



Differences in the thermal sensitivity and seminal quality of distinct ovine genotypes raised in tropical conditions

Ana Beatriz Bossois Moura ^a, Felipe Zandonadi Brandão ^a, Sérgio Novita Esteves ^b,
Guilherme Nunes de Souza ^c, Jeferson Ferreira da Fonseca ^d,
Messy Hannear Andrade Pantoja ^e, Narian Romanello ^f, Daniela Botta ^e, Alessandro Giro ^e,
Alexandre Rossetto Garcia ^{b,*}

^a Faculty of Veterinary Medicine, Fluminense Federal University, Niterói, RJ, Brazil

^b Laboratory of Animal Reproduction, Brazilian Agricultural Research Corporation, Embrapa Southeast Livestock (CPPSE/Embrapa), São Carlos, SP, Brazil

^c Embrapa Dairy Cattle (CNPGL/Embrapa), Juiz de Fora, MG, Brazil

^d Embrapa Goats and Sheep, Núcleo Regional Sudeste, (CEJHB-Embrapa Gado de Leite), Coronel Pacheco, MG, Brazil

^e Institute of Veterinary Medicine, Federal University of Pará, Castanhal, PA, Brazil

^f Faculty of Veterinary Medicine and Animal Science, University of São Paulo (FMVZ/USP), Pirassununga, SP, Brazil

ARTICLE INFO

Article history:

Received 4 May 2018

Received in revised form

10 September 2018

Accepted 26 September 2018

Available online 29 September 2018

Keywords:

Ovis aries

Testicular thermoregulation

Semen

Infrared thermography

ABSTRACT

For different ovine breeds to maximize their reproductive capacity in countries with tropical climate, it is important to evaluate their potential for thermal resilience and consequences on their reproductive traits. Therefore, the objective of this study was to evaluate the effect of thermal environment temperatures of climate seasons in a tropical climate region on the surface temperatures of the scrotum, testicular biometric characteristics, seminal quality and serum testosterone concentration of rams of different genotypes. Breeders of four different genotypes (Dorper, $n = 8$, Texel, $n = 8$, Santa Inês, $n = 9$ and Morada Nova, $n = 8$) were used throughout the four climate seasons. Higher thermal challenge was recorded in the spring and summer. In the summer increase in scrotal surface temperature was detected by infrared thermography ($P < 0.05$), mainly in the regions of the distal testicular pole and tail of the epididymis. The animals of the Texel genotype had higher rectal temperature in the summer. In spring, this genotype also had the highest testicular pole ($32.2 \pm 0.5^\circ\text{C}$; $P < 0.05$) and distal ($29.9 \pm 0.4^\circ\text{C}$; $P < 0.05$) temperatures and a higher mean testicular temperature ($31.7 \pm 0.4^\circ\text{C}$; $P < 0.05$). The Morada Nova genotype showed a higher surface temperature gradient between testicular poles ($2.96 \pm 0.1^\circ\text{C}$; $P < 0.05$), especially in spring. Genotype-dependent thermal sensitivity was detected for the thermal gradient between the testicular poles, reflecting the seminal quality. There was a positive correlation of the thermal gradient between testicular poles with sperm membrane integrity and negative correlation with total sperm defects. The Texel genotype showed less progressive motility and higher percentage of sperm defects. There was no difference in testosterone concentration between genotypes and in the different seasons ($P > 0.05$). Thus, the indigenous genotypes showed a greater capability to maintain the scrotum-testicular thermoregulation. Dorper animals resembled the indigenous sheep genotypes, in terms of seminal characteristics, unlike Texel animals, which showed lower adaptability and lower seminal quality.

© 2018 Elsevier Inc. All rights reserved.

1. Introduction

Recent climate changes associated with increasing global temperature have imposed new challenges on animal production [1]. By the year 2035, it is estimated that the atmospheric temperature will increase from 0.3 to 0.7°C [2]. The negative effect of high temperatures on animal production is already observed, mainly in

* Corresponding author. Laboratory of Animal Reproduction, Embrapa Pecuária Sudeste, Brazilian Agriculture Research Corporation, Rod. Washington Luiz, km 234, São Carlos, SP, 13560-970, Brazil.

E-mail address: alexandre.garcia@embrapa.br (A.R. Garcia).

tropical and subtropical regions [3], where the climate is predominantly hot and with high relative humidity [4]. Included in these regions are the countries of emerging economies that have significant animal protein production at global levels [5]. In the coming decades, these countries are expected to contribute with more than half of the world's meat export volume [6].

In tropical countries caloric stress is one of the constraints in ovine production [7]. This is because in order to dissipate heat, animals reduce food intake, and the energy that would be used for growth and reproduction is directed toward body thermoregulation [8]. Thus, although sheep of the natural tropical breeds are considered sexually active throughout the year, their reproductive activity may be reduced or interrupted in the hottest times of the year [9].

It is known that sheep breeders under temporary caloric stress conditions may present testicular degeneration [10]. In addition, when the testes and epididymis are subjected to high temperatures, there may be changes in seminal quality that can cause subfertility or even infertility [11]. Under these conditions, spermatozoa display a decrease in progressive motility, an increase in morphological anomalies and reduction in DNA integrity due to the excessive production of reactive oxygen species [12]. However, there is a substantial reduction in the number of spermatozoa in the ejaculate, due to the occurrence of germ cell apoptosis [13]. The somatic cells in the testis are also affected, negatively influencing the production of testosterone [14].

One of the alternatives to increase ovine production in the tropics is to identify and select genotypes with higher adaptive capacity to regulate body temperature and expression of their reproductive potential, given the adverse conditions due to high environmental temperatures [8,15]. Thus, it is important to evaluate the effect of climate related variations that the animals are naturally submitted to and their thermal resilience potential for reproductive characteristics in tropical regions. Therefore, the objective of the present study was to evaluate the effect of thermal environment of the different climate seasons on the scrotal surface temperatures, testicular biometric characteristics, seminal quality and serum testosterone concentration of rams of four different genotypes raised in tropical climate conditions.

2. Material and methods

2.1. Ethics

The Committee on Experimental Animal Use and Ethics of Embrapa Pecuária Sudeste approved this research (protocol approval: CEUA-CPPSE # 06/2013) and the experiment was carried out under the ethical principles of the National Council for Animal Experimentation Control (CONCEA-Brazil).

2.2. Location, climate characterization and experimental period

The study was conducted at the Embrapa Pecuária Sudeste, in São Carlos, SP, Brazil (21°58'30" S, 47°50'58" W and 911 m). The climate is the subtype Cwa, tropical of altitude, characterized by dry winter and hot summer according to Köppen-Geiger climate classification [16]. The climate seasons are well defined and comprise spring (September 23 to December 21), summer (December 22 to March 19), fall (March 20 to June 19) and winter (June 20 to September 22). In the hotter periods, the average maximum temperatures range from 27.8 to 32.0 °C, with peaks of up to 34.5 °C. The period of high relative air humidity coincides with the periods of high temperatures and their average values vary from 74.9 to 91.4%, with annual average rainfall of 1361.6 mm [17]. The experiment was carried out between November 2015 and July 2016, and

covered the four climate seasons.

2.3. Animals and handling

Thirty-three purebred rams of four genotypic groups (Dorper, $n = 8$, Texel, $n = 8$, Santa Inês, $n = 9$ and Morada Nova, $n = 8$) were used. Santa Inês and Morada Nova are indigenous sheep genotypes, while Dorper and Texel are exotic genotypes in Brazil, from South Africa and Holland, respectively. The animals were previously selected for clinical and reproductive status to allow age homogeneity (21.5 ± 0.5 months), weight (72.9 ± 1.6 kg) and body condition score (3.3 ± 0.1). The management adaptation period was two months before the beginning of the experiment.

The rams were kept in a single plot under equivalent nutritional and sanitary management conditions. The animals were kept in a covered shed with a sand bed that provided a shaded area of 6.0 m²/animal. The feed was supplied daily, containing 80% of roughage (corn silage) and 20% of concentrate. The animals had *ad libitum* access to automatic drinking trough and mineral salt.

2.4. Microclimate and thermal comfort indices

The characterization of the local microclimate included monitoring the air temperature (AT, °C), the relative air humidity (RH, %) and the black globe temperature (BGT, °C), obtained by automatic meteorological station installed in the animal housing shed. Then, the thermal comfort indices considered relevant in the test were calculated: the Temperature and Humidity Index (THI) and the Black Globe Temperature and Humidity Index (BGHI). The THI was calculated using equation $THI = AT - [(0.31 - 0.31RH)(AT - 14.4)]$ where AT is the air temperature obtained by the dry bulb thermometer (°C) and RH is the relative air humidity (RH%/100) [9]. The THI values obtained represent <22.2: absence of heat stress; 22.2 to <23.3: moderate heat stress; 23.3 to <25.6: severe heat stress and above 25.6: extreme stress [9]. The BGHI was calculated by the equation $BGHI = BGT + 0.36(DPT) + 41.5$ where BGT is the black globe temperature (°C) and DPT is the dew point temperature (°C) [18]. BGHI values represent <74: comfort condition; 74 to 78: alert; 79 to 84: danger condition and >84: emergency condition [19]. THI and BGHI were demonstrated for each climate season.

2.5. Scrotal circumference, testicular volume and consistency

Scrotal biometry and testicular parenchyma consistency were always evaluated by the same experienced technician every 30 days. The scrotal circumference (SC, cm) was obtained using flexible graduated tape at the greatest diameter point of the scrotum [20]. Testicular volume (V, cm³) was obtained by the equation $V = 4/3(\pi)(L/2)(W/2)^2$ where $\pi = 3.1415$, L is the length (cm) and W is the width (cm) of a single testicle [21], measured by a millimeter-scale steel pachymeter. Testicular consistency was determined by palpation and graded from one (flaccid) to five (firm) [20].

2.6. Scrotal surface temperatures

The scrotal surface temperatures were evaluated by infrared thermography, using 0.05 °C thermal sensitivity camera and 320 × 240 optical pixel resolution (FLIR T300, FLIR Systems, Portland, USA). The emissivity value was constant, of 0.98 [22]. The thermograms of each animal were generated monthly during the morning period (7h00 to 9h00), during the four climate seasons, in a place sheltered from direct solar radiation and rain. The camera was positioned 1.0 m away, oriented perpendicular to the scrotum. The thermograms were later analyzed with the FLIR Tools + software (FLIR Systems, Portland, USA), based on previous

studies. Spermatic funicle temperature (SFT, °C), proximal testicular pole (PPT, °C) and distal testicular pole (DPT, °C) were determined using rectangular analytical tracings [23]. The epididymal tail temperature (ETT, °C) was determined by circular tracing [23], and the mean testicular temperature (MTT, °C) was obtained by ellipsoid tracing [24] (Fig. 1). The results were presented as the mean of contralateral anatomical structures.

2.7. Temperature gradients

Immediately after the thermography, the rectal temperature (RT, °C) was measured with a clinical digital thermometer (Inco-term, São Paulo, Brazil). The temperature gradients (°C) of the scrotal regions of interest were calculated by the following equations: gradient between rectal temperatures and spermatic funiculus region ($Grad1 = RT - SFT$); gradient between rectal and distal testicular poles ($Grad2 = RT - DPT$); gradient between rectal and epididymis tail temperatures ($Grad3 = RT - ETT$); gradient between temperatures of the testicular poles ($Grad4 = PPT - DPT$) [25].

2.8. Evaluation of seminal quality

Among the breeders studied, 20 animals were used as semen donors, five from each genotype. The semen was collected monthly by artificial vagina from the same rams, during the four climate seasons, totaling nine ejaculates per animal and 180 semen samples analyzed. Seminal samples were always evaluated by the same examiner and maintained at 37 °C during the evaluations. The variables of interest considered were progressive sperm motility (PM, %), plasma membrane integrity (PMI, %), sperm morphology and DNA fragmentation (DNAf, %).

PM was evaluated by optical microscopy at 400x magnification (Nikon Eclipse E100, Tokyo, Japan) in five different fields of the glass slide covered with a coverslip containing 10 µL aliquot of semen diluted with ringer lactate solution (1:1) [20,26]. The

evaluation of the PMI used hypo-osmotic solution (100 mOsm/L) composed of D-fructose (Sigma-Aldrich, Saint Louis, USA) and sodium citrate dihydrate (Sigma-Aldrich, Saint Louis, USA) [27]. Two-hundred cells per sample were evaluated by phase contrast microscopy under 1000x magnification (Nikon Eclipse E200, Tokyo, Japan). The spermatozoa were classified as carriers of integral plasma membrane if they presented cellular swelling visualized by the coiled tail [28].

To evaluate the sperm morphology, an aliquot of 20 µL of semen was fixed in buffered formalin solution preheated to 37 °C. Wet preparation of spermatozoa were evaluated by phase contrast optical microscopy (Nikon Eclipse E200, Tokyo, Japan) under magnification of 1000x. 100 spermatozoa per sample were evaluated and classified as minor sperm defects (MiD, %), major defects (MaD, %) and total defects (TD, %) [29].

Sperm DNA fragmentation was evaluated on ethanol-acetic acid (3:1) solution smears for 1 min, followed by soaking in 70% ethanol for 3 min and subsequent acid hydrolysis with HCl (4 N) for 5 min. Once fixed, the samples were stained with 0.025% toluidine blue solution (Sigma-Aldrich, Saint Louis, USA). In each sample, 500 sperm cells under magnification of 1000x were evaluated by light microscopy (Nikon Eclipse E100, Tokyo, Japan). Light blue stained sperm cells were considered as carriers of intact DNA, while cells that showed dark blue to violet color were considered as chromatin abnormalities [30].

2.9. Testosterone dosage

Blood samples were collected monthly [14,31] at the same time of day (7h00 to 9h00) from each ram by jugular venipuncture using silicone vacuum tubes, without anticoagulant (Vacutainer, Nova Jersey, USA). The samples were centrifuged at $1350 \times g$ for 15 min for complete separation of the serum, which was fractionated into polypropylene microtubes and conditioned at –20 °C until analysis. Serum testosterone concentration was quantified by

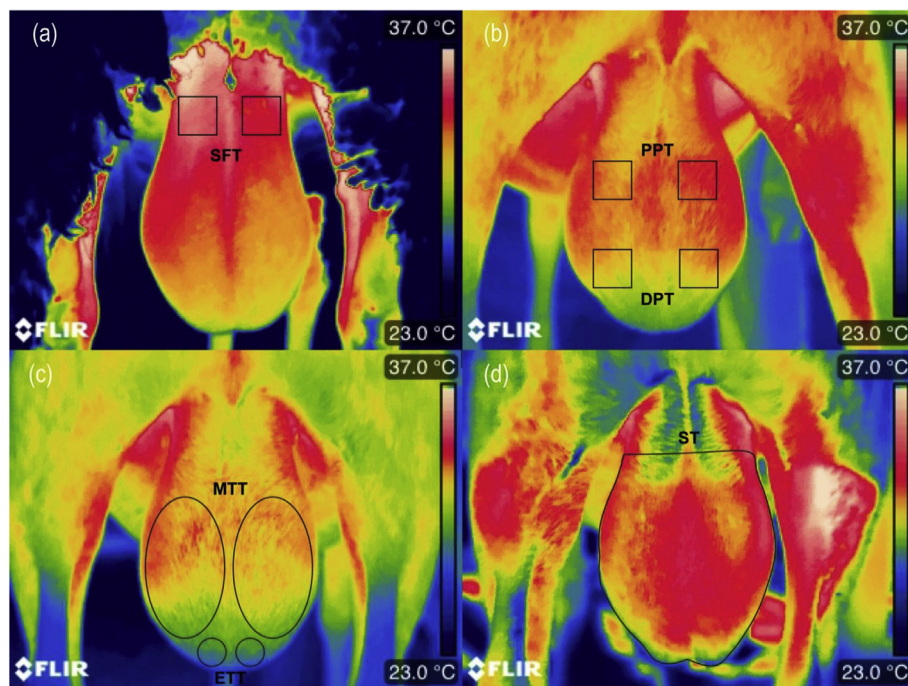


Fig. 1. Illustrative thermograms for determination of surface temperatures of (a) spermatic funiculus (SFT), (b) proximal testicular poles (PPT) and distal poles (DPT), (c) testicles (MTT) and tail of the epididymis (ETT), and (d) scrotum (ST) of rams. The tracings indicate the areas analyzed. Parameterized for rainbow color palette and thermal scale from 23.0 to 37.0 °C. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

radioimmunoassay (RIA) using the commercial ImmuChem™ Double Antibody Testosterone kit (MP Biomedicals, Inc., Diagnostics Division, Orangeburg, USA). The sensitivity and intra-assay coefficient (CV) were 0.03 ng/mL and 12.2%, respectively. The results of serum testosterone concentration were demonstrated as a mean value for each climate season.

2.10. Statistical analysis

The results were submitted to descriptive evaluation and analysis of variance using the generalized linear model-GLM procedure of SAS (SAS Institute, Cary, USA). The variables rectal temperature, scrotal surface temperatures, scrotal circumference, testicular consistency, testicular volume, progressive sperm motility, plasma membrane integrity, sperm morphology and DNA fragmentation were evaluated for the effect of the year-season (spring, summer, autumn and winter) and genotype (Dorper, Texel, Santa Inês and Morada Nova), as well as the effect of their interaction. Therefore, the statistical model $Y = \text{genotype} + \text{animal}(\text{genotype}) + \text{season} + \text{genotype} \times \text{season} + \text{error}$ was used in which the animal effect (*genotype*) was the error considered for testing the genotype. The comparison between means was performed by LSD. Correlations between the climatological indices and the animal response variables were determined by the Pearson's coefficient, and only the significant correlations were demonstrated. The level of significance previously determined for all analyses was 5%.

3. Results

The climate oscillations are presented by season (Fig. 2a). The THI and BGHI (Fig. 2b) values were significantly higher and critical in spring (22.2 ± 0.1 and 75.0 ± 0.1 , respectively) and summer (22.7 ± 0.1 and 75.3 ± 0.1 , respectively).

Higher values of SC and testicular volume were observed for the Dorper rams and lower in the Morada Nova animals (Supplementary Table 1). However, when performing the ratio between testicular volume and body weight, a higher proportion of testicular parenchyma by live weight was observed in Dorper ($2.6 \pm 0.1 \text{ cm}^3/\text{kg}$) and Morada Nova ($2.7 \pm 0.1 \text{ cm}^3/\text{kg}$), compared with Texel and Santa Inês, which presented values of 2.2 ± 0.1 and $2.2 \pm 0.1 \text{ cm}^3/\text{kg}$, respectively. Santa Inês animals showed no variation for SC and testicular consistency during the four climate seasons (Supplementary Table 1).

The mean RT showed lower values in winter, regardless of the genotype (Table 1). During summer the Texel animals presented higher rectal temperature. The mean ST was higher during the summer, while lower values were recorded in the winter. Regarding the temperatures of specific points of the scrotum (Table 2), it was observed that the SFT was lower in the winter. The Texel rams had maximum PPT, DPT, ETT and MTT values in spring and summer ($P < 0.05$). In relation to other genotypes, the Texel animals showed higher values of PPT, DPT and MTT during spring. Regardless of the genotype, DPT and ETT were higher in summer and lower in winter.

The temperature gradients were higher in winter (Fig. 3). The lowest values were verified in summer (9.3 ± 0.3 and $9.8 \pm 0.2 \text{ }^\circ\text{C}$ $P < 0.05$, respectively) for Grad2 and Grad3. Genotype influenced the behavior of Grad4, since Morada Nova animals showed higher values in spring ($3.24 \pm 0.2 \text{ }^\circ\text{C}$ $P < 0.05$), whereas Dorper rams presented lower values in the summer ($1.77 \pm 0.2 \text{ }^\circ\text{C}$ $P < 0.05$). Regardless of the climate seasons, animals of the Morada Nova differed from the other genotypes because they had higher Grad4 values (Morada Nova = $2.96 \pm 0.1 \text{ }^\circ\text{C}$; Dorper = $2.29 \pm 0.1 \text{ }^\circ\text{C}$; Texel = $2.50 \pm 0.2 \text{ }^\circ\text{C}$; Santa Inês = $2.62 \pm 0.2 \text{ }^\circ\text{C}$, $P < 0.05$).

The mean PM was lower in Texel, which presented values of $57.5 \pm 2.5\%$ ($P < 0.05$) (Fig. 4). The PMI and MiD did not change throughout the climate seasons ($P > 0.05$). However, there was a significant higher incidence of MaD in the summer ($13.3 \pm 2.0\%$) and lower TD values in the spring ($15.9 \pm 2.7\%$). Regardless of the climate season, the Texel animals had a higher percentage of spermatozoa with abnormal morphology ($30.7 \pm 3.0\%$), and higher incidence of MaD ($15.9 \pm 2.7\%$) ($P < 0.05$). There was no effect of season of the year and genotypes on the results of DNA fragmentation ($P > 0.05$). The serum concentration of testosterone showed no difference between genotypes within the seasons (Fig. 5). There was also no effect of the climate season on the plasma concentration, independently of the analyzed genotype ($P > 0.05$).

Considering the significant correlations, PM presented negative association with RT ($r = -0.16$), DNAf ($r = -0.23$), MaD ($r = -0.52$) and TD ($r = -0.38$). In contrast, PM was positively correlated with PMI ($r = 0.41$). However, PMI presented a negative correlation with MaD ($r = -0.28$) and TD ($r = -0.34$), and a positive correlation with Grad4 ($r = 0.29$). TD presented a positive correlation with RT ($r = 0.24$) and was inversely correlated with Grad4 ($r = -0.19$).

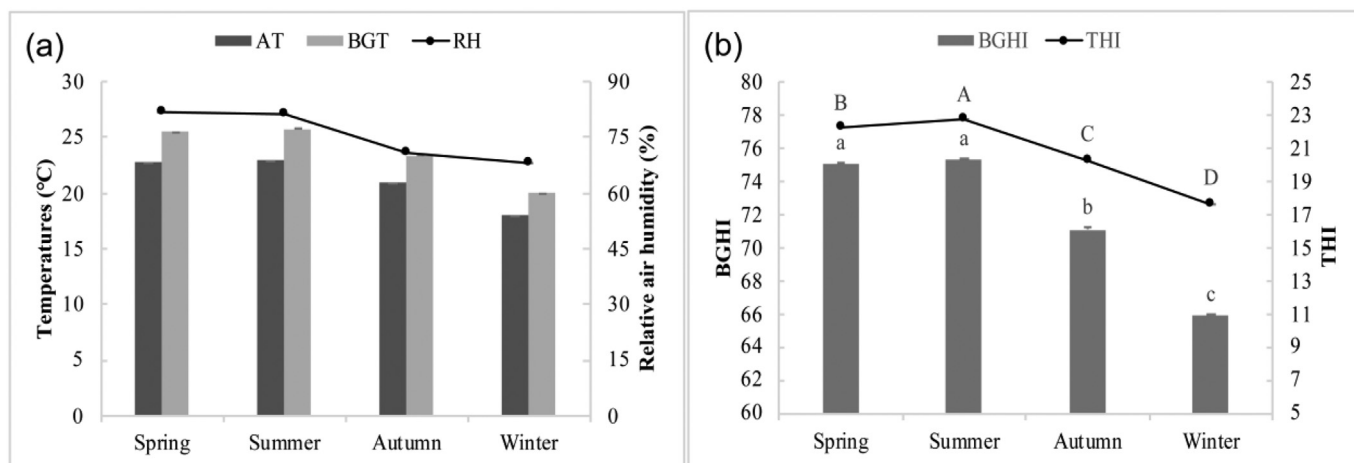


Fig. 2. Mean (and SEM) of the climate variables and the thermal comfort indices by climate season in a region of tropical climate. (a) Air temperature (AT), black globe temperature (BGT) and relative air humidity (RH). (b) Black Globe Temperature and Humidity Index (BGHI) and Temperature and Humidity Index (THI). ^{A,B,C,D} Indicates significant difference for THI between seasons ($P < 0.05$). ^{a,b,c} Indicates significant difference for BGHI between seasons ($P < 0.05$). SEM, standard error of the mean.

Table 1

Mean (and SEM) of the rectal and scrotal surface temperatures of 33 rams of different genotypes (Dorper, n = 8, Texel, n = 8, Santa Inês, n = 9 and Morada Nova, n = 8) during the four seasons of the year in tropical climate.

Season	Genotype				
	Dorper	Texel	Santa Inês	Morada Nova	Average
Rectal Temperature (RT, °C)					
Spring	38.5 ± 0.1 ^{ab}	38.9 ± 0.1 ^{aA}	38.5 ± 0.1 ^{abB}	38.8 ± 0.1 ^{aA}	38.7 ± 0.1 ^a
Summer	38.6 ± 0.1 ^{ab}	39.0 ± 0.1 ^{aA}	38.3 ± 0.1 ^{bC}	38.5 ± 0.1 ^{bB}	38.6 ± 0.1 ^a
Autumn	38.5 ± 0.1 ^{abC}	38.7 ± 0.1 ^{abAB}	38.8 ± 0.1 ^{aA}	38.4 ± 0.1 ^{bC}	38.6 ± 0.1 ^a
Winter	37.8 ± 0.1 ^{bB}	38.2 ± 0.1 ^{bAB}	38.6 ± 0.1 ^{aA}	38.6 ± 0.1 ^{abA}	38.3 ± 0.1 ^b
Average	38.4 ± 0.1 ^B	38.7 ± 0.1 ^A	38.6 ± 0.1 ^A	38.6 ± 0.1 ^A	
Scrotal Temperature (ST, °C)					
Spring	28.7 ± 0.4	29.6 ± 0.4	29.6 ± 0.4	29.4 ± 0.4	29.3 ± 0.2 ^b
Summer	30.4 ± 0.3	30.3 ± 0.3	30.7 ± 0.3	30.5 ± 0.4	30.5 ± 0.2 ^a
Autumn	30.4 ± 0.4	29.1 ± 0.4	30.0 ± 0.4	29.9 ± 0.5	29.9 ± 0.2 ^b
Winter	24.7 ± 0.4	23.6 ± 0.5	24.6 ± 0.4	25.0 ± 0.5	24.5 ± 0.2 ^c
Average	28.6 ± 0.2	28.1 ± 0.2	28.7 ± 0.2	28.7 ± 0.2	

^{A,B,C} different capital letters indicate significant difference in the rows ($P < 0.05$).

^{a,b,c} different lowercase letters indicate significant difference in the columns ($P < 0.05$).

Abbreviation: SEM, standard error of the mean.

4. Discussion

This is the first study that has reported on the referred ovine genotypes kept under the same environmental conditions in a tropical climate region, in which the climate implications on the reproductive characteristics were evaluated throughout the year. Also included is the use of infrared thermography as an auxiliary tool in the analysis of scrotal thermoregulation in these genotypes,

as well as the determination of seminal quality and serum testosterone concentration, characteristics considered relevant to evaluate the potential fertility of a breeder.

In order to evaluate the environmental conditions to which sheep are submitted, some thermal stress indices are used in order to adopt strategies to reduce heat related stress [3,7]. In the present study, the most difficult periods concerning thermal stress comprised the spring and summer seasons. The indices that estimate thermal stress showed average values that indicated the potential situation of moderate stress (22.2 to <23.3) or alert status (74–78), according to THI records [9] and BGHI [18], respectively. However, it seems that these environmental conditions were not able to override the thermolitic capacity of the animals [32], since the average rectal temperature remained within the range considered normal for sheep, from 38.3 to 39.9 °C [9]. Considering rectal temperature as an indicator of thermal equilibrium, indigenous sheep genotypes demonstrated a high thermoregulatory capacity, which, as observed in the literature [26], decreased their body temperature in the summer when the scrotal temperature increased by 1.1 °C.

It is known that thermal stress can lead to the reduction of testicular biometry [26], because it causes degeneration of the germinal epithelium and partial atrophy of the seminiferous tubules [14]. The possible thermal discomfort presumed by the THI and BGHI was not enough to raise the scrotal temperatures to the point of causing reduction in the scrotum-testicular biometry of the Dorper and Santa Inês rams. The absence of testicular tone variation was verified in Santa Inês rams, whose testicular parenchyma in previous studies did not demonstrate significant changes throughout the year in tropical regions [25] even under heat tolerance test [33].

Higher mean scrotal surface temperature was observed in the summer, and its effects were more expressive on the distal portions of the scrotum, verified by changes in DPT and ETT, a fact that is corroborated by the literature [34]. It is possible this effect results from the vertical orientation of the testicles within the scrotum and from the sintopia with the tail of the epididymis [35], which exhibit more distal positions in the scrotum and are subject to the incidence of direct solar radiation and infrared thermal radiation from the ground.

The lower testicular temperature relative to the body temperature is essential for the production and maturation of the male gamete, and occurs mainly due to the countercurrent mechanism, responsible for the transfer of heat from the arterial blood to the venous blood in the pampiniform plexus [36]. Equal efficiency in

Table 2

Mean (and SEM) of the surface temperatures of the scrotal region obtained by infrared thermography in 33 rams of different genotypes (Dorper, n = 8, Texel, n = 8, Santa Inês, n = 9 and Morada Nova, n = 8) during the four seasons of the year in tropical climate.

Season	Genotype				
	Dorper	Texel	Santa Inês	Morada Nova	Average
Spermathecal Funicle Temperature (SFT, °C)					
Spring	31.1 ± 0.6	32.0 ± 0.6	31.2 ± 0.5	31.8 ± 0.6	31.5 ± 0.3 ^a
Summer	32.3 ± 0.4	32.3 ± 0.5	31.9 ± 0.4	31.8 ± 0.5	32.1 ± 0.2 ^a
Autumn	31.9 ± 0.6	31.3 ± 0.6	31.7 ± 0.7	31.9 ± 0.6	31.7 ± 0.3 ^a
Winter	27.1 ± 0.6	25.7 ± 0.6	26.7 ± 0.5	26.2 ± 0.7	26.5 ± 0.3 ^b
Average	30.6 ± 0.3	30.3 ± 0.3	30.4 ± 0.3	30.4 ± 0.3	
Proximal Pole of Testis Temperature (PPT, °C)					
Spring	30.3 ± 0.5 ^{aC}	32.2 ± 0.5 ^{aA}	30.6 ± 0.5 ^{aBC}	31.5 ± 0.5 ^{aAB}	31.2 ± 0.2 ^a
Summer	31.5 ± 0.4 ^a	31.9 ± 0.4 ^a	31.7 ± 0.4 ^a	31.8 ± 0.5 ^a	31.7 ± 0.2 ^a
Autumn	31.5 ± 0.5 ^a	30.0 ± 0.5 ^b	31.5 ± 0.5 ^a	31.4 ± 0.6 ^a	31.1 ± 0.3 ^a
Winter	27.3 ± 0.6 ^b	25.8 ± 0.6 ^b	25.9 ± 0.5 ^b	26.8 ± 0.6 ^b	26.5 ± 0.3 ^b
Average	30.1 ± 0.3	29.9 ± 0.3	29.9 ± 0.2	30.4 ± 0.3	
Distal Pole of Testis Temperature (DPT, °C)					
Spring	28.6 ± 0.4 ^{aB}	29.9 ± 0.4 ^{aA}	28.4 ± 0.4 ^{aB}	28.3 ± 0.4 ^{aB}	28.8 ± 0.2 ^{ab}
Summer	29.7 ± 0.4 ^a	29.4 ± 0.4 ^a	29.3 ± 0.3 ^a	28.9 ± 0.4 ^a	29.3 ± 0.2 ^a
Autumn	29.1 ± 0.5 ^a	28.0 ± 0.5 ^b	28.8 ± 0.4 ^a	28.3 ± 0.5 ^a	28.6 ± 0.2 ^b
Winter	24.0 ± 0.5 ^b	22.4 ± 0.5 ^c	23.3 ± 0.4 ^b	24.4 ± 0.6 ^b	23.5 ± 0.2 ^c
Average	27.8 ± 0.2	27.4 ± 0.2	27.5 ± 0.2	27.5 ± 0.2	
Epididymal Tail Temperature (ETT, °C)					
Spring	27.4 ± 0.5 ^b	28.8 ± 0.5 ^a	27.7 ± 0.4 ^b	27.6 ± 0.5 ^a	27.8 ± 0.2 ^b
Summer	29.0 ± 0.4 ^a	28.8 ± 0.4 ^a	28.8 ± 0.4 ^a	28.5 ± 0.4 ^a	28.8 ± 0.2 ^a
Autumn	28.7 ± 0.5 ^a	27.3 ± 0.5 ^b	28.2 ± 0.5 ^{ab}	28.0 ± 0.5 ^a	28.0 ± 0.2 ^b
Winter	23.2 ± 0.5 ^c	21.5 ± 0.5 ^c	22.8 ± 0.4 ^c	23.6 ± 0.6 ^b	22.8 ± 0.3 ^c
Average	27.1 ± 0.2	26.6 ± 0.2	26.9 ± 0.2	26.9 ± 0.2	
Mean Testicular Temperature (MTT, °C)					
Spring	30.1 ± 0.5 ^{aB}	31.7 ± 0.4 ^{aA}	30.2 ± 0.4 ^{aB}	30.7 ± 0.4 ^{aB}	30.7 ± 0.2 ^a
Summer	31.1 ± 0.4 ^a	31.3 ± 0.4 ^a	31.2 ± 0.3 ^a	31.1 ± 0.4 ^a	31.2 ± 0.2 ^a
Autumn	31.1 ± 0.5 ^{aA}	29.8 ± 0.5 ^{bB}	31.3 ± 0.4 ^{aA}	30.7 ± 0.5 ^{aAB}	30.7 ± 0.2 ^a
Winter	26.6 ± 0.5 ^b	24.6 ± 0.5 ^c	25.7 ± 0.4 ^b	27.1 ± 0.6 ^b	26.0 ± 0.2 ^b
Average	29.4 ± 0.2	29.3 ± 0.2	29.6 ± 0.2	29.9 ± 0.2	

^{A,B,C} different capital letters indicate significant difference in the rows ($P < 0.05$).

^{a,b,c} different lowercase letters indicate significant difference in the columns ($P < 0.05$).

Abbreviation: SEM, standard error of the mean.

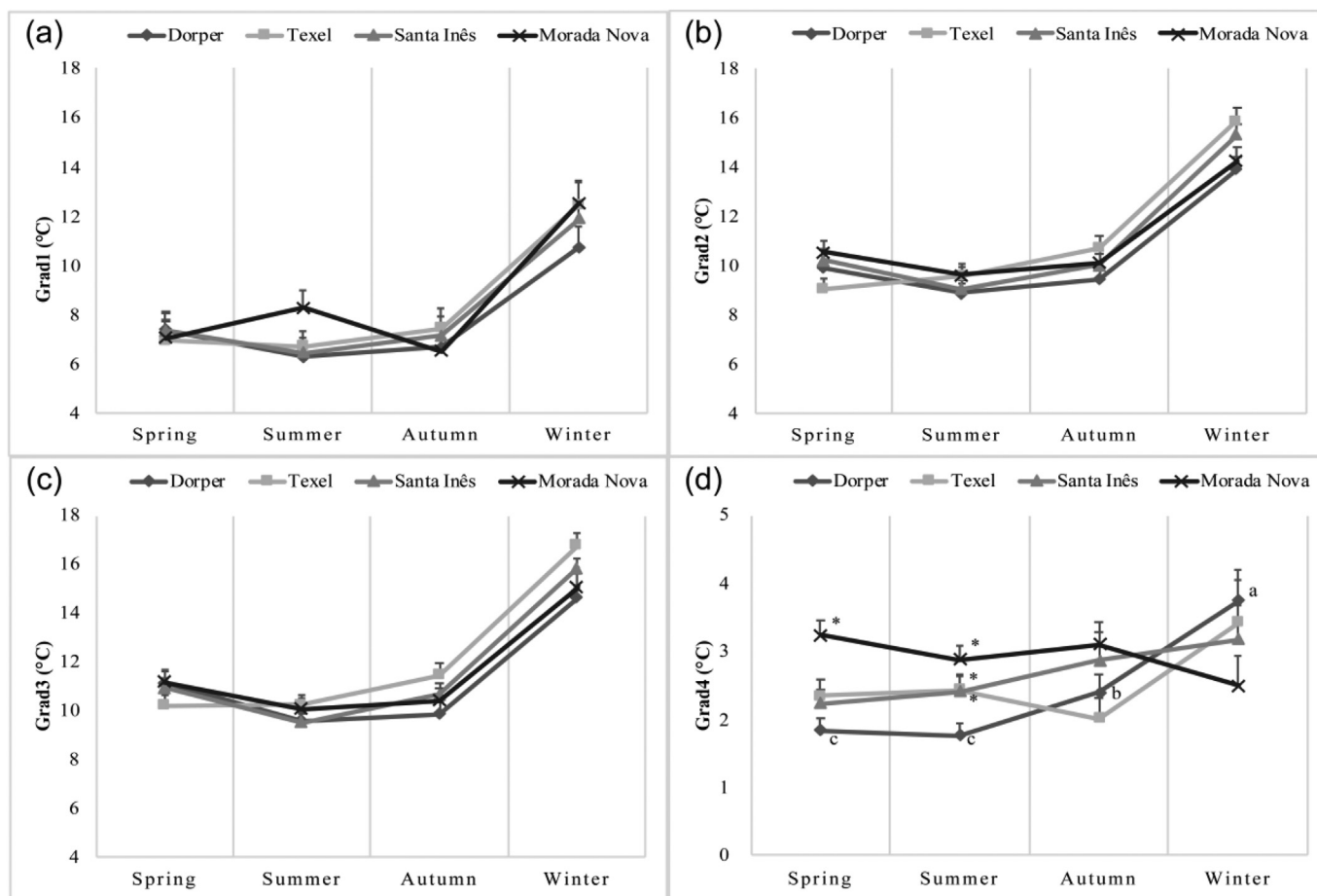


Fig. 3. Mean (and SEM) of surface temperature gradients measured in 33 sheep breeders of different genotypes (Dorper, $n = 8$, Texel, $n = 8$, Santa Inês, $n = 9$ and Morada Nova, $n = 8$) during the four seasons of the year in tropical region. (a) Grad 1 = gradient between rectal and spermatic funiculi temperatures. (b) Grad 2 = gradient between rectal and ventral testicular pole temperatures. (c) Grad 3 = gradient between rectal and tail of the epididymis temperatures. (d) Grad 4 = gradient between temperatures of the testicular poles. ^{a,b,c} Indicates significant difference for genotype between seasons ($P < 0.05$). * Indicates difference between genotypes within the season ($P < 0.05$). SEM, standard error of the mean.

this process was verified by the similar means of SFT and Grad1 among the genotypes. The gradient between body and scrotal surface temperatures assessed in the distal testicular pole region (Grad2) showed normal values for testicular thermoregulation, since it is recommended that the intratesticular temperature be maintained from 2 to 6 °C lower than the internal temperature [37] and that there is a positive correlation between the scrotal and intratesticular surface temperatures [38]. However, the studied genotypes showed a different behavior regarding the surface temperature gradient between the testicular poles (Grad4).

Morada Nova rams showed greater efficiency in testicular thermoregulation, as they exhibited constant Grad4 throughout the year, with higher values in the more challenging climate period and the highest average value among the genotypes. The Grad4 oscillations observed in Dorper rams given the change in climate showed less testicular heat dissipation capacity in the hotter periods. It is assumed that this difference in heat exchange with the external environment is attributed to the difference in the quantity, position, size and functionality of the sweat glands in the most distal portions of the scrotum, the color and thickness of the skin and the scrotal pelt [39], in addition to the anatomical difference of the scrotal-testicular vascularization, as observed in cattle [40].

In general, the negative correlation of Grad4 with TD and positive correlation of Grad4 with PMI demonstrates that the higher the thermal gradient between the testicular poles, the greater the

testicular ability to provide a more suitable thermal microenvironment for the production of the male gamete with morphology and normal functionality [23,41]. However, the Dorper and Texel genotypes showed a difference in the relationship between testicular thermostability and seminal quality. In fact, Dorper animals showed increasing Grad4 throughout the seasons due to their lower testicular heat dissipation capacity in the summer, but their seminal quality was not different in relation to the indigenous sheep genotypes. In contrast, the Texel genotype, whose Grad4 values over the seasons were constant and resembled the indigenous sheep genotypes, showed the lowest seminal quality among the evaluated genotypes. This unprecedented result may indicate that the genotypes have singular thermal sensitivity and require specific conditions of temperature variation between the testicular poles for the production of gametes.

The Texel breeders were more sensitive to the influence of climate on their seminal characteristics, indicating a lower adaptive capacity to tropical conditions than the other genotypes, especially in the hotter period. This was confirmed by the higher RT in summer and higher PPT, DPT and MTT in the spring, variables considered to be predictors of climate adaptation capacity and important for seminal quality [33]. The lower seminal quality of the Texel animals was perceived due to lower PM and higher incidence of MaD observed among genotypes. In general, it was possible to confirm by the correlation coefficient that higher values of MaD had

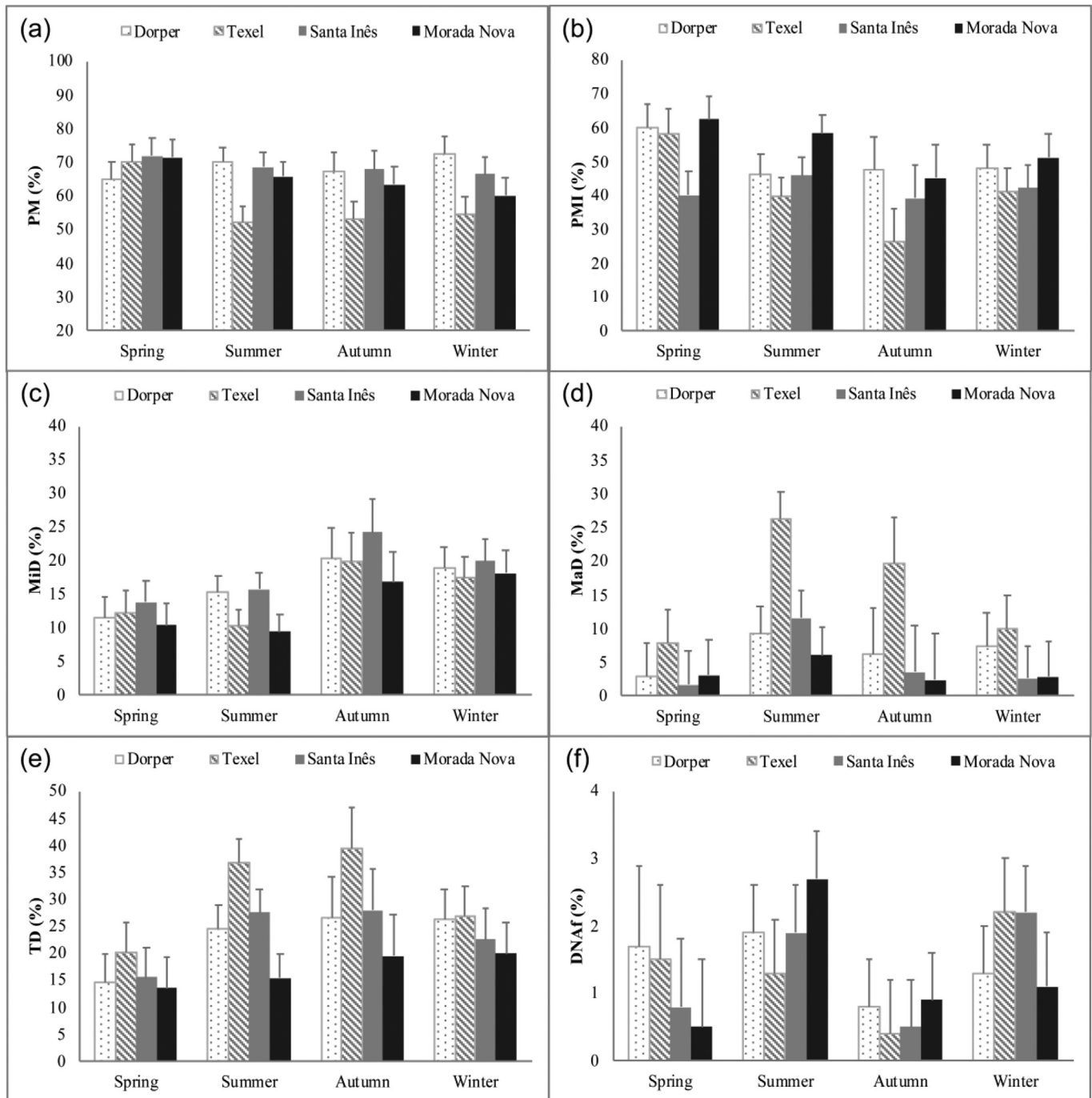


Fig. 4. Mean (and SEM) of the evaluated sperm characteristics of 20 rams of different genotypes (Dorper, $n = 5$; Texel, $n = 5$; Santa Inês, $n = 5$; Morada Nova, $n = 5$) during the four seasons of the year in tropical climate. (a) Progressive sperm motility (PM). (b) Plasma membrane integrity (PMI). (c) Minor sperm defects (MiD). (d) Major sperm defects (MaD). (e) Total sperm defects (TD). (f) Sperm DNA fragmentation (DNAf). SEM, standard error of the mean.

a negative influence on progressive sperm motility. In addition, the total morphological defects exhibited by the Texel breeders, mainly in the summer and autumn, exceeded 30%, the maximum acceptable limit for *in natura* ram semen [20]. This was possibly due to heat injury in spring and summer, as sperm defects can be manifested in the ejaculate of rams within 63 days after the testicular exposure at high temperatures, a period comprised of spermatogenesis and spermatozoa transit through the testis and epididymis [13].

The highest MaD value exhibited in summer, independently of

the genotype, can be associated to the thermal challenge that occurred in spring and summer. This can be inferred by the observed positive correlation of RT with MaD and TD, especially in the Texel genotype, which exhibited higher body temperature in these seasons. Although animals maintained body temperature within normal levels, there was an increase in MaD levels in the summer, probably because the energy that would be used for reproductive purposes was directed to body thermoregulation [8,42,43]. In addition, it was observed that the epididymis was also negatively impacted by heat, due to the typology of sperm defects

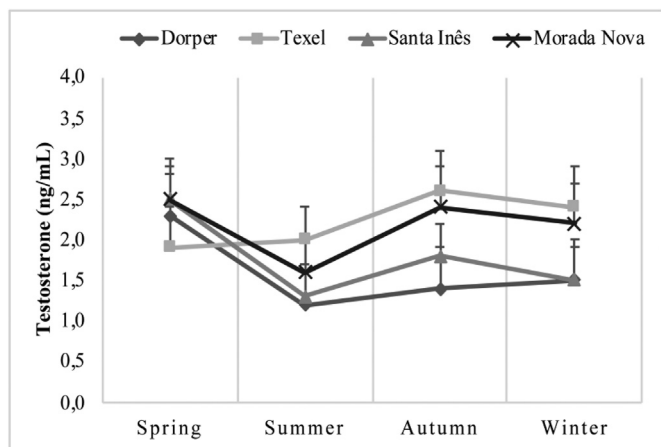


Fig. 5. Mean (and SEM) of plasma concentration of testosterone of 33 rams of different genotypes (Dorper, n = 8, Texel, n = 8, Santa Inês, n = 9 and Morada Nova, n = 8) grown in a tropical climate region during the four seasons of the year. SEM, standard error of the mean.

with higher occurrence in this study (for example, strongly bent tail and coiled tail), which are associated with epididymal dysfunction caused by heat related stress [44].

It is known that high temperatures caused by microclimatic fluctuations can affect the scrotum and cause disruption in the DNA of the spermatozoa. The phase of spermatogenic mitotic division is affected by heat stress and chromatin defects can be visualized in the ejaculate between 48 and 54 days after the thermal insult [45]. However, no difference was observed with regard to the incidence of sperm DNA damage throughout the seasons. It is probable that the temperatures the gametes were exposed to were not enough to unbalance the production of reactive oxygen species in the intra-tubular environment, which are mainly responsible for causing sperm DNA damage [11].

Another characteristic that can be compromised by the increase in testicular temperature is the permeability of the sperm membrane [42]. This cell envelope is especially sensitive in ovine and highly susceptible to oxidative damage due to the excessive increase of reactive oxygen species, in the case of thermal stress [12]. The absence of significant variation in the membrane integrity of the spermatozoa throughout the seasons can be explained by the adequate thermolitic capacity of the animals or by the efficient elimination of the cells damaged by apoptosis [11,12]. Moreover, the climatic environment probably was not stressful enough to affect the production of testosterone during the seasons of the year [42].

5. Conclusions

The indigenous genotypes showed a greater capability to maintain the scrotum-testicular thermoregulation, with emphasis on the Morada Nova genotype. Dorper animals resembled the indigenous sheep genotypes, in terms of seminal characteristics, unlike Texel animals, which showed lower adaptability and lower seminal quality. It was assumed that the distal portions of the scrotum are more susceptible to heat, especially in the hottest periods of the year. In addition, a specific genotype-dependent thermal sensitivity was detected for the thermal gradient between the testicular poles, with a seminal quality reaction. Based on the findings, it would be interesting to conduct further research using a larger number of animals in different environments in order to deepen knowledge about the genotype-environment interactions observed.

Acknowledgements

This project received financial support from Embrapa (BIOTEC Network-grant # 01.13.06.001.05.04), from the FAPERJ, CNPq and CAPES (Capes/Embrapa 15/2014). FZB is a CNPq fellow.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.theriogenology.2018.09.037>.

References

- [1] York L, Heffernan Rymer C, Panda N. A deterministic evaluation of heat stress mitigation and feed cost under climate change within the smallholder dairy sector. *Animal* 2016;11:1–10.
- [2] Intergovernmental panel on climate change. Climate change 2014: summary for policymakers. 2014 [accessed 14.02.17], https://www.ipcc.ch/pdf/assessment-report/ar5/syr/AR5_SYR_FINAL_SPM.pdf.
- [3] Renaudeau D, Collin A, Yhav S, Basilio V, Gourdiere JL, Collier RJ. Adaptation to hot climate and strategies to alleviate heat stress in livestock production. *Animal* 2012;6:707–28.
- [4] Garcia AR. Conforto térmico na reprodução de bubalinos criados em condições tropicais. *Rev Bras Reprod Anim* 2013;37:121–30.
- [5] Seo SN, McCarl BA, Mendelsohn R. From beef cattle to sheep under global warming? An analysis of adaptation by livestock species choice in South America. *Ecol Econ* 2010;69:2486–94.
- [6] Food and Agricultural Organization of the United Nations. FAO statistical pocketbook world food and agriculture. 2015 [accessed 14.02.17], <http://www.fao.org/3/a-i4691e.pdf>.
- [7] Silanikove N. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livest Prod Sci* 2000;67:1–18.
- [8] Hansen PJ. Effects of heat stress on mammalian reproduction. *Philos Trans R Soc Lond B Biol Sci* 2009;364:3341–50.
- [9] Marai IFM, El-darawany AA, Fadiel A, Abdel-hafez MAM. Physiological traits as affected by heat stress in sheep – a review. *Small Rumin Res* 2007;71:1–12.
- [10] Ridler AL, Smith SL, West DM. Ram and buck management. *Anim Reprod Sci* 2012;130:180–3.
- [11] Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. *Reprod Biomed Online* 2015;30:14–27.
- [12] Hamilton TRDS, Mendes CM, de Castro LS, de Assis PM, Siqueira AFP, Delgado JDC, et al. Evaluation of lasting effects of heat stress on sperm profile and oxidative status of ram semen and epididymal sperm. *Oxid Med Cell Longev* 2016;1687657:1–12.
- [13] Alves MBR, Andrade AFC de Arruda RP de, Batissaco L, Florez-Rodriguez SA, Oliveira BMM de, et al. Recovery of normal testicular temperature after scrotal heat stress in rams assessed by infrared thermography and its effects on seminal characteristics and testosterone blood serum concentration. *Theriogenology* 2016;86:795–805.
- [14] Rasooli A, Taha Jalali M, Nouri M, Mohammadian B, Barati F. Effects of chronic heat stress on testicular structures, serum testosterone and cortisol concentrations in developing lambs. *Anim Reprod Sci* 2010;117:55–9.
- [15] De K, Kumar D, Balaganur K, Kumar Saxena V, Thirumurugan P, Khurshed Naqvi SM. Effect of thermal exposure on physiological adaptability and seminal attributes of rams under semi-arid environment. *J Therm Biol* 2017;65:113–8.
- [16] Kottek M, Griener J, Beck C, Rudolf B, Rubel F. World map of the Köppen-Geiger climate classification updated. *Meteorol Z* 2006;15:259–63.
- [17] Empresa Brasileira de Pesquisa Agropecuária. Condições Meteorológicas da Estação da Embrapa Pecuária Sudeste. 2017 [accessed 10.03.17], <http://www.cnppe.embrapa.br/meteorologia/index.php?pg=inicio>.
- [18] Buffington DE, Colazon-Arocho A, Canton GH, Pitt D, Thatcher WW, Collier RJ. Black globe-humidity index (BGHI) as comfort equation for dairy cows. *Trans ASAE (Am Soc Agric Eng)* 1981;24:711–4.
- [19] Baeta FC. Responses of lactating dairy cows to the combined effects of temperature, humidity and wind velocity in the warm season. Columbia: Thesis, University of Missouri; 1985.
- [20] Colégio Brasileiro de Reprodução Animal. Manual para exame andrológico e avaliação de sêmen animal. third ed. Belo Horizonte: CBRA; 2013.
- [21] Bailey TL, Hudson RS, Powe TA, Riddell MG, Wolfe DF, Carson RL. Caliper and ultrasonographic measurements of bovine testicles and a mathematical formula for determining testicular volume and weight in vivo. *Theriogenology* 1998;49:581–98.
- [22] American Academy of Thermology. Veterinary guidelines for infrared thermography. 2013. <http://aathermology.org/organization/guidelines/veterinary-guidelines-for-infrared-thermography/>. [Accessed 15 October 2017].
- [23] Silva LKX, Sousa JS, Silva AOA, Lourenço JB, Faturi C, Martorano LG, et al. Testicular thermoregulation, scrotal surface temperature patterns and semen quality of water buffalo bulls reared in a tropical climate. *Andrologia* 2017;49:1–10.

- [24] Vencato J, Cestaro L, Vazzana I, Carrer G, Carlo E, Dara S, et al. Integrated evaluation of scrotal temperature and testosterone after GnRH administration in young bulls with low semen production. *Reprod Domest Anim* 2014;49:481–6.
- [25] Kahwage PR, Esteves SN, Jacinto MAC, Barioni Junior W, Machado R, Romanello N, et al. Assessment of body and scrotal thermoregulation and semen quality of hair sheep rams throughout the year in a tropical environment. *Small Rumin Res* 2018;160:72–80.
- [26] Rocha DR, Martins JA, van Tilburg MF, Oliveira RV, Moreno FB, Monteiro-Moreira AC, et al. Effect of increased testicular temperature on seminal plasma proteome of the ram. *Theriogenology* 2015;84:1291–305.
- [27] Bucak MN, Tuncer PB, Sariözkan S, Ulutas PB. Comparison of the effects of glutamine and an amino acid solution on post-thawed ram sperm parameters, lipid peroxidation and anti-oxidant activities. *Small Rumin Res* 2009;81:13–7.
- [28] Fonseca JF, Torres CAA, Maffili VV, Borges AM, Santos ADF, Rodrigues MT, et al. The hypoosmotic swelling test in fresh goat spermatozoa. *Anim Reprod* 2005;2:139–44.
- [29] Blom E. The ultrastructure of some characteristic sperm defects and a proposal for a new classification of the Bull spermogram. *Nord Vet Med* 1973;25:383–91.
- [30] Beletti ME, Mello MLS. Comparison between the toluidine blue stain and the Feulgen reaction for evaluation of rabbit sperm chromatin condensation and their relationship with sperm morphology. *Theriogenology* 2004;62:398–402.
- [31] Milczewski V, Chahad-Ehlers S, Spencoski KM, Morais RN, Soccol VT. Quantifying the effect of seasonality on testicular function of Suffolk ram in lower latitude. *Small Rumin Res* 2015;124:68–75.
- [32] Macías-Cruz U, López-Baca MA, Vicente R, Mejía A, Álvarez FD, Correa-Calderón A, et al. Effects of seasonal ambient heat stress (spring vs summer) on physiological and metabolic variables in hair sheep located in an arid region. *Int J Biometeorol* 2015;60:1279–86.
- [33] Kahwage PR, Esteves SN, Jacinto MAC, Barioni Junior W, Pezzopane JRM, Pantoja MHAP, et al. High systemic and testicular thermolytic efficiency during heat tolerance test reflects better semen quality in rams of tropical breeds. *Int J Biometeorol* 2017;61:1819–29.
- [34] Kastelic JP, Cook RB, Coulter GH. Contribution of the scrotum and testes to scrotal and testicular thermoregulation in bulls and rams. *J Reprod Fertil* 1996;108:81–5.
- [35] Schatten H, Constătescu GM. *Comparative reproductive biology*. first ed. Ames: Blackwell Publishing; 2007.
- [36] Kastelic JP, Byrne Cook R, Coulter GH. Contribution of the scrotum, testes and testicular artery to scrotal and testicular thermoregulation in bulls at two ambient temperatures. *Anim Reprod Sci* 1997;45:255–61.
- [37] Waites GMH. Testicular blood supply, lymphatic drainage, and secretion of fluid. In: Johnson AD, Gomes WR, Vandemark NL, editors. *The testis*, vol. I. New York: Academic Press; 1970. 101–239.
- [38] Coulter GH, Senger PL, Bailey RC. Relationship of scrotal surface temperature measured by infrared thermography to subcutaneous and deep testicular temperature in the ram. *J Reprod Fertil* 1988;84:417–23.
- [39] Paim TP, Borges BO, Lima PMT, Gomes EF, Dallago BSL, Fadel R, et al. Thermographic evaluation of climate conditions on lambs from different genetic groups. *Int J Biometeorol* 2013;57:59–66.
- [40] Brito LFC, Silva AEDF, Barbosa RT, Kastelic JP. 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* 2004;61:511–28.
- [41] Menegassi SRO, Barcellos JOJ, Dias EA, Koetz C, Pereira GR, Peripolli V, et al. Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. *Int J Biometeorol* 2015;59:357–64.
- [42] Setchell BP. The parkes lecture. Heat and the testis. *J Reprod Fertil* 1998;114:179–94.
- [43] Thundathil JC, Rajamanickam GD, Kastelic JP, Newton LD. The effects of increased testicular temperature on testis-specific isoform of Na⁺/K⁺-ATPase in sperm and its role in spermatogenesis and sperm function. *Reprod Domest Anim* 2012;47:170–7.
- [44] Cruz Júnior CA, Lucci CM, Peripolli V, Silva AF, Menezes AM, Morais SRL, et al. Effects of testicle insulation on seminal traits in rams: preliminary study. *Small Rumin Res* 2015;130:157–65.
- [45] Malama E, Bollwein H, Taitzoglou IA, Theodosiou T, Boscós CM, Kiossis E. Chromatin integrity of ram spermatozoa. Relationships to annual fluctuations of scrotal surface temperature and temperature-humidity index. *Theriogenology* 2013;80:533–41.