Journal of Thermal Biology 85 (2019) 102407

Contents lists available at ScienceDirect

Journal of Thermal Biology

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

Reproductive characteristics of bulls from two breed compositions and their correlations with infrared thermography

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ARTICLE INFO

Keywords:
Andrological examination
Crossbreeding
Dairy cattle
Girolando
Reproduction
Semen
Thermoregulation

ABSTRACT

The objective was to evaluate reproductive characteristics of crossbred Girolando (Gyr x Holstein) bulls from two breed compositions and correlate these results with infrared thermography data. Evaluations were performed considering sperm motility, vigor and morphology; scrotal circumference; body morphology and temperament. Infrared thermography was performed to determine surface temperatures of ocular and scrotal areas. Thermoregulation capacity was assessed by differences between air and rectal temperatures, air and maximum temperatures in ocular and scrotal areas, and dorsal and ventral lines of the scrotum. Data analysis was performed using a linear mixed model (breed composition as fixed effect and year of evaluation as random effect). Spearman correlation coefficient was used to associate thermography and reproductive data. Girolando 3/4 Holstein bulls had higher ($P \leq 0.001$) scrotal circumference and higher average body morphology and temperament, whereas 5/8 Holstein bulls had a higher ($P < 0.001$) percentage of major-type and total sperm defects. Girolando 3/4 Holstein bulls had scrotal temperatures 0.8 °C higher ($P < 0.001$) and 5/8 Holstein bulls had 9.8% and 10.6% higher differences on "rectal – scrotal area" and "ocular area – scrotal area" temperatures, respectively. Ocular area temperatures had negative correlations ($P \leq 0.1$) with sperm motility and vigor, and positive correlations ($P < 0.001$) with minor-type and total sperm defects. Ventral line scrotal temperatures had positive correlations ($P < 0.001$) with minor-type and total sperm defects. Girolando 3/4 Holstein bulls were found to be superior to 5/8 Holstein bulls for reproductive characteristics. Under non-stressing climatic conditions, semen characteristics of Girolando bulls were more influenced by breed composition than by the capacity for scrotal thermoregulation. Correlations between semen quality and scrotal temperatures can aid in the identification of bulls for breeding, particularly when a large number of animals are in the tests.

1. Introduction

Various breed compositions encompass the dairy production systems in Brazil, which is the world's fifth largest producer and consumer of bovine milk and its derivatives (USDA-NASS, 2017). The use of animals adapted to the tropical environment increases farmers' yields (Paranhos da Costa et al., 2015) and, because of their greater adaptability to the tropics and productive potential, the crossbreeding between Holstein and Gyr cattle, called Girolando, contributes to 80% of the milk produced in Brazil (FAO, 2017), with an estimated population close to 10 million Girolando animals (Oliveira Júnior et al., 2017).

Girolando breeding program has adopted progeny tests and encouraged the commercialization of semen. In 2015, 641,360 semen doses from Girolando bulls were sold, a value 70% higher than that of 2013. Mating with 3/4 Holstein and 5/8 Holstein bulls is important for

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https://doi.org/10.1016/j.jtherbio.2019.102407
Received 26 April 2018; Received in revised form 15 August 2019; Accepted 25 August 2019
Available online 26 August 2019
0306-4565/

obtaining the main breed compositions of Girolando (Silva et al., 2016). The proportion of taurine genes in breed compositions of Girolando (75% or 62.5% of Holstein genes for Girolando 3/4 and 5/8, respectively) may represent one of the factors responsible for reproductive (Godfrey et al., 1990) and productive (Silva et al., 2016) differences in progenies of bulls.

The fact that a single bull reproduces with several cows denotes the greater importance of bulls to imprint genetic traits of interest in herds (Franco et al., 2006). In order to increase reproductive capacity, bulls must produce morphologically normal and large numbers of sperm. For effective spermatogenesis, testes temperature must be between 2 and 6 °C below body temperature (Kastelic, 2014a). According to Brito et al. (2004), effects of testicular temperature elevation have direct reflexes in the decrease of sperm production and semen quality, as well as in bull fertility, resulting in economic loss that implies a lower pregnancy rate.

Infrared thermography is an indirect non-invasive method that can aid in the measurement of scrotal surface temperature in bulls (Brito et al., 2002). It is a promising alternative method to evaluate the capacity for testicular thermoregulation in bulls raised under high environmental temperatures and, consequently, can be used to identify animals adapted for breeding. However, there is still a lack of precise information to associate thermography with physiological or pathological phenomena in animal reproduction.

Based on the hypotheses: *i*) breed composition of Girolando bulls influences reproductive characteristics and *ii*) it is possible to use infrared thermography as a fast-track non-invasive auxiliary method of assessing the reproductive performance of bulls, the objective of this study was to evaluate the influence of breed composition of Girolando bulls on andrological and semen characteristics and to correlate these with infrared thermography data.

2. Material and methods

2.1. Experimental area

The study was conducted between February and April 2016 and 2017, involving animals of the Girolando testing program at the Federal Institute of Education, Science and Technology of Triângulo Mineiro (19°39′17.9″S 47°57′41.9″W), in Uberaba, MG, Brazil. All procedures involving animals were approved by the Embrapa Dairy Cattle Ethics Committee on Animal Use (protocol nº 2400161017).

Data of temperature and relative humidity were collected 9.2 km from where bulls were housed from the automatic meteorological station OMM:83577 located at EPAMIG (Agricultural Research Company of Minas Gerais) in Uberaba, MG (19°44′13.7″S 47°56′59.1″W), and were provided by the Brazilian National Institute of Meteorology (INMET, 2017). The minimum, average and maximum air temperatures (°C) and relative humidity (RH, %) for 2016 and 2017 were 14.6; 25.7 and 33.6 °C and 27.0%; 70.2% and 99.0%; and 19.2; 25.6 and 33.4 °C and 39.0%; 69.0% and 96.0%, respectively.

2.2. Management and selection of animals

The bulls belonged to herds associated to the Girolando breeding program and were kept on *Brachiaria* pastures (*Urochloa brizantha* cv. MG-5 Vitória) during the rainy season (from November to March). During the dry season (from April to October), bulls were supplemented with corn silage and concentrate composed of corn meal, soybean meal and mineral-vitamin mix at a 70:30 forage to concentrate ratio. All bulls were kept under the same feeding and management conditions, with free access to mineral supplement (PSAI Extra Fator P, Premix, Ribeirão Preto, SP, Brazil) and fresh water.

A total of seventy 3/4 Holstein bulls and fifty-eight 5/8 Holstein bulls were evaluated over two consecutive years (mean age 28.1 ± 6.61 m, mean body weight 579 ± 125 kg).

2.3. Data collection

2.3.1. Reproductive parameters

Scrotal circumference (SC) was evaluated according to Kastelic and Thundathil (2008), three times during each year. Two consecutive measurements were performed using a specific tape for measuring scrotal perimeter with a millimeter scale (Walmur Instrumentos Veterinários Ltda., Porto Alegre, RS, Brazil).

Semen analyses were performed according to procedures described by the Brazilian College of Animal Reproduction (CBRA, 2013). Samples were obtained by electrical stimulation using the Autojac-Neovet device (Autojac®, Neovet, Campinas, SP, Brazil). Monthly collections were carried out during the three-month test period of each year. Immediately after each collection, microscopic analysis of the semen was performed. Sperm individual motility (MOT) was examined with a bright-field microscope (LEICA Microsystems, Buffalo, NY, USA) at 400× magnification using ~10 μL aliquot of semen placed on a heated glass slide (37 °C) and covered with a coverslip. The MOT index was evaluated as the percentage of motile sperm (0–100%) at intervals of 5%. Sperm vigor (VIG) was evaluated using a 0 to 5 scale based on progressive sperm movement, where 0 = none, 1 = very weak, 2 = weak, 3 = intermediate, 4 = strong and 5 = very strong. These evaluations were done by visualizing at least three fields and the result expressed by the average of its results. An aliquot of semen was also diluted in buffered saline-formaldehyde solution (1:10) for evaluation of sperm morphology. These analyzes were performed under magnification 1,000× using a phase-contrast microscope (Meiji Techno, Chikumazawa, Japan). A wet chamber was prepared for reading (aliquot of 10 μL of semen between glass slide and coverslip), counting 200 cells per sample. Major-type sperm defects (MAJD) were considered as: acrosomal defect, abnormal head, folded head, dorsal cytoplasmic droplet, midpiece defect, accessory tail, and folded tail. Minor-type sperm defects (MIND) included ventral cytoplasmic droplet, abaxial insertion, folded tail, and large heads. Total defects (TOTD) were considered in 200 spermatozoa of each animal and sperm classification was based on the criteria adopted by Barth (2007).

2.3.2. Body morphology

Body morphology (MORP) test evaluated: body conformation and capacity (20%) - considering body depth, masculinity, body length, a strong back/loin, height at rump and breeding characteristics; dairy force (20%) - a combination of dairyness, chest girth and breast width; rear feet and legs (25%) - evaluating the legs seen from behind and the side, observing angles, hooves and rear feet; rump (10%) - involving its angle, width and length; and the whole reproductive tract (25%) - encompassing the scrotum and navel-sheath-foreskin combination. The average value from three subjective evaluators was considered, as described by Menezes and Ledic (2010).

2.3.3. Temperament

Temperament index (TEMP) was assessed adopting the procedures described by Paranhos da Costa et al. (2015), which involved evaluations of time required to enter the squeeze chute, reactivity in the squeeze chute, exit velocity and temperament score.

2.3.4. Infrared thermography

A FLIR T420 portable device (FLIR Systems Inc., Wilsonville, OR, USA) was used and the anatomical regions evaluated were: ocular (Fig. 1) and scrotal (Fig. 2). Cameras were positioned perpendicular (right side) to the bulls, to obtain a thermographic image in the ocular area, and behind the bulls focusing on the caudal surface of the scrotum to capture an image in the scrotal area. Distance between the thermograph and the anatomical region was standardized at ~1 m, reflectance of 20 °C, and emissivity value of 0.98, as recommended for biological tissues (Menegassi et al., 2015; Stewart et al., 2017). Files were processed and interpreted using the FLIR Tools 5.6 software (FLIR Systems,

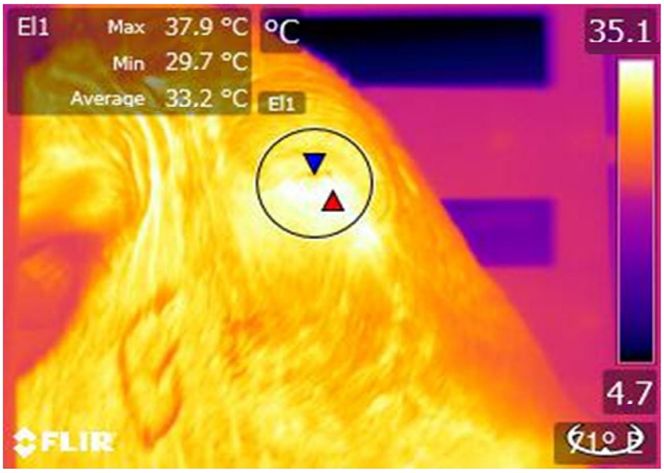

Fig. 1. Thermographic image of ocular area.

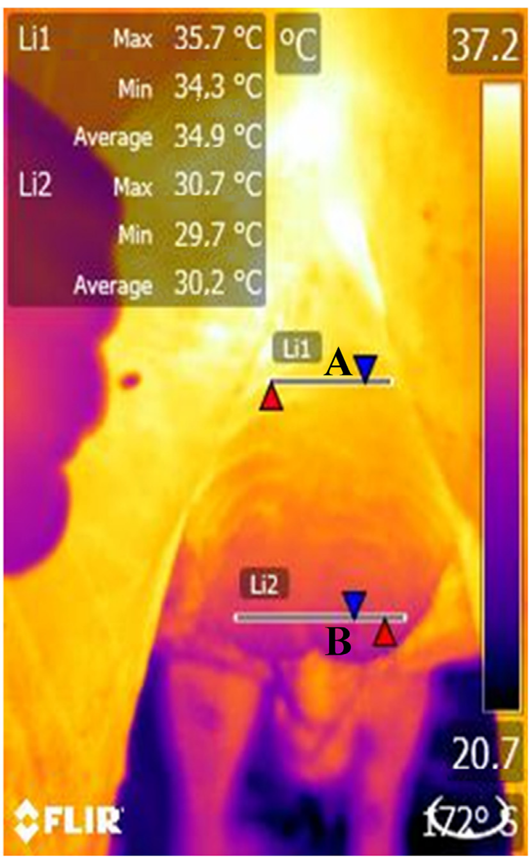

Fig. 2. Thermographic image of scrotal area, (A) - L1 (Line 1 representing the dorsal portion) and (B) - L2 (Line 2 representing the ventral portion).

Wilsonville, OR, USA). To obtain the maximum (MAX), minimum (MIN) and average (AVE) temperatures at the dorsal (Line 1, L1) and ventral scrotal portions (Line 2, L2), software was adjusted to the iron color pallet; the circle measurement tool was standardized at 57 × 57 mm for the ocular area (E), whereas the line measurement tool was used for the scrotal area to obtain (in pixels) the mean values of the maximum, minimum and mean values for the delimited regions in the dorsal (L1) and ventral (L2) portions of the scrotum.

Additionally, during the data collection of thermographic images, the bulls were weighed with an electronic scale (Valfrant®, VF - Premium model, Votuporanga, SP, Brazil) and the average of three weights was used.

2.4. Calculations

Temperature (^{0}C) and relative humidity (RH, %) data were evaluated during collection of thermographic images using a pinless Moisture Psychrometer with a built-in infrared thermometer and Bluetooth (FLIR Commercial Systems, Extech Division, model MO297, MeterLink™, Nashua, NH, USA). These data were used to calculate the Temperature and Humidity Index (THI) according to equation proposed by Thom and Thom (1958):

$$THI = 0.8\,x\ °C + RH\%x\ (°C - 14.4) + 46.4$$

Rectal temperature of all bulls was measured with a digital thermometer (Ombo Electronics, iColor®, G-Tech model, Shenzhen, China) in the range of 32.0–43.9 °C, immediately after obtaining a thermographic image. To evaluate the capacity for thermoregulation, temperature differences between ambient and rectal temperatures, and ambient and maximum temperatures of ocular and scrotal areas were calculated.

2.5. Statistical analysis

Effect of breed composition was evaluated using a mixed linear model, considering breed composition as a fixed effect and year of evaluation as a random effect. The GLIMMIX procedure was used in SAS statistical software (SAS Institute, Cary, NC, USA, version 9.4) according to the statistical model:

$$Yij = \mu + Gi + Aj + \varepsilon ij$$

where, Y_{ij} is the dependent variable, μ is the overall mean, G_i is the fixed effect of breed composition, A_j is the random effect of year of evaluation and ε_{ij} is the random error. Significance (F test) was considered adopting α equal to 0.05.

Data were evaluated using the Kolmogorov-Smirnov Normality Test (KS) to test its distribution before the correlation study. Reproductive parameters did not follow normal distribution. Thus, Spearman's rank correlation coefficient was used to associate thermography data with reproductive parameters. The CORR procedure in SAS (SAS Institute, Cary, NC, USA) was used. Spearman's rank correlation coefficient (ρ) was calculated by adopting α equal to 0.05.

3. Results

Age and body weight were similar ($P = 0.3$) between 5/8 and 3/4 Holstein bulls, and overall averages were 923 days and 598.5 kg, respectively. Girolando 3/4 Holstein bulls had higher averages ($P < 0.001$) for MORP, TEMP, and SC. There were no differences ($P > 0.05$) for VIG and MOT in relation to breed composition. Girolando 5/8 Holstein bulls had a higher percentage ($P < 0.001$) of MAJD and TOTD. There was no difference between the two breed compositions for MIND ($P = 0.064$) (Table 1).

There was no variation ($P = 0.76$) in ambient temperature during the collection of thermography data for different breed compositions. Although a change of only 0.1 °C was verified, the average rectal temperature was lower ($P < 0.001$) for 3/4 Holstein animals when compared to 5/8 Holstein. Average temperature of the ocular area (33.6 °C) did not differ ($P = 0.6$) between breed compositions. Girolando 3/4 Holstein bulls had scrotal temperatures 0.8 °C higher ($P < 0.001$) in relation to 5/8 Holstein. Temperature differences for “air - rectal” and “air – ocular area” did not show variation ($P > 0.4$) in relation to breed composition, although the difference of temperature for “air – scrotal area” ($P < 0.001$) was 10.7% higher for 5/8 Holstein in comparison to 3/4 Holstein. Comparing 5/8 Holstein bulls with the 3/4 Holstein bulls, variations of 9.8% and 15.6% were observed for differences of temperatures “rectal – scrotal area” and “ocular area – scrotal area”, respectively ($P < 0.001$) (Table 2).

Temperature and Humidity Index (THI) had a positive correlation

Table 1
Averages of age (days), body weight, body morphology, temperament, and semen characteristics in Girolando bulls according to breed composition.

Item	Breed composition		SEM [i]	*P*-value
	5/8 HOL	3/4 HOL		
Age (days)	931	914	34.3	0.359
Body weight, kg	601	596	21.7	0.997
MORP, % [a]	77.2	79.8	0.49	< 0.001
TEMP, % [b]	65.4	72.8	3.9	< 0.001
SC, cm [c]	35.2	36.7	0.26	< 0.001
VIG, (0–5) [d]	3.55	3.55	0.27	0.523
MOT, (0–100) [e]	65.6	66.8	4.22	0.926
MAJD, (0–100) [f]	4.36	3.20	0.23	< 0.001
MIND, (0–100) [g]	8.92	7.47	1.37	0.064
TOTD, (0–100) [h]	14.4	10.6	0.88	< 0.001

[a] MORP (Body morphology);
[b] TEMP (Temperament index);
[c] SC (scrotal circumference);
[d] VIG (Sperm Vigor);
[e] MOT (Sperm Individual Motility);
[f] MAJD (Major Sperm Defects);
[g] MIND (Minor Sperm Defects);
[h] TOTD (Total Sperm Defects);
[i] SEM (standard error of the mean).

Table 2
Air, rectal, ocular and scrotal areas temperatures and their differences in Girolando bulls according to breed composition.

	Breed composition		SEM [c]	*P*-value
	5/8 HOL	3/4 HOL		
Temperatures, °C [a]				
Air	21.2	21.1	4.02	0.755
Rectal	39.0	38.9	0.03	< 0.001
Ocular area	33.6	33.6	1.64	0.637
Scrotal area	28.8	29.6	1.69	< 0.001
Δ, °C [b]			SED [d]	
Air – rectal	−17.8	−17.7	4.03	0.664
Air – ocular area	−12.4	−12.5	2.39	0.419
Air – scrotal area	−7.55	−8.46	2.33	< 0.001
Rectal – scrotal area	10.29	9.28	1.69	< 0.001
Ocular area – scrotal area	4.80	4.05	0.12	< 0.001
Rectal - ocular area	5.47	5.24	1.64	0.059

[a] T° C (temperature in degrees Celsius) of the air, rectum, ocular and scrotal areas;
[b] Δ (temperature differences);
[c] SEM (standard error of the mean);
[d] SED (standard error of the difference).

($P < 0.001$) with MIND and TOTD, but this same index correlated negatively ($P < 0.001$) with MOT and VIG. Rectal temperature (RECT) had a positive correlation ($P < 0.05$) with MAJD, MIND and TOTD. Maximum (EMAX), average (EAVE) and minimum (EMIN) temperatures of the ocular area had negative correlations ($P \leq 0.01$) with MOT and VIG, and positive correlations ($P < 0.001$) with MIND and TOTD. For temperatures at the dorsal portion (L1), the correlations were positive ($P \leq 0.03$) for MAJD and TOTD. Conversely, for temperatures at the ventral portion on the surface of the testes (L2), the correlations were positive ($P < 0.001$) with MIND and TOTD, and negative ($P < 0.001$) with VIG (Table 3).

For AIRT, RECT and THI, the correlations were positive ($P < 0.001$) with the thermographic temperatures of EAVE, L1AVE, L2AVE and negative ($P < 0.001$) with the difference of thermographic temperature between line 1 and line 2 on the scrotal surface (DIF L1-L2). On the other hand, the correlations of the three average thermographic temperatures were negative for RH ($P < 0.001$) (Table 4).

Ventral line (L2) temperatures on the scrotal surface had positive

Table 3
Spearman's rank correlation coefficients (ρ), significance value (*P*-value), and number of observations (N) for climatic condition, ocular area and rectal temperatures, and semen characteristics in Girolando bulls.

		Motility		Spermatic Morphology		
		MOT [m]	VIG [n]	MAJD [o]	MIND [p]	TOTD [q]
AIRT [a]	ρ	**−0.17**	**−0.18**	−0.04	**0.21**	**0.15**
	P-value	< 0.001	< 0.001	0.38	< 0.001	< 0.001
	N	384	384	384	384	384
THI [b]	ρ	**−0.16**	**−0.17**	−0.05	**0.22**	**0.28**
	P-value	< 0.001	< 0.001	0.37	< 0.001	< 0.001
	N	384	384	384	384	384
RECT [c]	ρ	−0.01	0.00	**0.14**	**0.11**	**0.13**
	P-value	0.89	0.91	< 0.001	0.02	0.02
	N	379	379	379	379	379
EMAX [d]	ρ	**−0.17**	**−0.19**	−0.05	**0.23**	**0.14**
	P-value	0.01	< 0.001	0.34	< 0.001	< 0.001
	N	382	382	382	382	382
EMIN [e]	ρ	**−0.24**	**−0.23**	0.00	**0.19**	**0.16**
	P-value	< 0.001	< 0.001	0.98	< 0.001	< 0.001
	N	380	380	380	380	380
EAVE [f]	ρ	**−0.22**	**−0.22**	−0.06	**0.21**	**0.15**
	P-value	< 0.001	< 0.001	0.26	< 0.001	< 0.001
	N	381	381	381	381	381
L1MAX [g]	ρ	0.03	−0.02	**−0.15**	−0.03	**−0.11**
	P-value	0.59	0.72	< 0.001	0.47	0.02
	N	381	381	381	381	381
L1MIN [h]	ρ	0.02	−0.03	**−0.12**	0.00	−0.04
	P-value	0.74	0.57	0.03	0.98	0.33
	N	380	380	380	380	380
L1AVE [i]	ρ	0.00	−0.05	**−0.15**	0.00	−0.06
	P-value	0.99	0.35	< 0.001	0.93	0.17
	N	380	380	380	380	380
L2MAX [j]	ρ	**−0.16**	**−0.18**	−0.09	**0.14**	**0.22**
	P-value	< 0.0001	< 0.001	0.09	< 0.001	< 0.001
	N	378	378	378	378	378
L2MIN [k]	ρ	−0.11	**−0.14**	−0.09	**0.12**	**0.21**
	P-value	0.03	< 0.001	0.09	< 0.001	< 0.001
	N	378	378	378	378	378
L2AVE [l]	ρ	**−0.16**	**−0.18**	−0.08	**0.14**	**0.24**
	P-value	< 0.001	< 0.001	0.13	< 0.001	< 0.001
	N	381	381	381	381	381

[a] AIRT (Air temperature);
[b] THI (Temperature and Humidity Index);
[c] RECT (Rectal temperature);
[d] EMAX.
[e] EMIN
[f] EAVE (Maximum, minimum and average ocular area temperature, respectively);
[g] L1MAX,
[h] L1MIN
[i] L1AVE (Maximum, minimum and average temperature at Line 1, respectively);
[j] L2MAX,
[k] L2MIN
[l] L2AVE (Maximum, minimum and average temperature at Line 2, respectively);
[m] MOT (Sperm Individual Motility);
[n] VIG (Sperm Vigor);
[o] MAJD (Major-type defects);
[p] MIND (Minor-type defects);
[q] TOTD (Total defects).

correlations ($P \leq 0.0002$) with the MIND and TOTD in the general and specific evaluation of the 3/4 Holstein group. However, the temperature difference between the dorsal and ventral lines on the scrotal surface (DIF L1-L2) had negative correlations ($P \leq 0.001$) with MIND and TOTD (Table 5) in the general and specific evaluations of the 3/4 Holstein group.

There was variation between breed compositions ($P < 0.05$) for superficial scrotal temperatures, both for the dorsal (L1) and the ventral

Table 4
Spearman's rank correlation coefficients (ρ), significance value (*P*-value), and number of observations (N) for climatic condition indicators, ocular area and rectal temperatures, and thermographic temperatures in Girolando bulls.

		EAVE[e]	L1AVE [f]	L2AVE [g]	DIF L1-L2[h]
AIRT[a]	ρ	**0.9148**	**0.3553**	**0.7843**	**−0.6513**
	P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	N	381	381	381	377
RH[b]	ρ	**−0.2119**	**−0.4281**	**−0.2153**	0.0265
	P-value	< 0.001	< 0.0001	< 0.0001	0.6083
	N	381	381	381	377
THI[c]	ρ	**0.9126**	**0.3497**	**0.7832**	**−0.6529**
	P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	N	381	381	381	377
RECT[d]	ρ	**0.2809**	**0.3370**	**0.2129**	**−0.0776**
	P-value	< 0.0001	< 0.0001	< 0.0001	< 0.0001
	N	375	375	375	372

[a] AIRT (Air temperature);
[b] RH (relative humidity);
[c] THI (Temperature and Humidity Index);
[d] RECT (Rectal Temperature);
[e] EAVE (average thermographic temperature of the ocular area);
[f] L1AVE (average thermographic temperature at Line 1);
[g] L2AVE (average thermographic temperature at Line 2)
[h] DIF L1-L2 (Difference between thermographic temperatures at Line 1 and Line 2).

lines (L2) to the scrotum. There was also divergence between the breed compositions ($P < 0.0001$) for temperature differences between the dorsal and ventral lines of the scrotum (DIF L1-L2) (Table 6).

4. Discussion

The similarity in age and weight of bulls as a function of breed composition indicated the equality of experimental conditions necessary to compare these groups regarding semen quality. According to Mathevon et al. (1998), age and weight of animals are related to sexual maturity in bulls, affecting the parameters used to qualify the semen.

Higher values for MORP and TEMP in 3/4 Holstein bulls compared to the 5/8 Holstein bulls can be attributed to greater percentage of Holstein in the breed composition of 3/4 Holstein bulls, which according to García-Ruiz et al. (2016), has been selected for over a hundred years for functional, morphological and temperament traits.

The hypothesis *i)* "breed composition of Girolando bulls influences reproductive characteristics" was not rejected. The SC was 4.1% higher in 3/4 Hostein bulls, which may also be associated with greater

Table 6
Maximum temperature at Line 1 (dorsal) and at Line 2 (ventral) of scrotum and their differences in Girolando bulls according to breed composition.

	Breed composition		SEM [d]	P-value
	5/8 HOL	3/4 HOL		
Temperatures, °C				
L1MAX[a]	35.89	36.15	0.09	**0.0403**
L2 MAX [b]	29.84	30.80	1.48	< **0.0001**
DIF L1-L2[c]	6.04	5.36	1.41	< **0.0001**

[a] L1MAX (Maximum temperature at Line 1);
[b] L2MAX (Maximum temperature at Line 2);
[c] DIF L1-L2 (Difference between thermographic temperatures at Line 1 and Line 2);
[d] SEM (standard error of the mean).

Holstein breed contribution. Godfrey et al. (1990) reported lower sperm production, semen quality (higher incidence of sperm defects), and scrotal circumference in zebu cattle when compared to taurine bulls. Additionally, as the bulls were evaluated at an average age of over 30 months, the age was not relevant to influencing the reproductive characteristics (see Brito et al., 2002), which supports the hypothesis that these differences are related to variation in breed composition of the bulls.

Although different values for RECT were verified for both breed compositions, they were very similar and, regardless of the group, these averages were consistent with the physiological temperature proposed by Du Preez (2000), from 38.0 to 39.3 °C. This fact demonstrated the comparable capacity for internal thermoregulation of the two genetic groups under the environmental conditions throughout the study, as the air temperature averages during thermographic evaluations were similar. Consequently, similar values were observed for temperature of ocular area and temperature differences between "air - rectal" and "air - ocular area" between breed compositions. On the other hand, "air – scrotal area" temperature difference was greater for 3/4 Holstein bulls (10.75%).

Schaefer et al. (2012) and Stewart et al. (2008) verified that infrared thermography of the ocular area had a positive correlation with core body temperature in cows. Muller (1982) described that the thermoneutral zone, a condition where there are no significant physiological changes or loss of energy to maintain core body temperature, can reach 25 °C for Holstein cattle or up to 29 °C for zebu animals. Therefore, it indicated that the thermographic evaluations of the present study were conducted in thermoneutral conditions that did not impair the

Table 5
Spearman's rank correlation coefficients (ρ), significance value (P-value), and number of observations (N) for sperm morphology and thermographic temperatures in Girolando bulls.

		5/8 HOL			3/4 HOL			General		
		MAJD[d]	MIND[e]	TOTD[f]	MAJD	MIND	TOTD	MAJD	MIND	TOTD
L1MAX[a]	P	−0.09	−0.06	−0.08	−0.09	−0.01	−0.07	−0.10	−0.05	−0.09
	P-value	0.26	0.41	0.31	0.18	0.87	0.34	0.06	0.38	0.09
	N	165	165	165	195	195	195	360	360	360
L2 MAX [b]	P	−0.10	0.12	0.06	0.10	**0.39***	**0.38**	−0.02	**0.25**	**0.20**
	P-value	0.19	0.12	0.43	0.16	< .0001	< .0001	0.76	< .0001	0.0002
	N	164	164	164	193	193	193	357	357	357
DIF L1-L2 [c]	P	0.04	**−0.18**	−0.13	−0.11	**−0.38**	**−0.39**	−0.02	**−0.28**	**−0.25**
	P-value	0.59	0.02	0.11	0.11	< .0001	< .0001	0.69	< .0001	< .0001
	N	163	163	163	193	193	193	356	356	356

[a] L1MAX (Maximum temperature at Line 1);
[b] L2MAX (Maximum temperature at Line 2);
[c] DIF L1-L2 (Difference between thermographic temperatures at Line 1 and Line 2);
[d] MAJD (Major-type sperm Defects);
[e] MIND (Minor-type sperm defects);
[f] TOTD (Total sperm defects).

performance of the bulls.

Although no episodes of thermal stress occurred during the thermography study, the lower temperature averages observed at the surface of scrotum and for the "air - scrotum" temperature difference and the higher average temperature differences for "rectal – scrotal area" and "ocular area – scrotal area" in 5/8 Holstein bulls indicated the greater capacity of this genetic group for cooling their scrotal surface relative to the 3/4 Holstein group. This condition possibly occurred due to the greater contribution of zebu genes in the composition of 5/8 Holstein. Turner (1980) justified the adaptability of zebu cattle to high temperatures to the larger skin surface area in relation to body size and to the greater number of sweat glands per surface area, conditions that favor heat dissipation and contribute to the reduction of body surface temperature.

Reduction in seminal quality is more pronounced in *Bos taurus taurus* and crossbred bulls compared to *Bos taurus indicus* when exposed to thermal stress (Brito et al., 2004). However, as the present study was conducted at average temperatures below the threshold needed to promote stress in Holstein, Gyr and their crosses, it is estimated that climatic conditions were not predominant to influence semen quality.

Thermography results indicated that, under extreme climatic conditions (high temperatures and/or humid environments), 5/8 Holstein bulls might have greater ability to maintain the physiological conditions. Consequently, they might have advantages as to semen quality in relation to 3/4 Holstein bulls. This fact is supported by studies including Johnston et al. (1963), who reported a decrease in semen quality caused by thermal stress in bulls, and by Kastelic (2014b), who verified that the increased difference between the body temperature (higher) and testicular temperature (lower) favored spermatogenesis. Brito et al. (2004) also reported that semen quality decreases more quickly in taurine bulls compared to zebu cattle when they are subjected to high temperatures and humid conditions. Moreover, according to Johnston et al. (1963), in the tropics, semen quality of taurine and taurine-zebu crossbreed bulls declines during the warm season. However, variations in semen quality cannot be attributed exclusively to the variation in ambient temperature and the ability of animals to adapt to the stressful environmental conditions, because breed composition is one of the factors responsible for this characteristic (Johnston et al., 1963).

As the present study was conducted in non-stressful climatic conditions for the bulls and the variables MAJD, MIND and TOTD were 26.6%, 16.3% and 26.4% higher, respectively, in 5/8 Holstein bulls in relation to 3/4 Holstein bulls, these results indicate that breed composition influenced the parameters of semen quality.

Although the present study was not conducted under thermal stress conditions for the bulls, the observed negative correlations of MOT and VIG and positives of MIND and TOTD with THI and AIRT indicated that climatic conditions could have influenced the sperm quality in the bulls of this study, as observed previously by Everett and Bean (1982) and Brito et al. (2004).

Positive correlations between the ocular area thermography with RECT ($\rho = 0.2809$) and AIRT ($\rho = 0.9148$) indicate that the superficial temperature of ocular area was less subject to homeostatic control and was therefore more influenced by the environmental condition when compared to RECT. Therefore, measuring the ocular area temperature by infrared thermography may better reflect the effects of ambient temperature on the reproductive capacity of bulls. Negative correlations between ocular area thermography with MOT, VIG, and MAJD were observed, indicating the prospect for using thermography as an auxiliary methodology to evaluate the reproductive capacity of bulls. Thermography has potential to be a specially indicated approach when there are a large number of evaluations to be conducted, being used as a pre-test for initial screening of bulls for breeding. As high temperature and stress result in negative effects on spermatogenesis and can lead to reproductive problems in bulls, our inference is still supported by the results of Schaefer et al. (2012) and Stewart et al. (2008), who observed that the ocular area thermography had a direct relationship with core body temperature and stress in cattle.

In a study with Charolais, Angus, Hereford and Simental bulls, Kastelic et al. (1995) reported average temperatures of the scrotal surface at the dorsal and ventral portions of 30.4 °C and 28.8 °C, respectively, the temperature difference between the dorsal and ventral portions of the scrotum averaging 1.6 °C. These averages were similar to the temperature variations observed in the present study. According to Kastelic (2014b), this difference in temperature between portions is important for adequate spermatogenesis, guaranteed by the backflow of blood, as the scrotum is vascularized from top to bottom, whereas the opposite is true for the testes. It should be noted also that the sperm kinetic parameters (MOT and VIG) are limited when the proportion of sperm defects is higher and temperature differences between the dorsal and ventral poles of the surface of the testes relates to the need for cooling this organ which requires ranges of temperature below the internal temperature range of the body.

Negative correlations verified between L1 - L2 temperature differences with MIND and TOTD indicate that this parameter can be useful to evaluate the animal's capacity for cooling the testes and can also be indicative of sperm defects in bulls and route these bulls for more detailed examinations. The higher average value ($P < 0.0001$) of L1 - L2 temperature differences observed in 5/8 Holstein bulls indicated the higher capacity of this breed composition for cooling the scrotal surface compared to 3/4 Holstein bulls. The higher correlation between L1 - L2 temperature differences and MIND for 3/4 Holstein bulls in relation to that obtained for 5/8 Holstein pointed to the prospect for using this parameter as an indicative of occurrence of minor sperm defects due to the difficulty for cooling the scrotum, possibly due to increased taurine breed composition.

5. Conclusions

Under non-stressful climatic conditions, the 3/4 Holstein Girolando bulls were superior in terms of reproductive and morphological body traits to the 5/8 Holstein bulls, but with a lower capacity for scrotal thermoregulation.

Breed composition primarily influenced major-type sperm defects, while scrotal and rectal temperatures exerted a greater influence upon minor-type sperm defects.

Regardless of breed composition, rectal temperature was positively correlated with sperm defects and negatively with reproductive indices, indicating that poor thermoregulation capacity interferes with reproductive efficiency.

Infrared thermography can be used as an alternative, non-invasive and preliminary test for the reproductive evaluation of bulls, particularly when there are a large number of animals in the tests, which is common in the pre-selection phases of breeding programs.

Acknowledgments

The authors declare that they have no competing interests. The authors are grateful to the Brazilian Federal Agency for Post-graduate Education (CAPES), Brazilian National Council of Scientific and Technological Development (CNPq), Minas Gerais State Agency for Research and Development (FAPEMIG) and Brazilian Agricultural Research Corporation (Embrapa) for funding and the support of the Brazilian Girolando Breeders Association. Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the authors or their institutions.

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