DOI: 10.5433/1679-0359.2016v37n1p221

Digital infrared thermography of the scrotum, semen quality, serum testosterone levels in Nellore bulls (Bos taurus indicus) and their correlation with climatic factors

Termografia digital por infravermelho do escroto, qualidade do sêmen, níveis séricos de testosterona em touros Nelore (Bos taurus indicus) e suas correlações com os fatores climáticos

Felipe Rydygier de Ruediger[1*]; Marcelo George Mungai Chacur[2]; Francisco Carlos Platzeck Estrella Alves[3]; Eunice Oba[4]; Alcides de Amorim Ramos[4]

Abstract

The objective was to study the relationship of climatic conditions with the temperature of the scrotum surface and sperm quality through digital infrared thermography in Nellore bulls, raised extensively. In six bulls held with scrotal thermography Flir E40® cameras, blood samples for serum testosterone and semen collection were taken by electroejaculation every 10 days, with six replications. Climatic factors: ambient temperature, relative humidity, dry globe temperature and temperature of the wet globe were recorded using a globe thermometer (InstruTemp®, ITWTG-2000). Thermal images of the scrotum were analyzed with the Flir Tools® software for the temperatures of scrotal surface, the right and left sides of the scrotum lap thirds: dorsal, middle and ventral testicles; and tails of the epididymis. The semen data and thermograms were submitted to ANOVA and Tukey's test at 5%. Pearson correlation was used for the surface temperatures of the scrotum, rectal temperature, quantitative and qualitative characteristics of semen and climatic factors. There was a positive correlation ($P<0.05$) for sperm motility x scrotal temperatures; sperm concentration x scrotal temperatures; climatic factors x rectal temperature. There was a negative correlation ($P<0.05$) between ambient temperature x sperm concentration. It was concluded that the temperature of the scrotum surface and climatic factors, temperature and humidity, influence the quality of semen. Thermography is recommended as a supplementary examination for reproductive evaluation of bulls.
Key words: Climatic factors, scrotal thermogram, zebu bull, semen

Resumo

Objetivou-se estudar a relação dos fatores climáticos com a temperatura da superfície do escroto e qualidade seminal por meio da termografia digital de infravermelho em touros Nelore, criados extensivamente. Em seis touros realizaram-se termografia escrotal com câmera Flir E40®, colheita de sangue para a testosterona sérica e colheita de sêmen por eletroejaculação a cada 10 dias, com seis repetições. Os fatores climáticos: temperatura ambiente, umidade relativa do ar, temperatura do globo seco e temperatura do globo úmido foram registrados com globotermômetro (InstruTemp®,

[1] Discente de Doutorado em Biotecnologia Animal, Universidade Estadual Paulista "Júlio de Mesquita Filho", UNESP, Botucatu, SP, Brasil. E-mail: f_rydygier@hotmail.com
[2] Prof., Universidade do Oeste Paulista, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: chacur@unoeste.br
[3] Discente, UNOESTE, Presidente Prudente, SP, Brasil. E-mail: kikoplatzeckalves@outlook.com
[4] Profs., UNESP, Botucatu, SP, Brasil. E-mail: euniceoba@fmvz.unesp.br; aaramos@fmvz.unesp.br
[*] Author for correspondence

Recebido para publicação 10/03/15 Aprovado em 10/09/15

ITWTG-2000). Os termogramas do escroto foram analisados com o *software Flir Tools®* para as temperaturas da superfície escrotal, dos lados direito e esquerdo, colo do escroto, terços: dorsal, médio e ventral dos testículos; e caudas dos epidídimos. Os dados do sêmen e dos termogramas foram submetidos à análise de variância e teste de Tukey a 5%. Correlação de Pearson foi empregada para as temperaturas da superfície do escroto, temperatura retal, características quantitativas e qualitativas do sêmen e fatores climáticos. Houve correlação positiva (P<0,05) para motilidade espermática x temperaturas do escroto; concentração espermática x temperaturas do escroto; fatores climáticos x temperatura retal. Houve correlação negativa (P<0,05) entre temperatura ambiente x concentração espermática. Concluiu-se que as temperaturas da superfície do escroto e os fatores climáticos, temperatura e umidade do ar, influenciaram na qualidade do sêmen. A termografia é recomendada como exame complementar na avaliação reprodutiva de touros.

Palavras-chave: Fatores climáticos, termograma escrotal, touro zebu, sêmen

Introduction

To optimize the reproductive function for natural mating or artificial insemination, bulls should produce a large number of morphologically normal sperm. Knowledge of the testes of cattle and how they are evaluated is of great importance (KASTELIC, 2014). Several factors affect animal reproduction, including ambient temperature and relative humidity (HORN et al., 1997). According to Kastelic et al. (2001), a moderate increase in testicular temperature of the bulls submitted to scrotum insulation dramatically reduces sperm production, motility and the number of viable spermatozoa in the ejaculate, and increases the percentage of morphologically abnormrnal sperms.

The temperature of the bull testes between 4 and 5°C below the rectal temperature is essential for the efficient production of semen. Adverse effects of elevated testicular temperature have been reported in the production and semen quality, reducing fertility in ruminants (BRITO et al., 2004; COULTER et al., 1988). Local mechanisms, such as countercurrent heat exchange, blood flow regulation, the position of the testes and sweating, play an important role in the maintenance of testicular temperature (BRITO et al., 2004; GABALDI; WOLF, 2002).

Digital infrared thermography is a non-invasive imaging test with high accuracy. The scrotum surface temperature correlates with testicular temperature, providing detailed information about the bull's ability to maintain testicular temperature (COULTER et al., 1988). The zebu cattle predominate in the composition of the Brazilian herd, and their fertility is challenged by climatic factors, high temperature and relative humidity and the extensive management conditions. Therefore, it is of great importance to study the use of digital infrared thermography in zebu bulls bred in Brazil, this is a subject that has been little studied and reported in scientific articles. The objective was to study the relationship of climatic factors with the temperature of the scrotum surface and seminal quality by infrared digital thermal imaging in Nellore bulls, raised extensively.

Material and Methods

This project was approved by the Ethics Committee and Animal Use in Experimentation (CEUA) of the Universidade do Oeste Paulista - UNOESTE, under protocol 1700. The experiment was conducted from October to December of 2012 on a farm in the municipality of Piquerobi, SP, located at latitude 21º52'03" and longitude 51º43'43" with average rainfall of 1,300 mm/year, average annual temperature of 28°C, and a hot climate with a dry winter. Six Nellore bulls aged 33 ± 2 months, raised extensively in pasture of *Brachiaria decumbens* with mineral salt and water ad libitum were used.

The procedures described below were performed in the six bulls at 10-day intervals, with six replications. Climatic factors: wet-bulb globe temperature (WBGT), dry-bulb temperature (TA), globe thermometer temperature (TG) and relative humidity (RH) were recorded using a globe thermometer (InstruTemp®, ITWTG-2000) individually in each collection in the morning. The procedures were performed in the following order: scrotum infrared digital thermography, rectal temperature measurement, blood samples taken by jugular venipuncture and semen collection by electroejaculation.

The temperatures of the scrotal surface were recorded in thermograms via digital infrared thermography (thermal imager FLIR® E-40); the focus of the emitter unit was targeted in the caudal portion of each bull scrotum and oriented perpendicular, 1 meter away. The images were analyzed by FLIR Tools® software version 3.1.13080.1002 to obtain the temperatures of the regions at five points of the scrotal surface, and on the right (R) and left (L) side: scrotum neck (T1R and T1L), dorsal portion (T2R and T2L), medium portion (T3R and T3L) and ventral portion (T4R and T4L) of the testes and tails of the epididymis (T5R and T5L) (LUNSTRA; COULTER, 1997;. BRITO et al., 2012). The classification of infrared images (thermograms) of bull scrotum with ideal scrotal thermoregulation have a balanced left-right symmetry and a decrease in temperature from the top to the bottom of the testis portion, with patterns of images classified as satisfactory; random temperature patterns, lacking the left symmetry to the right and localized areas of temperature rise hot spot indicates abnormal thermoregulation of the testes and epididymides, and the image (thermogram) classified as doubtful or unsatisfactory (COULTER et al., 1988; PUROHIT et al., 1985; WOLFE et al., 1985). After thermography, rectal temperature (RT) with a digital clinical thermometer was measured.

Semen was collected by electroejaculation (Autoejac®, Neovet, Brazil) and incubated in a water bath between 32 and 35°C, for the analysis of quantitative and qualitative characteristics, according to standards of the Brazilian College of Animal Reproduction (GUASTI, 2013). Blood samples were taken by jugular venipuncture and then centrifuged at 1500g/15 minutes; the serum samples were stored in cryotubes at -20°C for the subsequent analysis of serum testosterone by radioimmunoassay (RIA) with DPC - Medlab® kit.

Data were collected as repeated measurements over time, the data of the temperatures of thermograms, semen and testosterone were subjected to analysis of variance and subsequent 5% Tukey test. Pearson correlation coefficients were used for semen variables and climatic factors, using the SAS (2005).

Results and Discussion

Concerning measurements of the organs of scrotal contents, the values of variables related to testicular morphometry, epididymal and scrotal circumference are shown in Table 1, showing a preserved anatomical pattern, as in the rules suggested by the Brazilian Animal Reproduction College (GUASTI, 2013). The production of testosterone was assessed by serum levels of the hormone, and did not vary between collections and animals ($p>0.05$), with mean and standard deviation of 253.51 ± 112.24 ng/dl, similar to that reported by Lezier (2004) in Nellore males.

For proper sperm production, transport, maturation and storage, one of the factors that should be considered is the preserved anatomy of the organs of scrotal contents, which is associated with the proper production of testosterone. Coulter et al. (1988) reported that the temperature of the testes of cattle should be below body temperature for the production of morphologically viable sperm cells and that elevated ambient temperatures cause a reduced quality of semen from bulls with incompetent testicular thermoregulation.

Table 1. Testicular and epididymal morphometric and scrotal circumference of Nellore bulls.

Animal	LENG L[1] (cm)	LENG R[2] (cm)	WIDTH L[3] (cm)	WIDTH R[4] (cm)	HEIG L[5] (cm)	HEIG R[6] (cm)	EPID L[7] (cm)	EPID R[8] (cm)	CE[9] (cm)
1	12.4	12	6.8	6.7	6.8	7.4	3.4	3.2	36
2	12.9	11.7	7.3	7.8	7.6	9	3.2	3	36
3	11.4	11.7	6.5	6.8	6.7	7.3	3.1	2.3	37
4	10.5	11.6	7.4	8.1	7.2	7.8	2.9	3	40
5	10.7	10.2	6.4	7.1	6.9	8.1	3.6	3.3	39
6	11.6	11.2	6.8	7.3	7.8	7.7	3.1	3.7	37.5

[1] Length of left testicle; [2] Length right testicle; [3] Left testicle width; [4] Right testicle width; [5] Left testicle height; [6] Right testicle height; [7] Left epididymis; [8] Right epididymis; [9] Scrotal circumference.

There was a significant difference (P<0.05) between the second and fourth collection, for motility (Table 2). Also, there was was no difference (P<0.05) among collections for the average characteristics of sperm concentration (Table 2). However, there was no difference (P>0.05) between collections, for sperm, minor, major and total defects (Table 2). Thus, the six bulls were approved for the activity of natural mating, according to the norms of the Brazilian College of Animal Reproduction (GUASTI, 2013).

Table 2. Mean values, standard deviation and variance analysis for the qualitative and quantitative characteristics of semen of Nellore bulls.

	VOL[1]	ASP[2]	GRO[3]	MOT[4]	VIT[5]	CONC[6]	ESPTO[7]	ESVIT[8]	LD[9]	MD[10]	TOD[11]
Mean	5.06± 1.65	1.80± 0.62	1.50± 1.63	69.30± 15.59	3.19± 0.88	23.62± 16.81	123.76± 118.43	88.95± 87.47	7.38± 3.55	6.09± 3.88	13.48± 4.68
Minimum	2.00	1.00	0	30.00	1	2	10	3	1.55	0.88	4.76
Maximum	8.50	3.00	5	90.00	5	69	645	451	15.50	15.71	21.48
Collection											
1	5.25^{a}	1.83^{a}	1.50^{a}	65.00^{ab}	3.33^{a}	13.91^{b}	75.04^{b}	48.30^{b}	7.38^{a}	5.90^{a}	13.28^{a}
2	3.66^{a}	2.16^{a}	1.66^{a}	80.00^{a}	3.33^{a}	50.91^{a}	254.42^{a}	192.91^{a}	9.17^{a}	6.35^{a}	15.52^{a}
3	5.33^{a}	1.83^{a}	1.33^{a}	65.83^{ab}	2.50^{a}	19.00^{b}	103^{ab}	72.25^{ab}	6.67^{a}	5.96^{a}	12.64^{a}
4	4.91^{a}	1.33^{a}	1.50^{a}	58.33^{b}	3.16^{a}	17.75^{b}	72.54^{ab}	44.84^{b}	6.10^{a}	6.59^{a}	12.70^{a}
5	6.16^{a}	1.83^{a}	1.50^{a}	73.33^{ab}	3.33^{a}	24.16^{b}	154.38^{b}	115.20^{ab}	6.66^{a}	7.09^{a}	13.75^{a}
6	5.08^{a}	1.83^{a}	1.50^{a}	73.33^{ab}	3.50^{a}	16.00^{b}	83.21^{ab}	60.22^{b}	8.32^{a}	4.65^{a}	12.97^{a}
Animal											
1	6.58^{a}	1.50^{a}	0.66^{bc}	65.00^{ab}	2.66^{bc}	15.75^{a}	174.17^{a}	114.78^{a}	4.10^{b}	2.73^{c}	6.84^{b}
2	4.50^{a}	1.66^{a}	1.66^{abc}	72.50^{ab}	3.33^{abc}	23.33^{a}	95.25^{a}	71.18^{a}	10.14^{a}	3.82^{c}	13.97^{a}
3	4.75^{a}	1.33^{a}	0.00^{c}	56.66^{b}	2.50^{c}	21.58^{a}	102.75^{a}	65.23^{a}	10.39^{a}	5.12^{bc}	15.51^{a}
4	5.58^{a}	1.83^{a}	0.83^{bc}	60.00^{b}	2.83^{abc}	27.66^{a}	153.04^{a}	103.93^{a}	5.99^{ab}	9.64^{ab}	15.63^{a}
5	4.33^{a}	2.16^{a}	2.50^{ab}	80.00^{a}	3.83^{ab}	29.08^{a}	105.63^{a}	84.94^{a}	6.48^{ab}	10.21^{a}	16.69^{a}
6	4.66^{a}	2.33^{a}	3.33^{a}	81.66^{a}	4.00^{a}	24.33^{a}	111.75^{a}	93.65^{a}	7.20^{ab}	5.02^{bc}	12.23^{ab}

Different letters in the same column (< P 0.05). [1] Volume (ml); [2] Aspect (1-2-Viscous, aqueous, 3-Creamy); [3] Gross Motility; [4] Motility (%); [5] Vitality; [6] Concentration (x 10^7 sperm/ml); [7] Sperm total (x 10^7 sperm/ml); [8] Sperm viable total (x 10^7 sperm/ml); [9] Larger defects (%); [10] Minor Defects (%); [11] Total defects (%).

With the use of digital infrared thermography of the scrotum technique, it was possible to measure temperatures at different points in the scrotal area. There were differences (P<0.05) between collections, for the temperatures of the scrotum, T1L, T2L, T3L, T4L, T1R, T2R, T3R and rectal temperature (Table 3). Note that there was no difference (P>0.05) between collections, for temperatures in the tail region of the epididymis (T5R and T5L), suggesting there is a more effective mechanism for maintaining the temperature equilibrium in this region. Probably, due to epididymal tail store, the sperm and maintain their quality until ejaculation. The effectiveness of the temperature balancing mechanism may be favored by the topographical anatomy of the tail of the epididymis in the ventral scrotum region with a large area of skin to make heat exchange with the external environment.

According to Coulter et al. (1988) and Kastelic et al. (2001), a moderate elevation of testicular temperature in the bull dramatically reduces sperm production, motility and the number of live sperm per ejaculate, and increases the percentage of morphologically abnormal sperms. The rise in testicular temperature leads to increased metabolism and testicular oxygen demand, but testicular blood flow remains stable and this increase does not offset the demand, resulting in hypoxia and alterations of spermatogenesis (SETCHELL, 2006).

Table 3. Mean values, standard deviation and variance analysis of temperatures (°C) of the scrotum measured by infrared thermography and rectal temperature in bulls bred extensively.

	T1L	T2L	T3L	T4L	T5L	T1R	T2R	T3R	T4R	T5R	RT
Mean	33.25± 1.08	32.59± 1.01	31.66± 0.96	31.34± 1.11	30.33± 1.28	32.44± 1.55	31.92± 1.33	31.28± 1.02	30.76± 0.93	30.09± 1.20	39.23± 0.32
Minimum	29.50	30.10	29.10	27.90	26.50	28.60	29.00	28.60	28.50	27.50	38.50
Maximum	35.30	34.40	33.30	33.20	32.90	36.30	34.30	33.30	32.60	32.40	40.10
Collection											
1	32.10^{b}	31.13^{b}	30.48^{b}	30.11^{b}	29.11^{a}	30.80^{b}	30.33^{b}	30.21^{b}	29.91^{a}	29.38^{a}	39.18^{bc}
2	33.56^{ba}	33.23^{a}	32.40^{a}	31.81^{a}	30.95^{a}	33.28^{a}	32.26^{a}	31.48^{ba}	31.01^{a}	30.30^{a}	38.98^{c}
3	33.56^{ba}	33.03^{a}	32.21^{a}	31.61^{ba}	30.56^{a}	32.63^{ba}	32.63^{a}	31.81^{a}	31.16^{a}	30.25^{a}	39.25^{bac}
4	32.95^{ba}	32.10^{ba}	31.28^{ba}	30.65^{ba}	29.53^{a}	32.48^{ba}	31.41^{ba}	30.75^{ba}	30.06^{a}	29.28^{a}	39.40^{ba}
5	34.23^{a}	33.31^{a}	32.10^{a}	31.95^{a}	30.90^{a}	33.51^{a}	32.33^{a}	31.70^{a}	31.08^{a}	30.58^{a}	38.98^{c}
6	33.08^{ba}	33.23^{a}	31.48^{ba}	31.93^{a}	30.96^{a}	31.96^{ba}	32.58^{a}	31.76^{a}	31.31^{a}	30.78^{a}	39.61^{a}
Animal											
1	32.65^{a}	32.08^{a}	31.15^{a}	30.56^{a}	29.76^{a}	31.85^{a}	31.58^{abc}	30.73^{b}	30.30^{a}	29.91^{a}	39.15^{a}
2	33.90^{a}	32.81^{a}	31.73^{a}	31.38^{a}	30.51^{a}	32.95^{a}	33.03^{a}	32.28^{a}	31.13^{a}	29.86^{a}	39.15^{a}
3	32.78^{a}	32.56^{a}	31.56^{a}	31.41^{a}	30.83^{a}	31.36^{a}	30.95^{c}	30.90^{b}	30.38^{a}	29.75^{a}	39.20^{a}
4	33.30^{a}	32.91^{a}	31.85^{a}	31.63^{a}	30.55^{a}	32.68^{a}	31.20^{bc}	31.06^{ba}	30.93^{a}	30.41^{a}	39.51^{a}
5	33.13^{a}	32.53^{a}	31.75^{a}	31.61^{a}	29.81^{a}	32.66^{a}	32.26^{abc}	31.45^{ba}	30.80^{a}	30.35^{a}	39.16^{a}
6	33.73^{a}	32.66^{a}	31.91^{a}	31.46^{a}	30.55^{a}	33.16^{a}	32.53^{ba}	31.30^{ba}	31.01^{a}	30.28^{a}	39.23^{a}

Different letters in the same column (< P 0.05). T1L to T5L - temperatures in parts of the left side of the scrotum; T1R to T5R - temperatures in parts of the right side of the scrotum; T1 - Spermatic Cord; T2 - Dorsal Third of the testis; T3 - middle third of the Testicle; T4 - Third Ventral of the testis; T5 - tail of the Epididymis.

High ambient temperatures are associated with reduced semen quality (COULTER et al., 1988). Significant differences (P<0.05) found in this study for WBGT, TA, TG and RH between collections

(Table 4) reveals climate variability among collections, influencing the rectal temperature (Table 3) and semen characteristics (Table 2). Anchieta et al. (2005) and Chacur et al. (2013) observed the negative effect of the rainy season, the hottest in Brazil on sperm motility, both in Zebu bulls, and in Europe kept in artificial insemination center.

There was significant difference (P<0.05) between collections to temperatures in the different surface points of the scrotum and the rectal temperature (Table 3). On the other hand, there was no difference (P>0.05) between animals for rectal temperature (Table 3), demonstrating similarity between the animals to maintain rectal temperature.

The positive correlation (P<0.05) between the temperature of the scrotum reveals the interdependence between the areas of the scrotum in testicular thermoregulation. There was no significance (P>0.05) between RT and the temperature of the scrotum. The means for scrotum surface temperature at different ambient temperatures were reported by Barros et al. (2009); in the dorsal and medial portions of the testes this temperature was elevated with increasing ambient temperature.

Table 4. Mean values, standard deviation and variance analysis of climate data obtained at the trial location by globe thermometer.

	WBGT[1]	TA[2]	TG[3]	RH[4]
Mean	23.14± 2.18	27.04± 2.95	27.36± 2.86	60.50±10.02
Minimum	18.40	22	22.10	49.20
Maximum	25.80	31.9	31.70	85.10
Collection				
1	20.42[c]	24.73[b]	25.86[b]	57.32[cb]
2	20.48[c]	23.98[b]	24.83[b]	56.13[cb]
3	22.85[b]	24.56[b]	24.68[b]	80.78[a]
4	24.37[a]	29.91[a]	29.52[a]	58.23[cb]
5	24.81[a]	29.10[a]	29.48[a]	59.15[b]
6	25.41[a]	30.80[a]	30.85[a]	52.56[c]
Animal				
1	22.40[a]	25.80[b]	26.16[a]	64.0[a]
2	23.03[a]	27.23[ba]	27.40[a]	59.58[a]
3	23.30[a]	27.43[ba]	27.61[a]	59.71[a]
4	23.35[a]	27.61[ba]	27.75[a]	60.35[a]
5	23.46[a]	27.78[a]	27.75[a]	59.03[a]
6	23.31[a]	26.41[ba]	27.48[a]	60.30[a]

Different letters in the same column (< P 0.05). [1] wet-bulb globe temperature (°C); [2] dry-bulb temperature (°C); [3] globe thermometer temperature (°C); [4] Relative humidity (%).

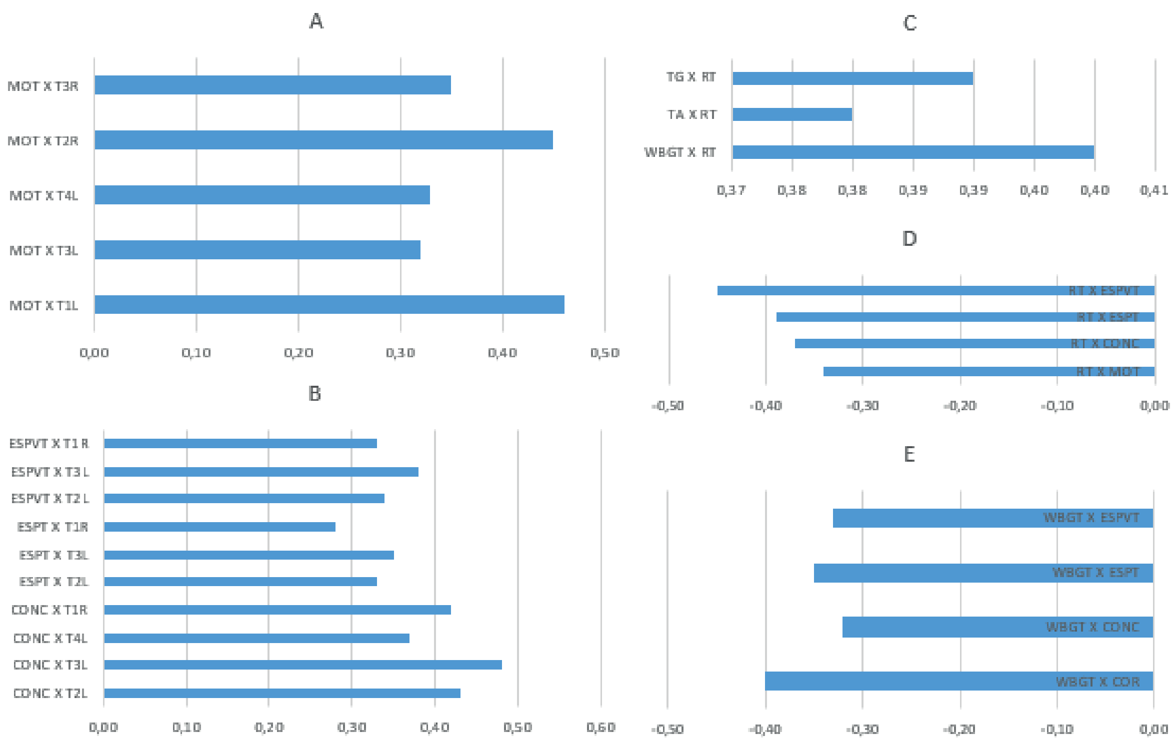
There was a positive correlation between semen motility and the temperature of the scrotum surface (Figure 1A, Table 5), similar to reporting Kastelic et al. (2001) where the increase in scrotal temperature influenced the improvement of sperm motility. There was a positive correlation (P<0.05) between sperm concentration, total sperm and total viable sperm and scrotal temperatures (Figure 1B, Table 5). Kastelic et al. (1995), in a study of *Bos taurus* bulls, reported that a moderate increase in testicular temperature on bulls can cause a marked reduction in sperm production and decrease the semen concentration.

A positive correlation (P<0.05) was observed between climatic factors WBGT, TA and TG compared to rectal temperature (Figure 1C, Table 5). Increased ambient temperature raises the rectal temperature. Extrinsic factors can influence the variation in the rectal temperature: time of day, ingestion of food and water, nutritional status, ambient temperature, density, shading, wind speed, season, exercise and sunlight (CARVALHO et al., 1995).

There was a negative correlation (P<0.05) between rectal temperature and MOT, CONC, ESPT and ESPVT (Figure 1D, Table 5), showing that the elevated rectal temperature results in decreased semen quality, probably due to heat stress with increased cortisol, corroborating the study by Horn et al. (1999).

In this study, statistically significant differences (P<0.05) were reported for different temperatures in the scrotum surface between collections (Table 3). Chacur et al. (2014) reported differences (P<0.05) in lower and total sperm defects in Nellore bulls in different seasons. Cook et al. (1994) observed that the increase in scrotal temperature is associated with the rise of sperm defects, while Lunstra and Coulter (1997) reported that there was a positive correlation between the temperature of the scrotum and tail defects of sperm.

Figure 1. Correlations between scrotal temperatures, rectal temperature, qualitative and quantitative characteristics of semen, and climate data.



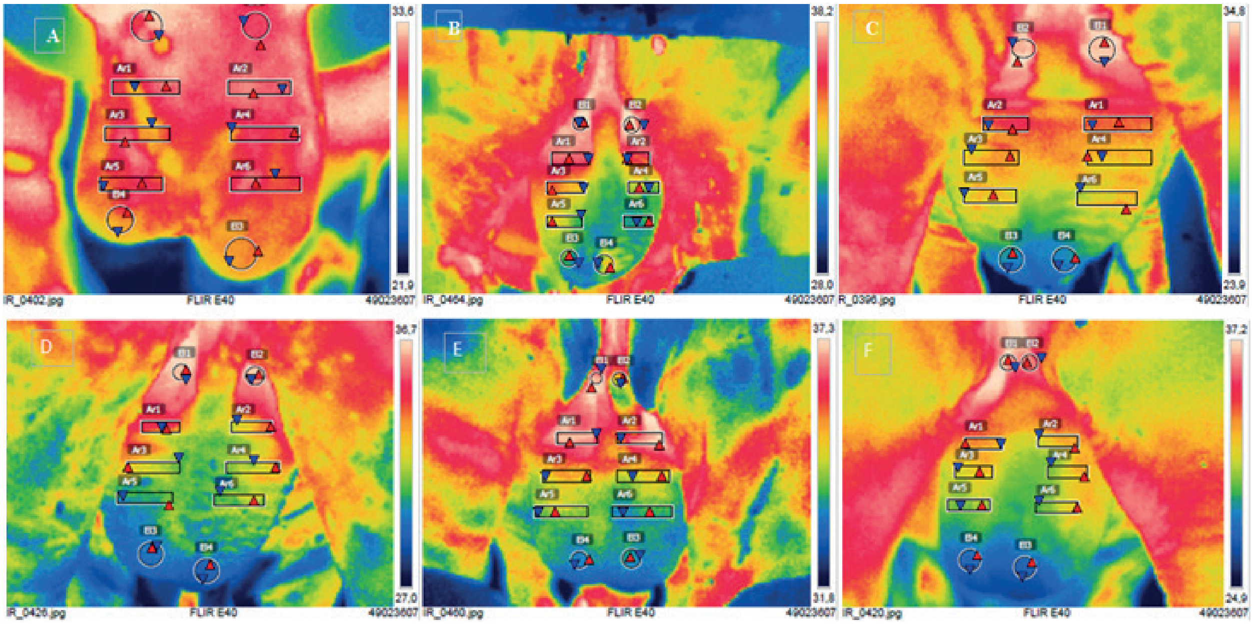
T1L to T5L - Temperatures in parts of the left side of the scrotum; T1R to T5R - temperatures in parts of the right side of the scrotum; T1 - Spermatic Cord; T2 - Dorsal Third of the testis; T3 - middle third of the Testicle; T4 - Third Ventral of the testis; T5 - tail of the Epididymis, RT - rectal temperature, MOT - motility, VIT - vitality, CONC - concentration, ESPVT - Sperm viable total, ESPT - Sperm total (x 10^7 sperm/ml), WBGT - wet-bulb globe temperature, TA - dry-bulb temperature, TG - globe thermometer temperature, RH - relative humidity.

The negative correlation ($P<0.05$) between WBGT, CONC, ESPT and ESPVT (Figure 1E, Table 5) shows that increasing the WBGT results in reduced concentration and sperm motility. This was similar to that described by Silva et al. (2009) between December and February in *Bos taurus*, which was related to reduced concentration and motility in heat stress suffered by animals in the period before semen collection.

Vandemark and Free (1970) described that the maintenance of scrotal skin temperature is affected by ambient temperature, humidity, body temperature, amount of heat lost by radiation from the scrotum, the animal's posture, anatomical variation in (scrotum with short spermatic funiculus, small scrotum), animal obesity degree (excess fat in the subcutaneous scrotum and spermatic cord) and integrity of the scrotum. According Kastelic et al. (1996) the ambient temperature had a great effect on the temperature of the lower region of the scrotum, a small effect on the temperature of the upper region and an intermediate effect on the temperature of the middle region of the scrotum.

In animals 2 and 6, the largest temperature differences were measured between the spermatic cord and dorsal third of the testicle (T1L-T2L; T1R-T2R), dorsal third of the testicle and middle third of the testicle (T2L-T3L; T2R-T3R), middle third of the testicle and third ventral testicle (T3L-T4L; T3R-T4R) and between the ventral third of testicle and epididymis tail (T4L-T5L; T4R-T5R); these were the animals with the best qualities of images 1 and 1.25, respectively. Animal 4 had the worst image quality among those studied, with a result of 3 (Figure 2), and was the one who showed the smallest temperature difference between the spermatic cord and dorsal third of the testicle (T1L-T2L; T1R-T2R), dorsal third of the testicle and middle third of the testicle (T2L-T3L; T2R-T3R), middle third of the testicle and third ventral testicle (T3L-T4L; T3R-T4R), between the ventral third of testicle and epididymis tail (T4L-T5L; T4R-T5R) and between the rectal temperature and the middle third of the testicle (RT-T3L; RT-T3R) (Table 6). This showed that the pattern of thermographic images changes with temperature differences between the points of the scrotum, having the best images in animals with the biggest differences and the worst standard of image was obtained between animals with smaller temperature differences between the points of the scrotum. Infrared thermograms of the scrotum of bulls with apparently normal scrotal/testicular thermoregulation feature of left-to-right symmetry but a decrease in temperature that varied from 4 to 6°C from the apex of the testicles toward the bottom of the scrotum. Thermographic images (thermograms) with temperature areas with a lack of symmetry from left to right, and localized areas with temperature increases in hot spots occur when abnormal testes or epididymides thermoregulation occurs, resulting in lower seminal quality in bulls (COULTER et al., 1988).

Figure 2. Images of infrared thermography of different classifications for image quality.



A - Thermographic image, animal 4, with classification 3 (Unsatisfactory). B - Animal 5, classification 2 (questionable). C - Animal 2, classification 1 (Satisfactory). D - Animal 1, classification 2 (questionable). E - Animal 3, classification 2 (questionable). F - Animal 6, classification 1 (Satisfactory).

Table 5. Correlations (л) and significance level (P) between the temperature of the scrotum, rectal temperature, qualitative and quantitative characteristics of semen, and climate data.

Continue ...

Correlations	Л	P
MOT X T1L	0.46	0.0045
MOT X T3L	0.32	0.05
MOT X T4L	0.33	0.05
MOT X T2R	0.45	0.0065
MOT X T3R	0.35	0.0368
CONC X T2L	0.43	0.0098
CONC X T3L	0.48	0.0029
CONC X T4L	0.37	0.0282
CONC X T1R	0.42	0.0100
ESPT X T2L	0.33	0.05
ESPT X T3L	0.35	0.0350
ESPT X T1R	0.28	0.0097
ESPVT X T2L	0.34	0.0398
ESPVT X T3L	0.38	0.0207
ESPVT X T1R	0.33	0.0479
WBGT X COR	-0.4	0.0158
WBGT X CONC	-0.32	0.05
WBGT X ESPT	-0.35	0.0382
WBGT X ESPVT	-0.33	0.0492
WBGT X RT	0.4	0.0148

... Continuation

TA X RT	0.38	0.0211
TG X RT	0.39	0.0180
RH X VIT	-0.39	0.0192
RT X MOT	-0.34	0.0410
RT X CONC	-0.37	0.0247
RT X ESPT	-0.39	0.0189
RT X ESPVT	-0.45	0.0060

T1L to T5L - Temperatures in parts of the left side of the scrotum; T1R to T5R - Temperatures in parts of the right side of the scrotum; T1 - Spermatic Cord; T2 - Dorsal Third of the testis; T3 - middle third of the Testicle; T4 - Third Ventral of the testis; T5 - tail of the Epididymis, RT - rectal temperature, MOT - motility, VIT - vitality, CONC - concentration, ESPVT - Sperm viable total, ESPT - Sperm total (x 10^7 sperm/ml), WBGT - wet-bulb globe temperature, TA - dry-bulb temperature, TG - globe thermometer temperature, RH - relative humidity.

Table 6. Differences between the temperature of the scrotum and image quality score by infrared thermography.

Animal	T1L-T2L	T1L-T3L	T1L-T4L	T1L-T5L	TR-T3L	T1R-T2R	T1R-T3R	T1R-T4R	T1R-T5R	RT-T3R	Quality
1	0.50	1.41	1.99	2.79	8.00	0.27	1.12	1.55	1.93	8.42	1.50
2	1.02	2.11	2.46	3.32	7.42	0.80	1.67	2.82	3.08	6.87	1.00
3	0.34	1.21	1.20	1.95	7.63	0.42	0.47	0.98	1.62	8.30	2.25
4	0.24	0.49	0.71	1.79	5.67	0.48	0.42	0.75	1.27	5.45	3.00
5	0.60	1.38	1.52	3.02	7.42	0.40	1.22	1.87	2.32	7.72	1.75
6	1.07	1.82	2.27	3.18	7.32	0.63	1.87	2.15	2.88	7.93	1.25

T1L to T5L - temperatures in parts of the left side of the scrotum; T1R to T5R - temperatures in parts of the right side of the scrotum; T1 - Spermatic Cord; T2 - Dorsal Third of the testis; T3 - middle third of the Testicle; T4 - Third Ventral of the testis; T5 - tail of the Epididymis; RT - rectal temperature; (-) minus sign between temperatures. Image quality: 1 (satisfactory), 2 (questionable) and 3 (unsatisfactory).

Conclusions

It is concluded that the surface temperature of the scrotum positively influences the motility and sperm concentration. The relative humidity negatively influences the force of the sperm. The infrared thermography is recommended for the additional examination in the reproductive evaluation of bulls.

References

ANCHIETA, M. C.; VALE FILHO, V. R.; COLOSIMO, E. Descarte e congelabilidade do sêmen de touros de raças zebuínas e taurinas em central de inseminação artificial no Brasil. *Arquivo Brasileiro de Medicina Vetrinária e Zootecnia,* Belo Horizonte, v. 57, n. 2, p. 196-204, 2005.

BARROS, C. M. Q.; OBA, E.; SIQUEIRA, J. B.; LEAL, L. S.; KASTELIC, J. P. Efeito da temperatura ambiente sobre as temperaturas escrotal, intratesticular, intravascular e fluxo sanguíneo testicular de touros. *Veterinária e Zootecnia,* Botucatu, v. 16, n. 2, p. 354-362, 2009.

BRITO, L. F.; BARTH, A. D.; WILDE, R. E.; KASTELIC, J. P. Testicular vascular cone development and its association with scrotal temperature, semen quality, and sperm production in beef bulls. *Animal Reproduction Science*, Philadelphia, v. 134, n. 3-4, p. 135-140, 2012.

BRITO, L. F.; SILVA, A. E.; BARBOSA, R. T.; KASTELIC, J. P. 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*, Philadelphia, v. 61, n. 3, 511-528, 2004.

CARVALHO, F. A.; LAMMOGLIA, M. A.; SIMÕES, M. J. Breed effects thermoregulation and epithelial morphology in imported and native cattle subjected to heat stress. *Journal of Animal Science,* Champaign, v. 73, n. 12, p. 3570-3573, 1995.

CHACUR, M. G. M.; MIZUSAKI, K. T.; GABRIEL FILHO, L. R. A.; OBA, E.; RAMOS. A. A. Seasonal Effects on Semen and Testosterone in Zebu and Taurine Bulls. *Acta Scientiae Veterinariae*, Porto Alegre, v. 41, n. 1, p. 1110-1114, 2013.

CHACUR, M. G. M.; REIS, J. D. A.; TAVARES, L. S.; SANCHES, K.; GUABERTO, L.; ALVES, V. C.; OBA, E.; RAMOS. A. A. Influência das épocas do ano na morfometria testicular e epididimária, características do sêmen e proteínas do sêmen em SDS-PAGE em zebus e taurinos. *Acta Scientiae Veterinariae*, Porto Alegre, v. 42, n. 1, p. 1174-1180, 2014.

COOK, R. B.; COULTER, G. H.; KASTELIC J. P. The testicular vascular cone, scrotal thermoregulation and their relationship to sperm production and seminal quality in beef bulls. *Theriogenology*, Philadelphia, v. 41, n. 3, p. 653-671, 1994.

COULTER, G. H.; SERENGER, P. L.; BAILEY, D. R. C. Relationship of scrotal surface temperature measured by infrared thermography to subcutaneous and deep testicular temperature in the ram. *Journal of Reproduction and Fertility,* Oxford, v. 84, n. 2, p. 417-423, 1988.

GABALDI, S. H.; WOLF, A. A importância da termorregulação testicular na qualidade do sêmen em touros. *Ciência Agrária e Saúde*, Andradina, v. 2, n. 2, p. 66-70, 2002.

GUASTI, P. N. *Manual para exame andrológico e avaliação de sêmen animal.* 3. ed. Belo Horizonte: CBRA, 2013. 93 p.

HORN, M. M.; MORAES, J. C. F.; GALINA, C. S. Qualidade de sêmen de touros Aberdeen Angus e Ibagé frente à degeneração testicular experimental. *Archivos Latinoamericanos de Producción Animal*, Maracaibo, v. 5, p. 356-359, 1997. Suplemento 1.

HORN, M.M.; MORAES, J.C.F.; GALINA, C.S. Qualidade do sêmen de touros das raças Aberdeen Angus e Brangus-Ibage em frente á degeneração testicular experimental induzida por dexametaxona. *Ciência Rural*, Santa Maria, v. 29, n. 3, p. 523-526, 1999.

KASTELIC, J. P. Understanding and evaluating bovine testes. *Theriogenology*, Philadelphia, v. 81, n. 1, p. 18-23, 2014.

KASTELIC, J. P.; COOK, R. B.; COULTER, G. H.; WALLINS, G. L.; ENTZ, T. Environmental factors affecting measurement of bovine scrotal surface temperature with infrared thermography. *Animal Reproduction Science,* Philadelphia, v. 41, n. 3-4, p. 153-159, 1996.

KASTELIC, J. P.; COOK, R. B.; PIERSON, R. A.; COULTER, G. H. Relationships among scrotal and testicular characteristics, sperm production, and seminal quality in 129 beef bulls. *Canadian Journal of Veterinary Research,* Ottawa, v. 65, n. 2, p. 111-115, 2001.

KASTELIC, J. P.; COULTER, G. H.; COOK, R. B. Scrotal surface, subcutaneous, intra-testicular and intra-epididymal temperatures in bulls. *Theriogenology*, Philadelphia, v. 44, n. 1, p. 147-152, 1995.

LEZIER, D. H. *Avaliação da biometria testicular, concentração plasmática de hormônios e minerais em bovinos Nelore variedade mocha dos 12 aos 24 meses de idade.* 2004. Tese (Doutorado em Medicina Veterinária) - Programa de Pós-Graduação em Reprodução Animal. Universidade Estadual Paulista, Botucatu.

LUNSTRA, D. D.; COULTER, G. H. Relationship between infrared temperature patterns and natural-mating fertility in beef bulls. *Journal of Animal Science,* Champaign, v. 75, n. 3, p. 767-774, 1997.

PUROHIT, R. C.; HUDSON, R. S.; RIDDELL, M. G.; CARSON, R. L.; WOLFE, D. F.; WALKER, D. F. Thermography of the bovine scrotum. *American Journal of Veterinary Research*, Schaumburg, v. 46, n. 4, p. 2388-2392, 1985.

STATISTICAL ANALYSIS SYSTEM INSTITUTE - SAS. SAS/STAT User`s Guide. SAS Inst. Inc. Cary. NC: SAS Inst. Inc., 2005. 214 p.

SETCHELL, B. P. The effects of heat on the testes of mammals. *Animal Reproduction,* Belo Horizonte, v. 3, n. 2, p. 81-91, 2006.

SILVA, A. R.; FERRAUDO, A. S.; RODRIGUES, L. H.; COSTA, M. Z.; LIMA, V. F. M. H. Efeito da idade e do período de colheita de sêmen sobre as características físicas e morfológicas do sêmen de bovinos europeus e zebus. *Revista Brasileira de Zootecnia*, Brasília, v. 38, n. 7, p. 1218-1222, 2009.

VANDEMARK, N. L.; FREE, M. J. Temperature effects. In: JOHNSON, A. D.; GOMES, W. R.; VANDEMARK, N. L. (Ed.). *The testis*. New York: Academic Press, 1970. p. 233-312.

WOLFE, D. F.; HUDSON, R. S.; CARSON, R. L.; PUROHIT, R. C. Effect of unilateral orchiectomy on semen quality in bulls. *Journal of American Veterinary Medical Association*, Schaumburg, v. 186, n. 3, p. 1291-1293, 1985.