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Scrotal thermography to assess the seminal quality in Nelore and Girolando bulls

Termografia escrotal e a relação com a qualidade seminal em touros Nelore e Girolando

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Highlights:
Nelore bulls sowed lower ocular globe and scrotum surface temperatures than Girolando bulls.
Nellore bulls had greater spermatic motility, vigor and fewer spermatic defects that Girolando bulls.
Nelore bulls showed greater adaptability to tropical climate than Girolando bulls.

Abstract

Bull selection by andrological examination aims to estimate the reproductive capacity of the male. Bulls of zebu origin adapt better to high temperatures than bulls of taurine origin, which may influence scrotum temperature and seminal quality due to the imbalance of testicular thermoregulation. The objective of this study was to investigate the relationship between bioclimatic variables, the temperature of body and scrotal areas, assessed with infrared thermography, and the quality of fresh and post-thawed semen in zebu Nelore bulls (*Bos taurus indicus*) and Girolando bulls (*Bos taurus taurus* x *Bos taurus indicus*). Bulls were kept in pickets with access to water, mineral mix and a diet supplemented with concentrate. Infrared thermographs of the scrotum, orbital globe and muzzle were performed twice a week with a Flir E40 thermal imager. For scrotal thermograms, we analyzed the temperatures of the spermatic cord, proximal and distal portion of the testes and tail of the epididymis using the Flir Tools software. Samples were collected using an artificial vagina and the ejaculates were processed and frozen in liquid nitrogen until further analyses. Data were analyzed with the Tukey test or the Kruskall-Wallis test, depending on their normal distribution. Our results showed differences ($p < 0.05$) between the two breeds regarding the temperature in the ocular globe, spermatic cord and proximal portion of the scrotum. Nelore bulls presented lower temperature in the body and in certain regions of the scrotum compared to Girolando bulls. Seminal characteristics varied between breeds, with the Nelore breed presenting better semen. Positive correlations were observed between minor sperm defects and ventral regions of the testes and tails of the epididymis in Girolando bulls. Nelore bulls were less influenced by climatic variables and presented lower temperature in skin surface areas in the infrared thermography examination compared to Girolando bulls. Nelore bulls presented superior semen quality in both fresh and thawed samples than Girolando bulls.

Key words: Infrared thermography. Testicular thermoregulation. Semen freezing. Bovine bulls.

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Resumo

A seleção de touros pelo exame andrológico visa estimar a capacidade reprodutiva do macho. Touros de origem zebuína apresentam melhor adaptabilidade às altas temperaturas, em relação aos touros de origem taurina, podendo influenciar na temperatura do escroto e na qualidade seminal pelo desequilíbrio da termorregulação testicular. Objetivou-se avaliar a influência das variáveis bioclimáticas e a relação de perfis de termogramas por infravermelho de áreas do corpo e escroto com a qualidade do sêmen fresco e descongelado em touros das raças Nelore e Girolando, mantidos em Central de Inseminação Artificial. Foram utilizados quatro touros Nelore e quatro Girolando, mantidos em piquetes com acesso a água e mistura mineral; e suplementação com concentrado. Termografias infravermelhas do escroto, globo ocular e mufla foram realizadas duas vezes por semana com termovisor Flir E40. Para os termogramas escrotais, as temperaturas do cordão espermático, porção proximal e distal dos testículos e cauda do epidídimo foram analisadas utilizando o *software* Flir Tools. Foram realizadas colheitas de sêmen com vagina artificial e os ejaculados processados e congelados em nitrogênio líquido e analisados na pós-descongelação. Os dados passaram pelo teste de normalidade e teste Tukey; e na ausência de normalidade pelo teste Kruskall-Wallis. Entre raças, foram observadas diferenças ($P < 0,05$) nas temperaturas do globo ocular, cordão espermático e porção proximal do escroto. Os touros Nelore apresentaram menor temperatura corpórea e de regiões do escroto, em relação aos touros Girolando. As características seminais variaram entre raças ($P < 0,05$), a raça Nelore apresentou sêmen de melhor qualidade. Correlações positivas ($P < 0,05$) foram observadas entre defeitos espermáticos menores e temperaturas da região ventral dos testículos e das caudas dos epidídimos. em touros Girolando. Os touros da raça Nelore sofreram menor influência das variáveis climáticas com menores temperaturas de áreas da superfície da pele examinada por termografia infravermelha, em relação aos touros da raça Girolando. Os touros da raça Nelore apresentaram qualidade do sêmen fresco e descongelado superiores, em relação aos touros da raça Girolando.

Palavras-chave: Termografia infravermelha. Termorregulação testicular. Congelação de sêmen. Touros bovinos.

Introduction

The Brazilian bovine herd is mainly composed of Indian races (*Bos taurus indicus*) and their mixed breeds, with the predominance of the Nelore genetic group. However, the herd shows low reproductivity and the birth rate is close to 50% (Instituto Brasileiro de Geografia e Estatística [IBGE], 2015). In extensive production systems in Brazil, natural mating occurs during the hottest months of the year, when bulls are subjected to bioclimatic and environmental variations that negatively interfere with the fertility of the herd (Menegassi, Barcellos, Lampert, Borges, & Peripolli, 2011).

The selection of bulls by the andrological exam is important to improve the herd, and the influence of the genetic composition is one of the requirements used by Artificial Insemination Centers to industrialize the semen of bulls for use in breeding programs (Chacur, Machado & Cristancho, 2006, Sirchia, 2008, Carvalhal & Costa, 2018).

Environments with high temperatures may compromise thermoregulation and heat dissipation mechanisms of the body, leading to thermal stress (Marai, El-Darawany, Fadiel, & Abdel-Hafez, 2008). Infrared thermography is used to evaluate scrotal thermoregulation and physiological responses of animals raised in regions of high temperatures (Knizkova, Kunc, Gurdil, Pinar, & Selvi, 2007) and since the 1980s, it is used as a noninvasive method to accurately measure the temperature of the surface skin of the scrotum (Coulter, Serenger, & Bailey, 1988). Nelore bulls have been shown to adapt to thermal stress, in particular due to the anatomical characteristics typical of *Bos indicus*, in which the pampiniform plexus and the heat exchange mechanism played an important role in the physiological requirements for their normal reproductive performance (Brito, Silva, Barbosa, & Kastelic, 2004).

One way of improving the productive performance in low latitudes regions is through genetic improvement, which is done by crossing zebu and taurine breeds. The resulting offspring will exhibit greater adaptation to the tropical climate and higher potential for production (Perotto, Kroetz, & Rocha, 2010).

The objective of this study was to investigate the relationship between bioclimatic variables, the temperature of body and scrotal areas, assessed with infrared thermography, and the quality of fresh and post-thawed semen in zebu Nelore bulls (*Bos taurus indicus*) and Girolando bulls (*Bos taurus taurus* x *Bos taurus indicus*).

Material and Methods

The project was approved by the Ethics and Animal Use Committee of the University of Oeste Paulista, under protocol 3336.

Animals and study site

During the month of May, semen samples from eight bulls were collected and frozen in liquid nitrogen: four Girolando bulls with ages ranging from 36 to 84 months, 770 ± 24 kg of body weight and 38 ± 2 cm of scrotal circumference, and four Nelore bulls aged 36 to 48 months, 735 ± 21 kg of body weight and 37 ± 1 cm of scrotal circumference. All bulls had been in a semen collection regime for 5 months and were suitable for breeding according to the norms of the Brazilian College of Animal Reproduction (Colégio Brasileiro de Reprodução Animal [CBRA], 2013). These animals were housed in an Artificial Insemination Center located in the municipality of Presidente Prudente-São Paulo, latitude 22º 07 'S, longitude 51º 23' W, with an average altitude of 472 meters above sea level. The city has a tropical climate with a climatic transition area and is under the influence of most atmospheric systems in South America. According to Köppen and Geiger (1928), the climate in this city is classified as Cfa, which is characterized by humid temperate with hot summer. The climate includes a hot and rainy period between October and March, and a mild and dry period between April and September. The average temperature is 21.6°C with an annual average rainfall of 1,207 mm.

During the study, all bulls were kept in pickets of 15x30 meters, formed with *Brachiaria decumbens* with access to the covered 4x4 meters bay with water troughs and mineral mix *ad libidum* and supplementation of 5kg/ bull/day of concentrate with 14% crude protein and 10 kg/bull/day Tifton hay.

Study design

Six data collections were performed, all of them repeated between 7am and 9am with intervals of 72 to 96 hours. In each collection, bioclimatic factors were measured using infrared thermograms of the scrotum, ocular globe and muzzle. Semen samples were then frozen until further analyses.

Bioclimatic factors

The monitoring of room temperature (RT), wet-bulb globe temperature (WBGT, which represents the thermal sensation or total stress in the animals), relative humidity (RH) and dry black bulb temperature (T_{db}), which represents insolation or thermal irradiation), was performed at study site at 7am and 9am with a portable digital globe thermometer (model HT-30, InstruTemp®, São Paulo, Brazil).

Subsequently, the temperature and humidity index (THI) was calculated, according to Thom (1959):

$$THI = 0,8 \times T_{db} + RH\ (T_{db} - 14,4) + 46,4$$

where:

T_{db} = dry black bulb temperature (ºC)

RH = relative humidity (in decimals).

Infrared digital thermography

Infrared digital thermography (Thermovisor model E40®, FLIR Stockholm, Sweden) was performed on the surface skin of the scrotum, ocular globe and muzzle, with the emitter focus of the device aligned in the caudal portion and head of each bull and perpendicularly oriented, at approximately 1.5 meters away from the bull. Using the FLIR Tools software (version 2.0.11333.1001), each thermogram was analyzed from the captured image to determine the average temperature of the spermatic cord regions, proximal and distal regions of the scrotum and tail of the epididymis, on both the sides (Figure 1).

Thermography was chosen for this study because it provides a non-invasive examination without causing containment stress in bulls and because, according to Weschenfelder et al. (2013), the temperature of the ocular globe has a high correlation with body temperature.

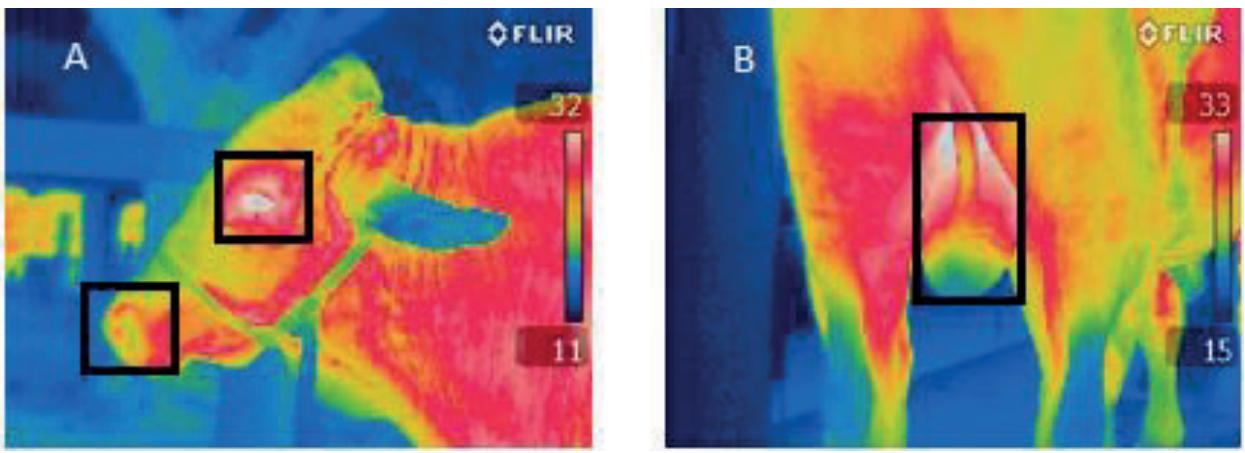

Figure 1. Infrared thermography of the orbital area, muzzle (A) and scrotum (B) of bulls.

Collection and analysis of fresh semen

Semen collection was obtained from bulls using the artificial vagina method, which causes less stress to the animal (Carvalhal & Costa, 2018). Each bull was put together with a cow for a few minutes to induce excitation. When the bull jumped on the cow, its penis was diverted and introduced into the artificial vagina, where ejaculation occurred. The internal mucosal temperature of the artificial vagina, after being filled with water, was standardized at 42-45°C.

The procedures for semen collection using an artificial vagina and the analyses of the quantitative and qualitative characteristics of the semen were performed according to the norms of the Brazilian College of Animal Reproduction (CBRA, 2013).

Samples were incubated in a water bath (Model 100®, FANEM, São Paulo, Brazil) at 32-37ºC during the analyses. We recorded the volume (mL) of the ejaculate and analyzed it under a microscope (model Eclipse 200®, Nikon, Tokyo, Japan) for sperm motility (0 to 100%), sperm vigor (0 to 5) and sperm whirlwind (0 to 5). Semen was diluted in a buffered saline formaldehyde (BSF) solution in a ratio of 1:100 (semen: BSF) for further calculation of sperm concentration in the Neubauer chamber. Sperm morphology was analyzed by phase contrast microscopy (model Eclipse E200®, Nikon, Tokyo, Japan), with counts of 200 spermatozoa per ejaculate.

Semen freezing and post-thawing analysis

Semen freezing was performed using TRIS, egg yolk and citric acid medium, according to the rules of the Brazilian College of Animal Reproduction (CBRA, 2013). Samples were frozen in 0.5 mL French straws at a concentration of 30 x 106 spermatozoa per standardized straw. Sperm motility (%), sperm vigor (0 to 5) and sperm defects (%) were analyzed after semen thawing in a water bath (37ºC/30 seconds).

Statistical analysis

Infrared temperatures, seminal parameters and bioclimatic variables were analyzed using the SAS® statistical software, version 9.3 (Statistical Analysis Software, Cary, NC). Variables were analyzed by the mixed model (MIXED procedure), considering the effects of the animals as random effects, and the means of variation factors were compared using the Tukey test or the Kruskall-Wallis test, depending on their normal distribution. The relationships between infrared temperatures, seminal parameters and bioclimatic variables were analyzed using the CORRELATION procedure, and Spearman's linear correlation coefficients were calculated. Differences were considered statistically significant when $p \leq 0.05$. Correlations were determined and classified according to Levine, Berenson, & Stephan 2012.

Results and Discussion

In the present study, infrared thermographies were performed between 15 and 20 days. Examples of the areas examined are presented in Figure 1. As this period is shorter than the spermatogenesis period (57 days on average), thermography was used to accurately measure the temperature of the surface skin of the scrotum just prior to semen collection. According to Brito et al. (2004) if the temperature of the scrotum skin remains high and outside a range of 3 to 6ºC below body temperature, spermatogenesis and androgenesis are affected. Another study reported that infrared scrotal thermography can also be used as a complementary screening test for andrological examination in field bulls, which reliably reflects the scrotal thermoregulation capacity of animals (Chacur et al., 2016).

A single thermographic image of the scrotum of each bull was obtained at each data collection, shortly after semen collection. The analysis of temperature at the six sample collections performed in Girolando and Nelore bulls showed variations in the temperatures of the body and scrotum areas. In both breeds, seminal characteristics were maintained during the samplings (Tables 1 and 2). According to Moreira (2016), environmental factors, especially temperature, may interfere with the spermatogenic cycle and semen quality. Thus, the ideal temperature of the testicles should be 3 to 6°C below that of the body, which occurs through thermoregulation provided by numerous physiological mechanisms, and temperatures above thermoregulation limits could compromise fertility.

Table 1

Mean ± standard deviation of the temperatures obtained by thermography (°C) in the ocular globe (OG), muzzle, spermatic cord (SPER.CORD), proximal portion of the testis, distal portion of the testis, and tail of the epididymis on the right and left sides of the scrotum, and macroscopic and microscopic characteristics of the ejaculate of each sperm collected from Girolando bulls

GIROLANDO										
		LEFT					RIGHT			
SAMPLING	OG	MUZZLE	SPER.CORD	PROXIMAL	DISTAL	EPIDIDYMIS	SPER. CORD	PROXIMAL	DISTAL	EPIDIDYMIS
1	34.62 ± 1.52^{A}	29.20 ± 3.47^{A}	33.92 ± 1.87^{A}	34.00 ± 2.12^{A}	32.32 ± 2.32^{A}	30.30 ± 2.90^{A}	33.92 ± 2.32^{A}	33.45 ± 2.36^{A}	32.02 ± 2.24^{A}	30.42 ± 2.83^{A}
2	32.40 ± 1.16^{A}	23.22 ± 2.86^{B}	30.37 ± 1.15^{A}	31.45 ± 1.70^{B}	29.80 ± 1.06^{B}	26.15 ± 1.64^{BC}	30.77 ± 1.21^{AB}	31.70 ± 2.24^{A}	29.12 ± 1.10^{AB}	25.92 ± 2.09^{BC}
3	32.70 ± 1.14^{A}	25.75 ± 1.43^{AB}	32.90 ± 1.29^{A}	32.95 ± 0.83^{AB}	30.75 ± 0.37^{AB}	28.35 ± 1.20^{ABC}	32.32 ± 1.33^{AB}	33.00 ± 0.87^{A}	30.07 ± 0.58^{AB}	28.32 ± 0.84^{ABC}
4	33.62 ± 2.00^{A}	29.47 ± 2.20^{A}	33.10 ± 0.84^{A}	33.30 ± 0.55^{AB}	31.50 ± 1.10^{AB}	30.07 ± 1.69^{AB}	33.05 ± 0.83^{AB}	32.60 ± 1.28^{A}	30.97 ± 1.39^{A}	29.37 ± 1.67^{AB}
5	33.10 ± 2.00^{A}	26.25 ± 1.61^{AB}	32.12 ± 1.19^{A}	32.25 ± 0.81^{AB}	30.47 ± 0.64^{AB}	27.90 ± 1.34^{ABC}	31.22 ± 2.16^{AB}	31.90 ± 1.53^{A}	29.90 ± 0.69^{AB}	27.75 ± 1.71^{ABC}
6	31.97 ± 1.57^{A}	24.47 ± 4.00^{AB}	30.75 ± 2.30^{A}	30.77 ± 3.59^{B}	28.00 ± 4.09^{B}	24.52 ± 3.66^{C}	29.25 ± 2.41^{B}	30.00 ± 3.59^{A}	27.32 ± 3.36^{B}	24.47 ± 3,09^{C}

SAMPLING	VOL. (mL)	[ ] (x10^{6}/ mL)	MOT. (%)	VIGOR	TOTAL (%)	MAJOR DEFECTS (%)	MINOR DEFECTS (%)	TTR MOT. (%)	TTR VIGOR
1	9.34 ± 2.47^{A}	1653.50 ± 394.46^{A}	50.00 ± 13.93^{A}	3.5±0.6^{A}	19.75 ± 13.30^{A}	16.50 ± 12.31^{A}	3.25 ± 6.49^{A}	17.50 ± 16.35^{A}	1.50 ± 1.48^{A}
2	7.63 ± 2.44^{A}	1089.22 ± 488.59^{A}	52.50 ± 9.57^{A}	3.5±0.58^{A}	13.75 ± 12.28^{A}	13.25 ± 12.84^{A}	0.50 ± 1.00^{A}	16.25 ± 18.87^{A}	1.50 ± 1.73^{A}
3	8.46 ± 4.36^{A}	1560.50 ± 481.92^{A}	55.00 ± 12.91^{A}	3.75±0.5^{A}	16.25 ± 14.52^{A}	15.75 ± 13.82^{A}	0.50 ± 1.00^{A}	15.00 ± 17.32^{A}	0.75 ± 1.50^{A}
4	9.10 ± 4.37^{A}	1152.70 ± 289.40^{A}	52.50 ± 17.08^{A}	3.5±0.58^{A}	18.75 ± 18.55^{A}	7.75 ± 8.92^{A}	11.00 ± 11.60^{A}	15.00 ± 17.32^{A}	0.75 ± 1.50^{A}
5	7.46 ± 3.78^{A}	906.22 ± 247.98^{A}	45.00 ± 12.91^{A}	3.5±0.58^{A}	16.25 ± 13.77^{A}	15.75 ± 13.52^{A}	0.50 ± 1.00^{A}	16.25 ± 18.87^{A}	1.50 ± 1.73^{A}
6	9.62 ± 2.96^{A}	1081.00 ± 147.11^{A}	52.50 ± 15.00^{A}	3.5±0.58^{A}	15.50 ± 14.64^{A}	15.00 ± 15.23^{A}	0.50 ± 1.00^{A}	16.25 ± 18.87^{A}	1.50 ± 1.73^{A}

VOL = volume of the ejaculate; [ ] = sperm concentration in the ejaculate; MOT. = sperm motility; TOTAL, MAJOR and MINOR = total, major and minor sperm defects; TTR MOT. = sperm motility after the thermo-resistance test; TTR VIGOR = sperm vigor after the thermo-resistance test. * different letters in each column indicate $p<0.05$.

Table 2

Mean ± standard deviation of the temperatures obtained by thermography (°C) in the ocular globe (OG), muzzle, spermatic cord (SPER.CORD), proximal portion of the testis, distal portion of the testis, and tail of the epididymis on the right and left sides of the scrotum, and macroscopic and microscopic characteristics of the ejaculate of each sperm collected from Nelore bulls

NELORE										
			LEFT				RIGHT			
SAMPLING	OG	MUZZLE	SPER.CORD.	PROXIMAL	DISTAL	EPIDIDYMIS	SPER. CORD	PROXIMAL	DISTAL	EPIDIDYMIS
1	33.52 ± 1.50^{A}	29.52 ± 4.52^{A}	33.25 ± 0.90^{A}	33.60 ± 0.80^{A}	31.40 ± 1.28^{A}	30.85 ± 0.52^{A}	33.00 ± 1.07^{A}	32.40 ± 1.08^{A}	31.52 ± 0.99^{A}	30.95 ± 0.62^{A}
2	30.75 ± 1.34^{BC}	23.92 ± 2.12^{BC}	29.60 ± 1.23^{BC}	29.77 ± 2.02^{B}	28.80 ± 1.47^{A}	26.10 ± 0.42^{CD}	28.72 ± 0.56^{B}	28.45 ± 0.42^{B}	28.95 ± 0.45^{BC}	26.32 ± 0.50^{CD}
3	32.75 ± 0.59^{AB}	24.77 ± 1.24^{BC}	30.20 ± 0.75^{BC}	30.60 ± 0.91^{B}	30.22 ± 0.72^{A}	27.80 ± 0.64^{BC}	30.55 ± 0.66^{BC}	30.15 ± 0.37^{AB}	29.77 ± 1.08^{AB}	27.67 ± 1.17^{BC}
4	32.95 ± 1.13^{AB}	26.82 ± 1.89^{B}	32.70 ± 0.61^{AB}	32.42 ± 0.52^{AB}	30.87 ± 0.99^{A}	29.92 ± 1.23^{AB}	32.10 ± 0.73^{AB}	31.95 ± 1.35^{AB}	30.90 ± 1.01^{AB}	30.05 ± 1.27^{AB}
5	31.65 ± 1.71^{ABC}	27.90 ± 2.14^{AB}	31.02 ± 2.00^{ABC}	31.12 ± 1.35^{AB}	30.60 ± 0.82^{A}	28.05 ± 0.57^{B}	30.32 ± 1.22^{BC}	30.32 ± 1.45^{AB}	30.37 ± 0.97^{AB}	28.32 ± 1.54^{ABC}
6	29.37 ± 1.02^{C}	20.67 ± 2.45^{C}	29.10 ± 1.37^{C}	29.55 ± 1.36^{B}	27.60 ± 0.51^{A}	25.25 ± 1.00^{D}	27.90 ± 1.99^{C}	28.95 ± 3.20^{B}	27.12 ± 0.63^{C}	25.35 ± 1.03^{D}

SAMPLING	VOL. (mL)	[ ] (x10^{6}/mL)	MOT. (%)	VIGOR	TOTAL (%)	MAJOR DEFECTS (%)	MINOR DEFECTS (%)	TTR MOT. (%)	TTR VIGOR
1	4.45 ± 2.42^{A}	1453.80 ± 866.03^{A}	62.50 ± 9.57^{A}	4.00 ± 0.00^{A}	8.25 ± 4.99^{A}	5.75 ± 3.77^{A}	2.50 ± 1.91^{A}	32.50 ± 2.89^{A}	3.00 ± 0.00^{A}
2	4.43 ± 2.16^{A}	1378.90 ± 646.89^{A}	57.50 ± 12.58^{A}	3.75 ± 0.50^{A}	8.25 ± 3.77^{A}	3.75 ± 2.75^{A}	4.50 ± 4.12^{A}	26.25 ± 17.50^{A}	2.25 ± 1.50^{A}
3	4.50 ± 0.33^{A}	2053.00 ± 793.23^{A}	52.50 ± 9.57^{A}	3.75 ± 0.50^{A}	9.75 ± 4.65^{A}	6.75 ± 6.50^{A}	3.00 ± 2.58^{A}	25.00 ± 16.83^{A}	2.25 ± 1.50^{A}
4	4.27 ± 1.38^{A}	1852.50 ± 670.53^{A}	52.50 ± 15.00^{A}	4.00 ± 0.00^{A}	4.75 ± 0.96^{A}	4.75 ± 0.96^{A}	0.00^{A}	27.50 ± 18.48^{A}	2.25 ± 1.50^{A}
5	5.37 ± 2.31^{A}	1909.40 ± 816.45^{A}	50.00 ± 21.60^{A}	3.50 ± 0.58^{A}	7.00 ± 1.41^{A}	5.50 ± 1.73^{A}	1.50 ± 1.91^{A}	22.50 ± 15.00^{A}	2.25 ± 1.50^{A}
6	5.21 ± 0.69^{A}	1396.57 ± 693.09^{A}	55.00 ± 12.91^{A}	4.00 ± 0.00^{A}	5.50 ± 1.73^{A}	4.50 ± 2.89^{A}	1.00 ± 2.00	23.75 ± 16.01^{A}	2.25 ± 1.50^{A}

VOL = volume of the ejaculate; [ ] = sperm concentration in the ejaculate; MOT. = sperm motility; TOTAL, MAJOR and MINOR = total, major and minor sperm defects; TTR MOT. = sperm motility after the thermo-resistance test; TTR VIGOR = sperm vigor after the thermo-resistance test. * different letters in each column indicate $p<0.05$.

In addition to the differences observed in body and scrotal temperatures between samplings, we also observed numerical climatic variations, altering room temperature, wet-bulb globe temperature, dry black bulb temperature and relative humidity (Table 3). We observed a positive medium-to-strong correlation between THI and body temperature measured by thermography (ocular globe and muzzle) and temperatures of the scrotum (spermatic cord, proximal and distal region of the testis and tail of the epididymis) on the right and left sides (Table 4, $P<0.05$). Nogueira et al. (2013) emphasized that room temperature and thermal comfort provided to the animals during the use of the equipment directly influence the thermogram results. In the present work, the absence of variations in seminal characteristics between collections indicate that oscillations in the temperature of scrotum surface in the two breeds were controlled, without affecting the seminal profile of the animals (Ruediger, Chacur, Alves, Oba, & Ramos, 2016). Kastelic (2014) reported that a moderate increase in testicular temperature in bulls drastically reduces sperm production, progressive sperm motility and live sperm count per ejaculate, and increases the percentage of morphologically abnormal spermatozoa.

All thermograms of the scrotum and ocular globe were obtained when their surfaces were dry. The skin of the scrotum on rainy days (wet with water) can influence the temperature of the surface of the scrotum and cause its considerable decrease, and 30 minutes are required for the temperature to stabilize after the skin is dry (Chacur et al., 2016).

Comparing both breeds, differences in the temperature of the eyeball, spermatic cord and proximal portion of the scrotum were observed on both the right and left sides, with Nelore bulls presenting lower temperature in the scrotum and ocular globe than Girolando bulls. Seminal characteristics also varied among these breeds. Although Nelore bulls presented lower volume of ejaculate, it had higher sperm concentration, sperm motility and sperm vigor, lower incidence of sperm defects and greater TTR resistance than Girolando bulls (Table 5). Ruediger et al. (2018) observed that body surface temperatures measured with the thermograph can be used to identify heat stress due to the positive correlation of these points with rectal temperature and plasma cortisol levels. Weschenfelder et al. (2013) found that imaging the eye surface allows the detection of temperature changes associated with physiological conditions. Stewart (2008) correlated the increase in ocular temperature with the elevation of thermal stress. Therefore, it has been demonstrated that Girolando bulls are more susceptible to high temperatures than Nelore bulls, and that the latter presents greater adaptability to tropical climate.

Although the Girolando and Nelore breeds have a distinct adaptation to climate factors, these factors influence spermatogenesis. However, this influence can be minimized by using strategies to improve thermal comfort, such as natural or artificial shading of the pastures (Renaudeau et al., 2012).

Table 3
Values of room temperature (RT), relative humidity (RH), wet-bulb globe temperature (WBGT), dry black bulb temperature (T_{db}) and temperature and humidity index (THI) at the start and end of each semen collection from Girolando and Nelore bulls

SAMPLING	RT		AIR HUMIDITY		WBGT	T_{db}	THI		
	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	INITIAL	FINAL	
1	23.80	25.70	64.50%	55.90%	20.30	21.40	23.70	26.20	73.23
2	21.80	19.10	63.90%	83.20%	18.30	17.80	20.70	19.30	65.57
3	22.40	21.40	71.20%	77.70%	19.70	19.60	21.60	21.90	68.93
4	22.70	23.10	73.90%	79.50%	20.70	21.20	23.80	22.90	71.77
5	22.90	22.60	71.00%	74.40%	20.20	20.60	22.60	23.10	70.55
6	20.90	19.80	53.80%	60.50%	16.50	16.50	19.60	20.20	65.46

Table 4
Correlation between temperature and humidity index (THI) with temperatures of the ocular globe (GO), muffle, spermatic cord (SC), proximal portion of the scrotum (PROX), distal portion of the testis (DIST) and tail of the epididymis (EPI) on the left and right sides ($p<0.05$)

	GO	MUZZLE de	SC left	SC right	PROX left	PROX right	DIST left	DIST right	EPI left	EPI right
THI	0.69	0.72	0.77	0.84	0.78	0.71	0.74	0.84	0.91	0.88

Table 5

Mean ± standard deviation of the temperatures obtained by thermography (°C) in the ocular globe (OG), muzzle, spermatic cord (SPER.CORD.), proximal portion of the testis, distal portion of the testis, and tail of the epididymis on the right and left sides of the scrotum, and macroscopic and microscopic characteristics of the ejaculate, comparing Girolando and Nelore bulls

	LEFT						RIGHT			
BREED	OG	MUZZLE	SPER. CORD.	PROXIMAL	DISTAL	EPIDIDYMIS	SPER. CORD	PROXIMAL	DISTAL	EPIDIDY-MIS
Girolando	33.07 ± 1.63^{A}	26.39 ± 3.31^{A}	32.19 ± 1.75^{A}	32.45 ± 1.89^{A}	30.47 ± 2.21^{A}	27.88 ± 2.75^{A}	31.75 ± 2.14^{A}	32.11 ± 2.11^{A}	29.91 ± 2.06^{A}	27.71 ± 2.63^{A}
Nelore	31.83 ± 1.83^{B}	25.61 ± 3.74^{A}	30.97 ± 1.91^{B}	31.17 ± 1.84^{B}	29.91 ± 1.61^{A}	27.99 ± 2.11^{A}	30.43 ± 2.07^{B}	30.37 ± 2.04^{B}	29.77 ± 1.66^{A}	28.11 ± 2.21^{A}

BREED	VOL. (mL)	[ ] (x10^{6}/ mL)	MOT. (%)	VIGOR (1 a 5)	TOTAL (%)	MAJOR DEFECTS (%)	MINOR DEFECTS (%)	TTR MOT. (%)	TTR VIGOR
Girolando	8.61 ± 3.37^{A}	1240.54 ± 432.01^{A}	51.25 ± 12.95^{A}	3.54 ± 0.58^{A}	16.71 ± 13.79^{A}	14.00 ± 12.42^{A}	2.71 ± 5.88^{A}	16.04 ± 16.48^{A}	1.25 ± 1.51^{A}
Nelore	4.71 ± 1.61^{B}	1674.02 ± 721.01^{B}	55.00 ± 13.18^{B}	3.83 ± 0.38^{B}	7.25 ± 3.42^{B}	5.16 ± 3.31^{B}	2.08 ± 2.61^{A}	26.25 ± 14.01^{B}	2.37 ± 1.24^{A}

VOL = volume of the ejaculate; [ ] = sperm concentration in the ejaculate; MOT. = sperm motility; TOTAL, MAJOR and MINOR = total, major and minor sperm defects; TTR MOT. = sperm motility after the thermo-resistance test; TTR VIGOR = sperm vigor after the thermo-resistance test. * different letters in each column indicate $p<0.05$.

If the environment is inadequate and does not favor physical processes (radiation, conduction, convention) (Despopoulos, 2003), the physiological mechanisms need to adjust according to the climate, thereby increasing the sweating, respiratory rate and peripheral vasodilatation (Swenson, Reece & Dukes, 1996). However, when these mechanisms become inefficient due to the environment, the animal undergoes thermal stress, which reduces fertility. In addition, thermal stress decreases food intake by the animal, which leads to a reduction in heat dissipation and metabolism (Bernabucci et al., 2010), reduced somatotropin production (Rhoads et al., 2009), and increased insulin (Wheelock, Rhoads, Van Baale, Sanders, & Baumgard, 2010) and serum cortisol levels (Chacur et al., 2010), affecting growth and reproductive efficiency.

Another important aspect is the adaptation of zebu breeds to tropical climate (i.e. temperature, frequent solar irradiation and high relative humidity of the air). This was demonstrated in Brahman and Nelore bulls (Chacur, Miyasaki, Oba, Souza, & Gabriel, 2017; Ruediger, Chacur, Alves, Oba, & Ramos, 2016), which present as main adaptive characteristics skin thickness, dark coloration, white pelt, higher concentration of sweat glands and greater skin irrigation than mixed or taurine animals (Silva & Pocay, 2001). It is known that the thermoregulation mechanisms of the male reproductive organ are not totally efficient (Losano et al., 2018). In the case of thermal stress, the increase in scrotal/testicular temperature occurs, thereby increasing cellular metabolism in the testicles, which leads to a higher O_2 need (Kastelic, Wilde, Rizzoto, & Thundathil, 2017). Testicular blood supply is limited and will not be enough to supply the increase in O_2 demand in testicular cells, which leads to cell death and testicular degeneration, affecting both spermatogenesis and androgenesis (Paul, Teng, & Saunders, 2009). Due to a lower adaptability to tropical climate, Girolando bulls may suffer from chronic thermal stress, which may result in subfertility or infertility.

A medium positive correlation between the ventral portion of the left testicle and sperm vigor after the thermoresistence test ($r=0.44$, $p<0.05$) was observed in the Nelore breed when correlating thermography temperatures with sperm quality. In the Girolando breed, a medium positive correlation was found between the temperature of the ventral portion of the left testicle and sperm concentration, as well as with smaller spermatic defects. Similar results were observed between the ventral portion of the right testicle and the right and left side of the epididymis with smaller spermatic defects (Table 6). Similarly, Souza et al. (2014)' extensively observed correlations of the different regions of the scrotum of Nelore bulls with seminal quality, reinforcing the importance of testicular thermoregulation in spermatogenesis and androgenesis.

Table 6
Correlations between the temperatures measured by infrared thermography and seminal characteristics of Girolando bulls. Only significant correlations are shown ($p<0.05$)

	Minor defects	Sperm concentration
Ventral portion of the testicle	0.49	0.42
Ventral portion of the testicle	0.45	
Tail of the epididymis	0.53	
Tail of the epididymis	0.52	

Conclusions

Lower temperatures were measured in certain areas of the surface skin of the scrotum examined by infrared thermography in Nelore and Girolando bulls. The quality of fresh and post-thawed semen was higher in the Nelore breed. Bioclimatic parameters influenced more broadly the temperature at the surface skin of the scrotum and the quality of fresh semen and post-thawing in the Girolando breed.

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