al., 2013; Silva et al., 2011a). Thus, the small RR and HR oscillation associated with maintaining the RT over the periods shows the bulls leaving the thermoneutral zone and activating their thermoregulatory system; however, a state of thermal stress was not observed during the experiment.

It is known that the ocular globe region is considered to be sensitive to variations in the ambient temperature (Schaefer et al., 2007). However, there was no significant difference in the OGT in the different periods, indicating that the change in temperature did not change this. In contrast, in cattle bred in a humid subtropical region, the OGT values were higher in the summer and spring, a time known for higher air temperatures and pluviosity (Menegassi et al., 2015). Interestingly, in this study, the OGT oscillations followed the variations observed in the RT very closely.

The increased SCT in the less rainy period may have been caused by factors such as higher direct and indirect solar radiation, due to fewer cloud formations associated with higher ambient temperature, which is characteristic for this time of year (Moraes et al., 2005). The increase in temperature can also lead to high scrotal surface temperatures of bovine bulls (Berry et al., 2011), and these directly reflect the temperature of the corresponding intrascrotal structures (Coulter et al., 1988). The average surface temperature of the spermatic cord region indicates that, regardless of the period, this anatomical region has a high thermoregulatory capacity. As the average air temperature varied, the difference favoured thermal exchanges between the funicular surface and the environment, in addition to

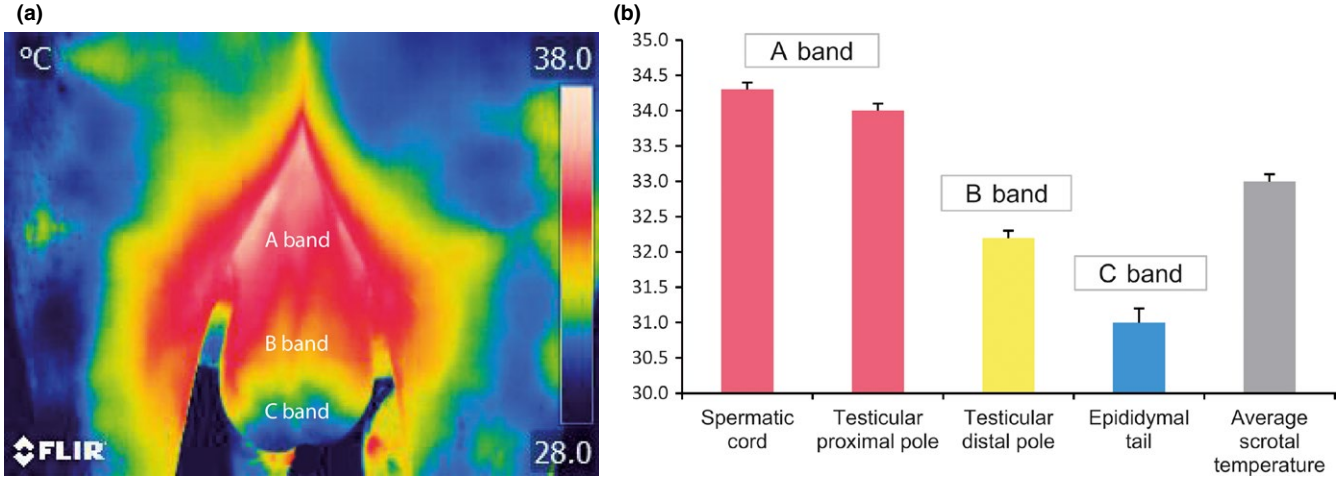

FIGURE 1 Illustrative thermogram of the buffalo bulls' scrotum in thermal comfort. (a) Thermographic image showing decreasing temperature pattern from the area of the testicular vascular cone to the tail of the epididymis, in three different horizontal bands (bands A, B and C); scale 28.0–38.0°C. (b) Graph of surface temperatures (mean ± *SD*) of the different regions of bulls' scrotum with normal thermogram

the internal mechanisms that activate the heat exchange (Kastelic, 2014).

The PPT observed was higher than that recorded for bovine bulls, ranging between 28.5 and 30.4°C (Kastelic, Cook, Coulter, Wallins, & Entz, 1996a; Kastelic et al., 1995). Maintaining similar PPT in the rainiest and less rainy periods indicates analogous possibility of heat loss by countercurrent mechanism during the blood flow through the vascular cone (Brito, Barth, Wilde, & Kastelic, 2012) in the different periods. However, it has been shown that the bovine species has higher PPT values in the summer and the seasonal increase in PPT presents a negative correlation with sperm motility (Menegassi et al., 2015). The results observed demonstrate that the temperatures for both the spermatic cord and the dorsal testicular pole are higher than other scrotal structures because they are closer to the abdominal cavity. Even so, a favourable and progressive temperature decrease was observed from the region of the spermatic cord to the tail of the epididymis, which was confirmed because three different bands of decreasing temperature were detected in the scrotal thermograms. A similar phenomenon was previously reported for bovine (Kastelic, 2014), but not for buffalo bulls.

Earlier reports have indicated that ambient temperature variations have the greatest effect on the ventral testicular pole (Kastelic et al., 1996a) and the tail of the epididymis. These anatomical structures are more susceptible due to increased environmental exposure and its high temperature is proportionate to the increase in air temperature (Sealfon & Zorgniotti, 1991). However, this effect was not observed in our study, because the DPT and ETT variations in the climate periods and daytime were negligible, which favoured maintaining sperm quality.

The relative temperature stability of the tail of the epididymis observed between the seasons and daytime periods may be regarded as an advantageous feature in buffalos, given that changes in epididymal temperature interfere in the time required for sperm maturation (Bedford & Yanagimachi, 1991). The STAvg was higher than that observed in bovine bulls (Kastelic et al., 1995). It is known that this temperature is moderately affected by ambient temperature (Kastelic et al., 1996a). The absence of a significant difference in the STAvg, STMin and STMax in the different periods demonstrates that even with the environmental heat increase, the buffaloes were efficient in their testicular and scrotal thermoregulation.

The temperature decrease observed between the dorsal and distal poles of the testis, which comprises the GRAD TEST, was in average 1.7°C in the rainy season, 1.4°C in the transitional and 1.9°C in the less rainy season. The GRAD for bovine bulls with normal thermograms was 1.6°C (Kastelic et al., 1995), a fact that was also observed in our study. Therefore, the temperature of the testes is higher when it is closer to the arterial supply point and decreases after the dorsal testicular pole (Brito, Silva, Barbosa, & Kastelic, 2004; Kastelic, Cook, Coulter, & Saacke, 1996b). Moreover, the GRAD TEST relates directly to the progressive motility and epididymal sperm reserve (Berry et al., 2011), therefore of fundamental interest in the thermographic evaluation of the scrotum. As the GRAD TEST is inversely related to ambient temperature (Kastelic et al., 1996a) and as it remained within a satisfactory range, it was observed that even with the high THI, the animals efficiently activated their thermoregulatory system and did not undergo thermal stress, thereby preserving their sperm quality.

The normal testicular function is temperature-dependent for maintaining the scrotal temperature between 2 and 6°C below normal body temperature; otherwise, it may damage the testicular parenchyma and consequently reduce the production of fertile sperm and semen quality (Garcia et al., 2010; Kastelic, 2014). The lower GRAD RESC, GRAD RSCO, GRAD RPP and GRAD RTE values in the less rainy season indicate that this period is challenging to animals. However, maintaining semen quality of buffalo bulls within normal parameters (Barros et al., 2015; CBRA - Colégio Brasileiro de Reprodução Animal,

TABLE 5 Scrotal surface gradient temperatures (mean ± *SD*) of buffalo bulls reared in a tropical climate in different climate periods

	Period			
Daytime	Rainiest	Transitional	Less rainy	Mean
GRAD RSCO				
Morning	4.0 ± 0.3	3.9 ± 0.1	3.4 ± 0.1	3.8 ± 0.1
Afternoon	4.5 ± 0.2	3.9 ± 0.1	3.4 ± 0.1	3.9 ± 0.1
Mean	4.3 ± 0.7[a]	3.9 ± 0.7[a]	3.4 ± 0.6[b]	
GRAD RPP				
Morning	4.2 ± 0.2	4.4 ± 0.1	3.9 ± 0.1	4.2 ± 0.1
Afternoon	4.5 ± 0.2	4.4 ± 0.1	3.8 ± 0.1	4.2 ± 0.1
Mean	4.4 ± 0.9[a]	4.4 ± 0.7[a]	3.9 ± 0.5[b]	
GRAD RDP				
Morning	4.9 ± 0.2	4.8 ± 0.2	4.5 ± 0.2	4.7 ± 0.1
Afternoon	5.0 ± 0.2	4.7 ± 0.2	4.4 ± 0.1	4.7 ± 0.1
Mean	4.9 ± 0.8	4.7 ± 0.8	4.5 ± 0.6	
GRAD RTE				
Morning	7.7 ± 0.4	6.8 ± 0.2	6.7 ± 0.2	7.1 ± 0.1
Afternoon	7.5 ± 0.3	6.8 ± 0.2	6.9 ± 0.2	7.0 ± 0.1
Mean	7.6 ± 0.7[a]	6.8 ± 0.9[b]	6.8 ± 0.8[b]	
GRAD RESC				
Morning	5.0 ± 0.2	5.0 ± 0.1	4.8 ± 0.1	4.9 ± 0.1[A]
Afternoon	4.9 ± 0.1	4.5 ± 0.1	4.0 ± 0.1	4,5 ± 0,1[B]
Mean	4.9 ± 0.1[a]	4.7 ± 0.1[a]	4.4 ± 0.1[b]	
GRAD TEST				
Morning	2.0 ± 0.1	1.5 ± 0.1	2.0 ± 0.1	1.8 ± 0.1[A]
Afternoon	1.5 ± 0.1	1.3 ± 0.1	1.9 ± 0.1	1.6 ± 0.1[B]
Mean	1.7 ± 0.6[ab]	1.4 ± 0.3[b]	1.9 ± 0.6[a]	

Different capital letters in the same column and different lower case letters in the same line indicate statistical difference ($p < .05$).
GRAD RSCO, rectal-spermatic cord temperature gradient (°C); GRAD RPP, rectal-proximal pole temperature gradient (°C); GRAD RDP, rectal-distal pole temperature gradient (°C); GRAD RTE, rectal-tail of the epididymis temperature gradient (°C); GRAD RESC, rectal-average scrotal temperature gradient (°C); GRAD TEST, testicular temperature gradient (°C).

2013; Ohashi et al., 2011; Sansone et al., 2000; Vale et al., 2008) over the periods provides evidence that the gradients observed, especially GRAD RESC and GRAD RTE, were sufficient to maintain normal spermatogenesis and sperm maturation.

The positive correlations of THI with RT and THI with OGT show that temperature and relative humidity can directly affect the body temperature of buffalos. Similar results were reported for bovine bulls, which showed a positive correlation between RT and THI (Lunstra & Coulter, 1997), and THI and OGT (Menegassi et al., 2015, 2016). Interestingly, animals with no correlation or negative correlation between THI and body temperature appear to be better adapted to tropical climate; consequently, this characteristic is of interest in the screening and genetic improvement programmes (Marcondes et al., 2010).

In turn, the strong correlation between OGT and RT makes the former a highly important variable in the remote monitoring of buffaloes, with very favourable future prospects to accurately estimate the internal body temperature of animals without having to perform physical restraint and eliminating invasive tests. The high correlations of OGT with ETT and OGT with STMin suggest that complementary studies should be done. This is because the ETT and STMin showed negative correlations with seminal volume, sperm motility and sperm membrane integrity, characteristics regarded as crucial for animal fertility concerning reproductive breeding (Ahmed, Andrabi, Anwar, & Jahan, 2016). As expected, the STAvg showed a negative correlation with sperm membrane integrity and a positive correlation with total sperm defects, confirming that the increase in scrotal temperature deteriorates sperm quality (Berry et al., 2011).

In conclusion, the present study demonstrated that buffaloes were efficient to dissipate thermal energy, even at high temperature and humidity levels. Therefore, testicular and scrotum thermoregulation was not impaired and the bulls showed scrotal thermograms defined by decreasing temperature from the spermatic cord to the tail of the epididymis. These effects favoured the maintenance of normal seminal parameters and may contribute to select more heat-resilient buffalo bulls.

TABLE 6 Raw semen variables (mean ± *SD*; *n* = 176 ejaculates) of buffalo bulls reared in a tropical climate in different climate periods

	Period			
Variable	Rainiest	Transitional	Less rainy	CV (%)
VOL (ml)	2.2 ± 0.2	2.2 ± 0.1	2.6 ± 0.1	56.7
CONC (×10^9 sptz/ml)	1.3 ± 0.8	1.3 ± 0.6	1.2 ± 0.6	4.5
PM (%)	67.0 ± 2.1	68.6 ± 1.7	66.7 ± 1.6	4.1
PMI (%)	66.5 ± 2.0	68.5 ± 1.6	68.1 ± 1.5	3.6
MajABN (%)	14.3 ± 0.9	13.8 ± 0.8	12.2 ± 0.7	11.9
MinABN (%)	6.7 ± 0.7	5.6 ± 0.6	4.7 ± 0.6	41.5
TotABN (%)	21.0 ± 1.4	19.5 ± 1.1	17.0 ± 1.1	10.1

VOL, semen volume; CONC, sperm concentration; PM, progressive motility; PMI plasma membrane integrity; MajABN, major defects; MinABN, minor defects; TotABN, total defects.
Different lower case letters in the same line indicate statistical difference ($p < .05$).

TABLE 7 Linear correlation between scrotal surface temperatures, thermal comfort indices, physiological and seminal variables of buffalo bulls reared in a tropical climate

	SCT	ETT	STAvg	STMin	STMax
THI	.26*	.25*	.27	.42**	.20
BTCI	.26*	.25*	.13	.11	.11
RT (°C)	.48**	.52**	.48**	.51**	.41**
OGT (°C)	.41**	.65**	.56**	.61**	.46**
VOL (ml)	−.34*	−.54**	−.17	−.41**	−.13
CONC ($\times 10^9$ sptz/ml)	.35**	.08	.17	.08	.31**
PM (%)	−.26*	−.44**	−.34**	−.45**	−.29**
PMI (%)	−.02	−.34**	−.21*	−.31**	−.14
MajABN (%)	−.19	−.07	.02	.11	.05
MinABN (%)	−.09	.06	.18	.26**	.17
TotABN (%)	−.20	−.02	.11*	.21	.13

SCT, spermatic cord temperature; ETT, epididymal tail temperature; STAvg, average scrotal temperature; STMin, minimum scrotal temperature; STMax, maximum scrotal temperature; THI, Temperature and Humidity Index; BTCI, Benezra's Comfort Index; RT, rectal temperature; OGT, ocular globe temperature; VOL, seminal volume; CONC, sperm concentration; PM, progressive motility; PMI, plasma membrane integrity; MajABN, major defects; MinABN, minor defects; TotABN, total defects.
*Significant correlation ($p < .05$).
**Significant correlation ($p < .01$).

ACKNOWLEDGEMENTS

This project was financially supported by Embrapa-Brazilian Agricultural Research Corporation (grants BIOTEC Network #01130600105 and PECUS #01100600107), by the Federal University of Pará (FADESP) and by CAPES-Brazilian Coordination for Enhancement of Higher Education.

CONFLICT OF INTEREST

The authors declare they have no conflict of interest.

ETHICAL APPROVAL

The experiment complies with the current Brazilian laws, and all procedures performed were approved by the Committee on Experimental Animal Use and Ethics (Protocol CEUA-CPATU #01/2015).

AUTHORS' CONTRIBUTIONS

The conception of this study was undertaken by A.R.G. and L.K.X.S., A.R.G., J.S.S., A.O.A.S., J.B.L.J., L.G.M. and I.M.F. designed the final study work and planning of analyses. A.R.G. and J.S.S. coordinated the acquisition of data, and all authors participated in data collection. C.F. conducted the statistical analysis. L.K.X.S., A.R.G., M.H.A.P. and J.B.L.J. wrote the manuscript. All authors contributed to discussions, revised critically the content and approved the submitted manuscript.

REFERENCES

Aggarwal, A., & Singh, M. (2008). Changes in skin and rectal temperature in lactating buffaloes provided with showers and wallowing during hot-dry season. *Tropical Animal Health and Production*, *40*, 223–228.

Ahmed, H., Andrabi, S. M., Anwar, M., & Jahan, S. (2016). Use of post-thaw semen quality parameters to predict fertility of water buffalo (*Bubalus bubalis*) bull during peak breeding season. *Andrologia*, *49*, e12639. doi:10.1111/and.12639

Alvares, C. A., Stape, J. L., & Sentelhas, P. C. (2013). Köppen's climate classification map for Brazil. *Meteorologische Zeitschrift*, *22*, 711–728.

Barros, D. V., Silva, L. K. X., Lourenço Junior, J. B., Silva, A. O. A., Silva, A. G. M., Franco, I. M., … Garcia, A. R. (2015). Evaluation of thermal comfort, physiological, hematological, and seminal features of buffalo bulls in an artificial insemination station in a tropical environment. *Tropical Animal Health and Production*, *47*, 805–813.

Bedford, J. M., & Yanagimachi, R. (1991). Epididymal storage at abdominal temperature reduces the time required for capacitation of hamster spermatozoa. *Journal of Reproduction and Fertility*, *91*, 403–410.

Benezra, M. V. (1954). A new index measuring the adaptability of cattle to tropical conditions. *Journal of Animal Science*, *13*, 1015.

Berry, D. P., Evans, R. D., & Parland, S. M. C. (2011). Evaluation of bull fertility in dairy and beef cattle using cow field data. *Theriogenology*, *75*, 172–181.

Bloom, E. (1973). The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the bull spermiogram. *Nordisk Veterinaermedicin*, *25*, 383–391.

Brito, L. F., Barth, A. D., Wilde, R. E., & Kastelic, J. P. (2012). Testicular vascular cone development and its association with scrotal temperature, semen quality, and sperm production in beef bulls. *Animal Reproduction Science*, *134*(3–4), 135–140.

Brito, L. F., Silva, A. E., Barbosa, R. T., & Kastelic, J. P. (2004). Testicular thermoregulation in *Bos indicus*, crossbred and *Bos taurus* bulls: Relationship with scrotal, testicular vascular cone and testicular morphology, and effects on semen quality and sperm production. *Theriogenology*, *61*, 511–528.

CBRA - Colégio Brasileiro de Reprodução Animal (2013). *Manual para Exame Andrológico e Avaliação de Sêmen Animal*, 3rd ed. Belo Horizonte: Colégio Brasileiro de Reprodução Animal.

Coulter, G. H., Cook, R. B., & Kastelic, J. P. (1997). Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. *Journal of Animal Science*, *75*, 1048–1052.

Coulter, G. H., Senger, P. L., & Bailey, D. R. C. (1988). Relationship of scrotal surface temperature measured by infrared thermography to subcutaneous and deep testicular temperature in the ram. *Journal of Reproduction and Fertility*, *84*, 417–423.

Garcia, A. R. (2013). Estratégias para elevar a eficiência reprodutiva de búfalos em ambientes tropicais. *Proceedings of the 10th Brazilian Congress of Buiatrics* (pp. 1–11). Belém, Pará: Brazilian Buiatrics Association.

Garcia, A. R., Matos, L. B., Lourenço Junior, J. B., Nahúm, B. S., Araújo, C. V., & Santos, A. X. (2011). Variáveis fisiológicas de búfalas leiteiras criadas sob sombreamento em sistemas silvipastoris. *Pesquisa Agropecuaria Brasileira*, *46*, 1409–1414.

Garcia, O. S., Vale, W. G., Garcia, A. R., Ribeiro, H. F. L., Ferro, R. S., Rolim Filho, S. T., & Sousa, E. M. (2010). Experimental stud of testicular insulation in buffalo. *Revista Veterinária*, *21*, 889–891.

Gaughan, J. B., Bonner, S. L., Loxton, I., & Mader, T. L. (2013). Effects of chronic heat stress on plasma concentration of secreted heat shock protein 70 in growing feedlot cattle. *Journal of Animal Science*, *91*, 120–129.

Gudev, D., Popova-Ralcheva, S., Moneva, P., Aleksiev, Y., Peeva, T., & Penchev, P. (2007). Physiological indices in buffaloes exposed to sun. *Archiva Zootechnica*, *10*, 127–133.

Haque, N., Ludri, A., Hossain, S. A., & Ashutosh, M. (2012). Comparative studies on temperature threshold for heat shock protein 70 induction in young and adult Murrah buffaloes. *Journal of Animal Physiololgy & Animal Nutrition*, *96*, 920–929.

Hoffmann, G., Schmidt, M., Ammon, C., Rose-Meierhöfer, S., Burfeind, O., & Heuwieser, W. (2013). Monitoring the body temperature of cows and calves using video recordings from an infrared thermography camera. *Veterinary Research Communications*, *37*, 91–99.

Iqbal, M., Aleem, M., Ijaz, A., Rehman, H., & Yousaf, M. S. (2010). Assessment of buffalo semen with the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra zolium bromide reduction assay. *Journal of Animal Science*, *88*, 922–925.

Kahwage, P. R. (2015). *Respostas termolíticas e qualidade seminal de ovinos naturalizados criados em ambiente tropical*. Belém: Universidade Federal do Pará.

Kastelic, J. P. (2014). Understanding and evaluating bovine testes. *Theriogenology*, *81*, 18–23.

Kastelic, J. P., & Brito, L. F. (2012). Ultrasonography for monitoring reproductive function in the bull. *Reproduction in Domestic Animals*, *47*, 45–51.

Kastelic, J. P., Cook, R. B., Coulter, G. H., & Saacke, R. G. (1996b). Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology*, *45*, 935–942.

Kastelic, J. P., Cook, R. B., Coulter, G. H., Wallins, G. L., & Entz, T. (1996a). Environmental factors affecting measurement of bovine scrotal surface temperature with infrared thermography. *Animal Reproduction Science*, *41*, 153–159.

Kastelic, J. P., Coulter, G. H., & Cook, R. B. (1995). Scrotal surface, subcutaneous, intratesticular, and intraepididymal temperatures in bulls. *Theriogenology*, *44*, 147–152.

Lunstra, D. D., & Coulter, G. H. (1997). Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. *Journal of Animal Science*, *75*, 767–774.

Mader, T. L., Davis, M. S., & Gaughan, J. B. (2007). Effect of sprinkling on feedlot microclimate and cattle behavior. *International Journal of Biometeorology*, *51*, 541–551.

Malama, E., Bollwein, H., Taitzoglou, I. A., Theodosiou, T., Boscos, C. M., & Kiossis, E. (2013). Chromatin integrity of ram spermatozoa. Relationships to annual fluctuations of scrotal surface temperature and temperature-humidity index. *Theriogenology*, *80*, 533–541.

Marai, I. F. M., El-Darawany, A. A., Fadiel, A., & Abdel-Hafez, M. A. M. (2008). Reproductive performance traits as affected by heat stress and its alleviation in sheep. *Tropical and Subtropical Agroecosystems*, *8*, 209–234.

Marai, I. F. M., & Haeeb, A. A. M. (2010). Buffalo's biological functions as affected by heat stress: A review. *Livestock Science*, *127*, 89–109.

Marcondes, C. R., Vozzi, P. A., Cunha, B. R. N., Lôbo, R. B., Araújo, C. V., & Marques, J. R. F. (2010). Variabilidade genética de búfalos em rebanho-núcleo com base na análise de pedigree. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *62*, 706–711.

Menegassi, S. R. O., Barcellos, J. O. J., Dias, E. A., Koetz, C. Jr., Pereira, G. R., Peripolli, V., … Lopes, F. G. (2015). Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. *International Journal of Biometeorology*, *59*, 357–364.

Menegassi, S. R. O., Pereira, G. R., Dias, E. A., Koetz, C. Jr., Lopes, F. G., Bremm, C., … Barcellos, J. O. J. (2016). The uses of infrared thermography to evaluate the effects of climatic variables in bull's reproduction. *International Journal of Biometeorology*, *60*, 151–157.

Moraes, B. C., Costa, J. M. N., Costa, A. C. L., & Costa, M. H. (2005). Variação espacial e temporal da precipitação no estado do Pará. *Acta Amazonica*, *35*, 207–214.

Ohashi, O. M., Santos, S. S. D., Miranda, M. S., Cordeiro, M. S., Costa, N. N., & Silva, T. V. G. (2011). Morfologia do sistema genital, distúrbio reprodutivo e manejo do macho bubalino (*Bubalus bubalis*). *Revista Brasileira de Reprodução Animal*, *35*, 88–94.

Rhoads, R. P., Baumgard, L. H., & Suagee, J. K. (2013). Metabolic priorities during heat stress with an emphasis on skeletal muscle. *Journal of Animal Science*, *91*, 2492–2503.

Sansone, G., Nastri, M. J., & Fabbrocini, A. (2000). Storage of buffalo (*Bubalus bubalis*) semen. *Animal Reproduction Science*, *62*(1–3), 55–76.

Santos, A. X., Kahwage, P. R., Faturi, C., Quinzeiro Neto, T., Lourenço Junior, J. B., Joele, M. R. S. P., & Garcia, A. R. (2014). Feed supplementation with palm kernel cake-based concentrate increases the quality of water buffalo semen. *Animal Reproduction*, *11*, 85–95.

Schaefer, A. L., Cook, N. J., Church, J. S., Basarab, J., Perry, B., Miller, C., & Tong, A. K. W. (2007). The use of infrared thermography as an early indicator of bovine respiratory disease complex in calves. *Research in Veterinary Science*, *83*, 376–384.

Sealfon, A. I., & Zorgniotti, A. W. (1991). A theoretical model for testis thermoregulation. *Advances in Experimental Medicine and Biology*, *286*, 123–135.

Silanikove, N. (2000). Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science*, *67*, 1–18.

Silva, J. A. R., Araújo, A. A., Lourenço Junior, J. B., Santos, N. F. A., Garcia, A. R., & Nahúm, B. S. (2011a). Conforto térmico de búfalas em sistema silvipastoril na Amazônia Oriental. *Pesquisa Agropecuaria Brasileira*, *46*, 1364–1371.

Silva, J. A. R., Araújo, A. A., Lourenço Junior, J. B., Viana, R. B., Santos, N. F. A., & Garcia, A. R. (2011b). Perfil hematológico de búfalas da raça Murrah, criadas ao sol e à sombra, em clima tropical da Amazônia Oriental. *Acta Amazonica*, *41*, 425–429.

Silva, G. R., Garcia, A. R., Faturi, C., Lourenço Junior, J. B., Nahúm, B. S., Gonçalves, A. A., … Meneses, A. M. C. (2014). Adição de óleo de palma na dieta sobre a lipidemia e a qualidade do sêmen de bubalinos (*Bubalus bubalis*). *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*, *66*, 152–160.

Somparn, P., Gibb, M. J., Markvichitr, K., Chaiyabutr, N., Thummabood, S., & Vajrabukka, C. (2004). Analisys of climatic risk for cattle and buffalo production in northeast Thailand. *International Journal of Biometeorology*, *49*, 59–64.

Thom, E. (1959). The discomfort index. *Weatherwise*, *12*, 57–60.

Vale, W. G. (2002). Reproductive management of buffalo male aiming semen production for artificial insemination. In *Proceeding of the 1st Buffalo Symposium of Americas, Belém* (pp. 156–171). Belém: Associação Paraense de Criadores de Búfalos.

Vale, W. G., Ribeiro, H. F. L., Sousa, J. S., Silva, A. O. A., Barbosa, E. M., & Rolim Filho, S. T. (2008). Selection and breeding soundness evaluation in the male buffalo. *Revista Brasileira de Reprodução Animal*, *32*, 141–155.

How to cite this article: Silva LKX, Sousa JS, Silva AOA, et al. Testicular thermoregulation, scrotal surface temperature patterns and semen quality of water buffalo bulls reared in a tropical climate. *Andrologia*. 2017;00:e12836. https://doi.org/10.1111/and.12836