




Infrared Thermography and Testicular Morphometry in Rams of Different Breeds: Implications for Reproductive Fitness in Tropical Environments

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Abstract

The Brazilian sheep industry relies on extensive production systems, where rams are frequently exposed to high ambient temperatures and low relative humidity, potentially compromising reproductive efficiency. Testicular thermoregulation is critical for spermatogenesis, requiring scrotal temperature to be 2°C-6°C below core body temperature. This study aimed to evaluate testicular shape and infrared thermography (IRT) in 38 rams from four different breeds (Poll Dorset, Dorper, Santa Inês, and Suffolk) managed under tropical extensive conditions in Botucatu, Brazil. Testicular morphometry (scrotal circumference—SC, width, length, volume) and IRT of scrotal regions (dorsal, medial, ventral), as well as rectal, eye, and nose temperatures, were assessed. Significant differences were found in SC among breeds ($P = 0.024$), with Dorper rams having larger SC. IRT revealed significant variations in the ventral temperature of the right testicle ($P = 0.039$), dorsal temperature of the left testicle ($P = 0.006$), and mean dorsal temperature of both testicles ($P = 0.025$). Poll Dorset, a woolled breed, exhibited higher mean dorsal testicular temperatures, consistent with wool's insulating effect hindering heat dissipation. Conversely, Suffolk rams, despite being woolled, showed lower dorsal testicular

temperatures, suggesting complex breed-specific thermoregulatory mechanisms or anatomical adaptations. Dorper rams (hair, white-coated) showed lower ventral scrotal temperatures, indicating better heat dissipation. Nose temperature also varied significantly ($P < 0.001$), reinforcing breed-specific thermal regulation. These findings underscore the influence of breed characteristics, particularly coat type and color, on testicular thermoregulation and overall heat management in rams, providing valuable insights for selecting resilient breeding stock in challenging tropical environments.

Keywords

Sheep, Thermography, Testicular Thermoregulation, Heat Stress

1. Introduction

The Brazilian sheep industry largely relies on extensive production systems, characterized by vast pastures and significant exposure to environmental challenges. In such tropical climates, where high ambient temperatures and low relative humidity persist even during rainy seasons [1], the reproductive management of sheep flocks primarily occurs in the field. This necessitates a rigorous selection of breeding rams based not only on their genetic merit but also on their resilience and adaptability to the tropical conditions prevalent in the Southern Hemisphere [2].

The neuroendocrine system primarily controls spermatogenesis, the process of sperm production. However, this intricate process is highly sensitive to testicular temperature, with optimal spermatogenesis requiring the scrotal temperature to be maintained 2°C to 6°C below core body temperature [3] [4]. In sheep, the testes are located in the inguinal region, and their thermoregulation is achieved through three main mechanisms: apocrine sweat glands in the scrotal epidermis facilitate cooling through perspiration; the tunica dartos and cremaster muscle regulate testicular proximity to the body; and the pampiniform plexus, comprising the testicular artery and veins, enables efficient countercurrent heat exchange to cool arterial blood [5].

Maintaining efficient testicular thermoregulation is crucial for optimal reproductive performance, especially during periods of elevated environmental temperature and reduced relative humidity. Such conditions can limit reproductive potential and negatively impact spermatogenesis and semen quality. Notably, male sheep are often more susceptible to heat stress than females, exhibiting altered behavior and physiological parameters [6]. Prolonged exposure to high ambient temperatures can lead to a reduction in reproductive efficiency in rams due to the deleterious effects of heat on gametogenesis and libido [7]. The temperature gradient between the dorsal and ventral testicular poles in fertile rams, typically ranging from 2.3°C to 2.9°C [8], is inversely related to total morphological sperm defects and positively correlated with sperm plasma membrane integrity [9]. A

greater thermal gradient reflects enhanced testicular ability to maintain an optimal environment for sperm production and function. Even a 1 °C increase in scrotal surface temperature can significantly compromise sperm quality [10], leading to reduced mass movement, vigor, and progressive motility, decreased sperm concentration and viability, and an increase in major and/or minor sperm defects, potentially culminating in azoospermia in severe cases [2] [11] [12].

Phenotypic differences among sheep breeds, such as the presence or absence of wool, and variations in coat and skin color, can significantly influence their adaptability to tropical environments [13]. For instance, darker-coated animals tend to be more susceptible to heat stress under direct solar radiation in extensive systems than lighter-coated ones [13]. Studies have shown that white-coated Santa Inês sheep exhibit lower respiratory rates than dark-coated counterparts under the same temperature gradients [14], highlighting how phenotypic traits can influence the reproductive system's response to heat. Moreover, scrotal circumference, a widely used selection criterion, may not fully capture testicular thermoregulatory capacity. Rams with longer testicular shapes, for example, may possess a larger surface area for heat exchange, improved blood vessel distribution, and consequently, more efficient thermoregulation [15], suggesting a need for more nuanced assessment methods.

Infrared thermography (IRT) is a non-invasive technique that accurately measures surface temperature, thanks to advancements in camera technology [16]. This technique has been successfully applied to assess scrotal temperature in humans [17], bulls [9] [18], and rams [19], offering a valuable tool for investigating animal physiology and enhancing reproductive evaluations [20]. Despite its potential, comprehensive studies comparing testicular thermography across different sheep breeds in tropical extensive systems, especially considering variations in testicular morphology, are still limited.

Therefore, the objective of this study was to evaluate testicular shape and infrared thermography in rams from different breeds, aiming to provide insights into their thermoregulatory capacity and its implications for reproductive fitness in tropical environments.

2. Materials and Methods

2.1. Experimental Location and Environmental Data

The experiment was conducted at the “Laboratory for Applied Biotechnology in Sheep and Goat Reproduction” (LEBRAOC) within the Department of Animal Reproduction and Veterinary Radiology, School of Veterinary Medicine and Animal Science (FMVZ), São Paulo State University (UNESP), Botucatu, São Paulo, Brazil. The geographical coordinates of the experimental site are 22° 53'09" S latitude and 48° 26'42" W longitude, at an altitude of 804 meters above sea level. All experimental procedures involving animals were approved by the Ethics Committee on Animal Use (CEUA) of FMVZ-UNESP, under protocol number (129/2022).

Ambient temperature and relative humidity were obtained from the National

Institute of Meteorology (INMET) throughout the entire experimental period. These data were subsequently used to calculate daily values for the Temperature-Humidity Index (THI).

2.2. Animals and Management

A total of 38 clinically healthy rams, with no reproductive tract pathologies detected at the time of evaluation, were used in this study. The animals were divided into four breed groups: Dorper (n = 10): Hair sheep with predominantly white coats; Poll Dorset (n = 11): Woolled sheep with white fleeces; Santa Inês (n = 8): Hair sheep with dark coats; Suffolk (n = 10): Woolled sheep with white fleeces and black skin.

All rams were maintained extensively on natural pasture. They had free access to shaded areas, received *ad libitum* mineral supplementation, and had continuous access to fresh water.

2.3. Scrotal Infrared Thermography (IRT)

Digital infrared thermography of the scrotum was performed using a Flir® E53 24° infrared camera (Flir Systems Inc., Wilsonville, OR, USA). Thermograms were recorded with the camera positioned approximately 1 meter away from the animal. For each ram, images were captured focusing on three distinct scrotal regions: dorsal, medial, and ventral, as described by [21]. The acquired thermal images were then analyzed using the dedicated software provided with the thermographer (Flir Tools+).

Immediately following thermographic image acquisition, rectal temperature (RT) was measured. A digital clinical thermometer was carefully inserted 5 cm into the animal's rectum, and the reading was recorded.

2.4. Testicular Morphometry and Shape Evaluation

Testicular morphometry was assessed for both testes, measuring scrotal circumference (SC), testicular width (TW), testicular height (TH), testicular length (TL), and testicular volume (TV).

Scrotal circumference (SC) was measured using a flexible measuring tape placed around the widest part of the scrotum.

Testicular width (TW), testicular height (TH) and testicular length (TL) were measured using a universal caliper. For TL, the epididymal tail was excluded from the measurement. TW was measured at the medial portion of the testicle in a latero-medial direction, following Louvandini *et al.* (2008) [22].

Testicular volume (TV) was calculated using the formula described by El-Zelaky *et al.* (2011): $TV = 0.0396 \times (\text{Mean TL}) \times (\text{SC})$.

2.5. Statistical Analysis

Statistical analyses and graph generation were performed using GraphPad Prism version 9.3.0 (GraphPad Software Inc., San Diego, CA, USA). Data were presented

as means \pm standard deviation (SD). The normality of data distribution was assessed using the Shapiro-Wilk test. Variables were compared across groups using One-Way Analysis of Variance (ANOVA), followed by Tukey's post-hoc test for multiple comparisons.

3. Results

3.1. Testicular Morphometry

Regarding testicular morphometric evaluations, significant differences were observed only in scrotal circumference (SC) among the breeds ($P = 0.024$). No significant differences were found for testicular height (right and left testes), length (right and left testes), width (right and left testes), or overall testicular volume among the studied breeds ($P > 0.05$).

The statistical graph presents the scrotal circumference (SC) values observed among the Poll Dorset (PD), Dorper (D), Santa Inês (SI), and Suffolk (SU) breeds can be seen in supplementary (**Figure 1**).

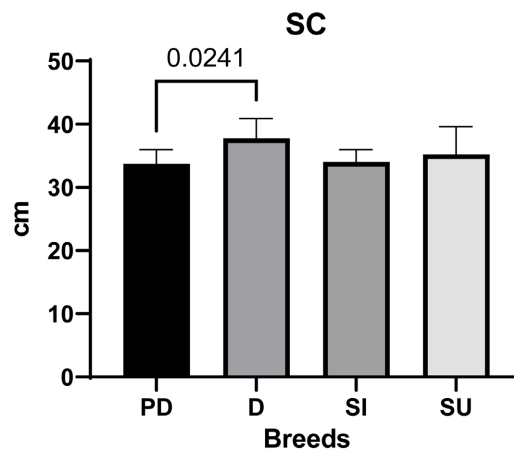


Figure 1. Scrotal Circumference (SC) values among the Poll Dorset (PD), Dorper (D), Santa Inês (SI) and Suffolk (SU) breeds.

3.2. Scrotal and Body Temperature (Infrared Thermography and Rectal Temperature)

Infrared thermography revealed significant differences in specific temperature parameters among the breeds. Significant variations were found for the ventral temperature of the right testicle ($P = 0.039$), dorsal temperature of the left testicle ($P = 0.006$), mean dorsal temperature of both testicles ($P = 0.025$), and nose temperature ($P < 0.001$). No significant differences were observed for other scrotal regions, mean medial and ventral temperatures of both testicles, rectal temperature, or eye temperature ($P > 0.05$).

In addition to the main results presented, the supplementary material provides visual information that reinforces the physiological differences among the evaluated breeds. **Figure 2** highlights the significant differences between Poll Dorset (PD), Dorper (D), Santa Inês (SI), and Suffolk (SU) breeds, while **Figure 3** shows

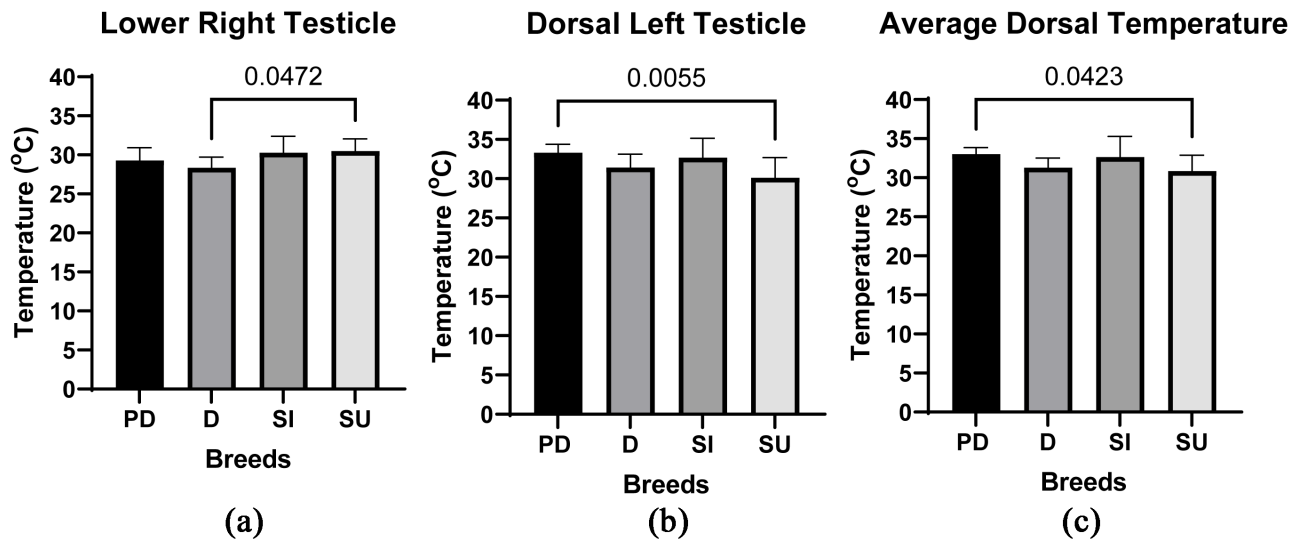


Figure 2. Significant differences found between the Poll Dorset (PD), Dorper (D), Santa Inês (SI) and Suffolk (SU) breeds.

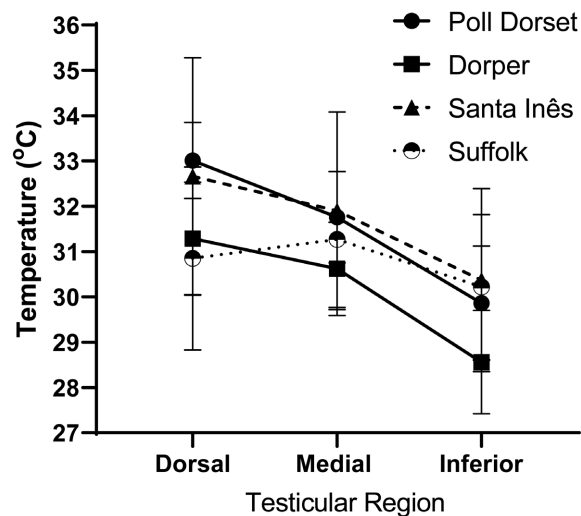


Figure 3. Average testicular temperature of the testicular regions of each breed.

the average testicular temperature of the different testicular regions in each breed. **Images 1-4** illustrate, through infrared thermography, the eye, muzzle, and testicular regions of representative animals from each breed, revealing variations in body surface temperature patterns. These images complement the quantitative data, allowing a clear visualization of the thermal particularities observed among the experimental groups.

4. Discussion

Testicular thermographic evaluation is crucial for assessing reproductive efficiency in animals, particularly during periods of elevated ambient temperatures, as heat stress significantly impairs spermatogenesis [6]. Our study found a significant difference in scrotal circumference (SC) among the breeds ($P = 0.024$), with Dorper rams exhibiting a larger SC compared to Poll Dorset (**Table 1**). While SC

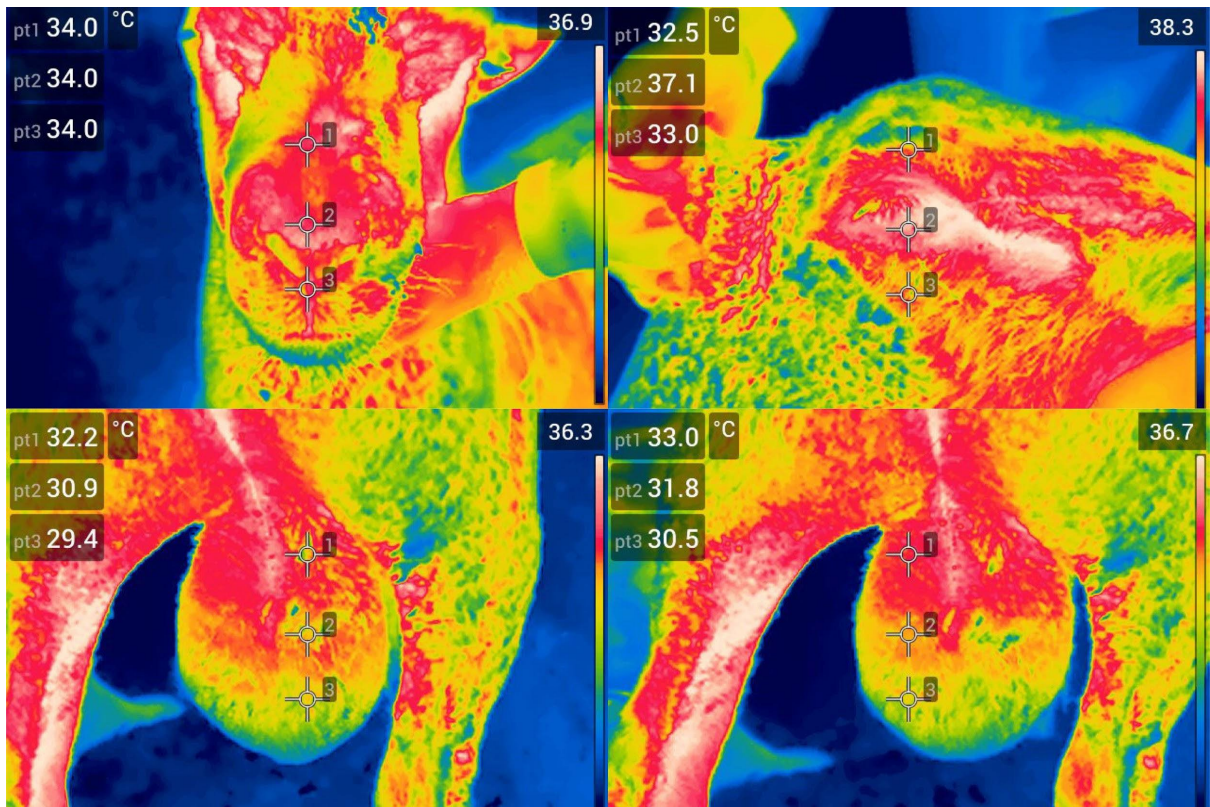


Image 1. Thermographic images of the eye, muffle and testicles of a Poll Dorset breed animal.

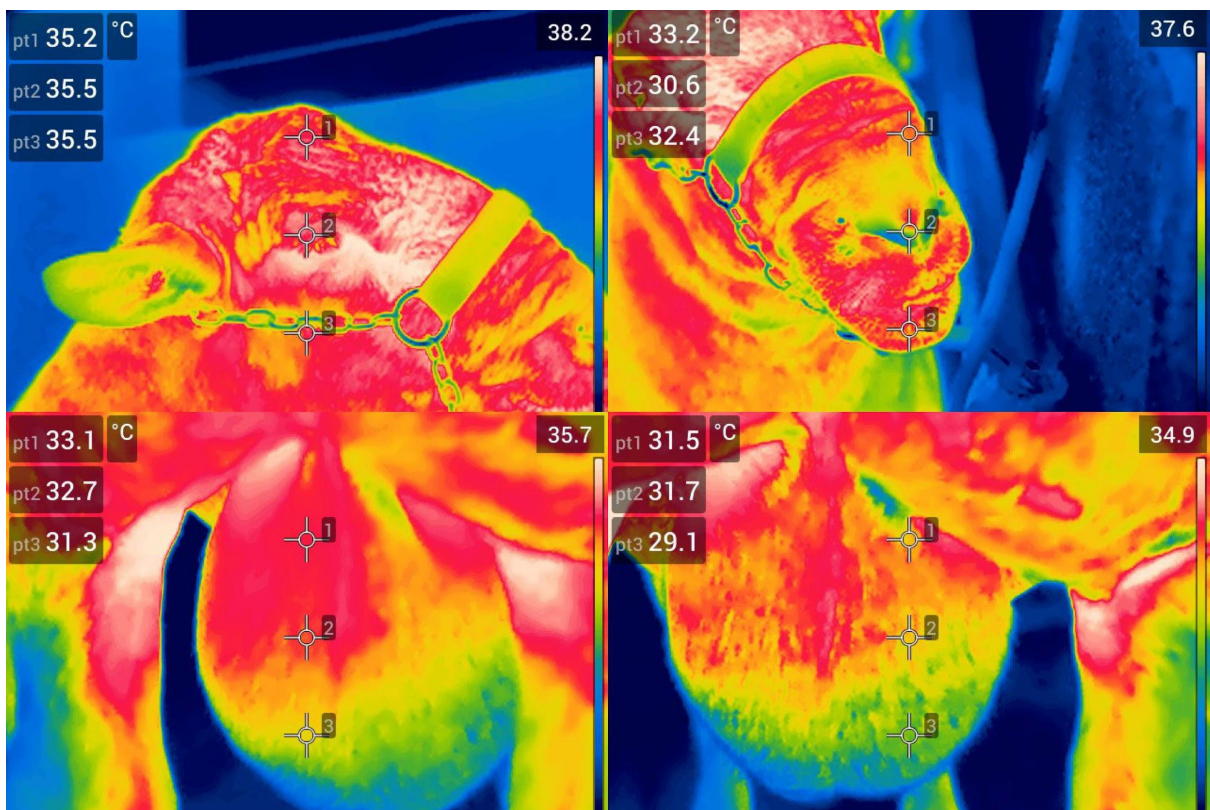


Image 2. Thermographic images of the eye, muffle and testicles of a Dorper animal.

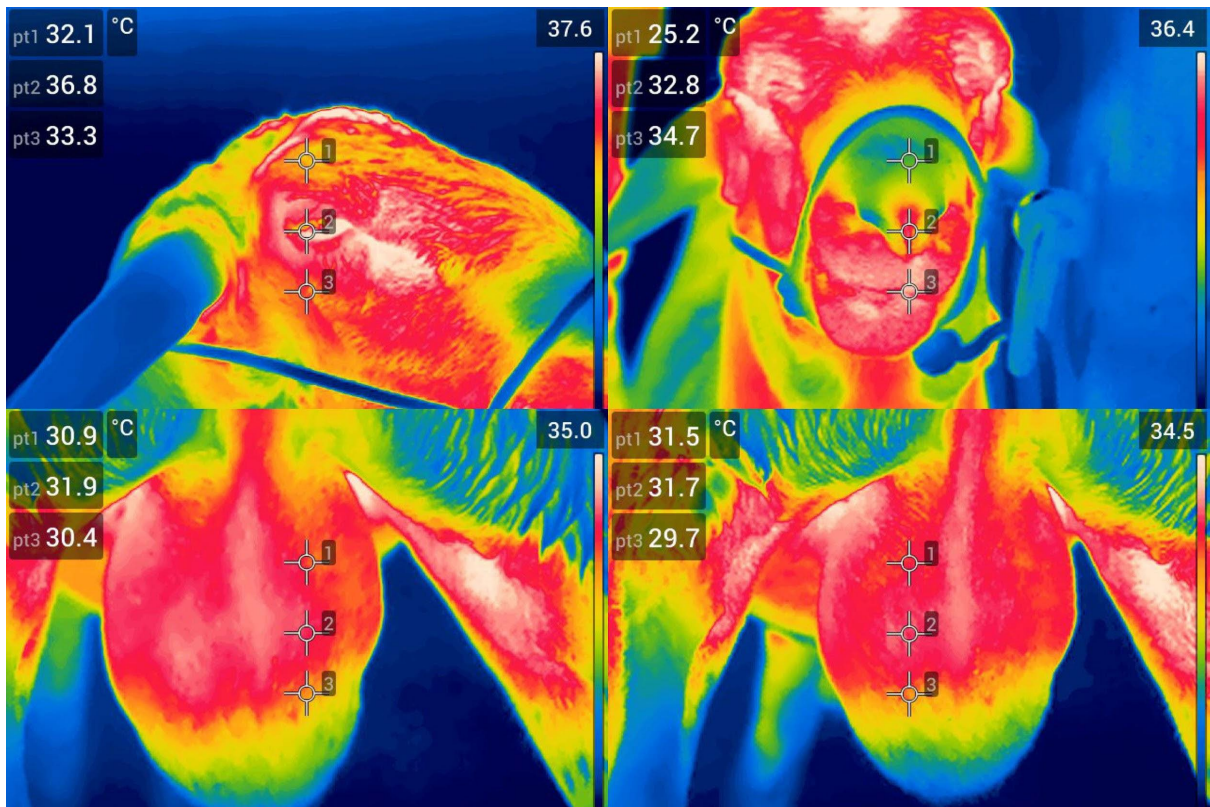


Image 3. Thermographic images of the eye, muffle and testicles of a Santa Inês animal.

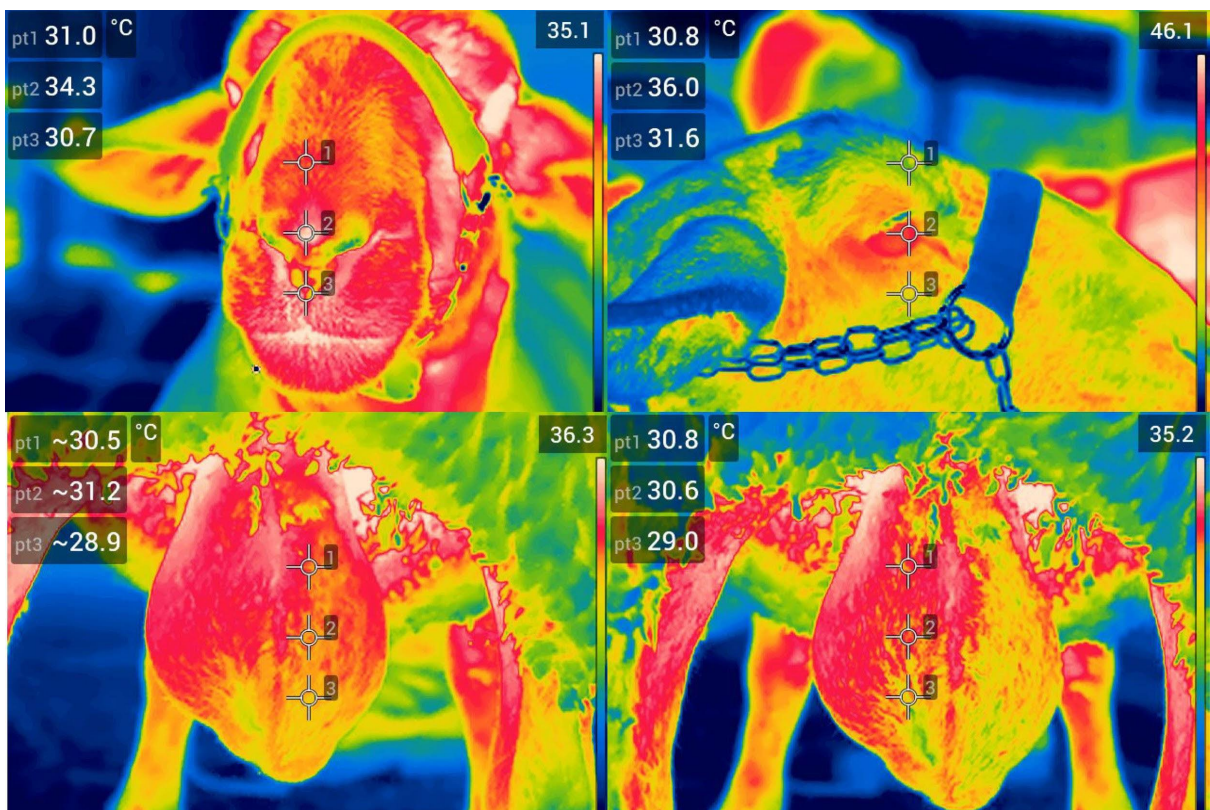


Image 4. Thermographic images of the eye, muffle and testicles of a Suffolk breed animal.

Table 1. Mean \pm standard deviation of testicular morphometric evaluations across different sheep breeds.

Variable	Poll Dorset	Dorper	Santa Inês	P-value
SC (cm)	33.7 \pm 2.3 ^b	37.7 \pm 3.1 ^a	34.0 \pm 1.9 ^{ab}	0.024
Height Right (cm)	10.2 \pm 1.4	9.7 \pm 1.4	9.1 \pm 1.0	ns
Height Left (cm)	10.3 \pm 1.2	9.7 \pm 1.3	9.2 \pm 0.8	ns
Length Right (cm)	6.2 \pm 0.7	6.7 \pm 0.7	6.8 \pm 0.5	ns
Length Left (cm)	6.4 \pm 0.6	5.5 \pm 0.7	6.6 \pm 0.5	ns
Width Right (cm)	6.1 \pm 0.5	6.4 \pm 0.8	5.6 \pm 0.3	ns
Width Left (cm)	6.2 \pm 0.5	6.2 \pm 1.0	5.8 \pm 0.3	ns
Testicular Volume (cm ³)	72.5 \pm 20.1	104.5 \pm 34.2	79.1 \pm 27.4	ns

^{a,b}: Means within a row with different superscripts differ significantly ($P < 0.05$). ns: non significant.

is a widely used indicator of testicular development and potential sperm production, its relationship with thermoregulatory capacity is complex. Previous research suggests that testicular shape, which was not significantly different among breeds in our study (testicular length, width, height, and volume), might play a more crucial role in heat dissipation by influencing the surface area available for heat exchange and blood vessel distribution [15]. Our findings suggest that while SC varies, other morphometric aspects might be more uniform across these breeds under the studied conditions, or that SC alone does not fully predict thermoregulatory efficiency.

Our infrared thermography results revealed significant differences in mean dorsal temperature of both testicles ($P = 0.025$) and dorsal temperature of the left testicle ($P = 0.006$) among the breeds. Specifically, Poll Dorset rams, a woolled breed, showed higher mean dorsal testicular temperatures compared to Suffolk, Santa Inês, and Dorper breeds (Table 2). This observation aligns with the insulating properties of wool, which, while beneficial in cold climates [23] [24], can impede heat dissipation from the testicles, leading to higher superficial temperatures. Elevated temperatures in the dorsal region of the testis can particularly affect the caput epididymis, a critical site for sperm maturation, where biochemical modifications to the plasma membrane and chromatin stabilization occur, enabling spermatozoa to bind to the zona pellucida for fertilization. Therefore, such temperature increases in this area may compromise subsequent sperm fertilizing ability. It is important to acknowledge that assessing the full extent of heat stress impact on gametogenesis requires additional examinations, including detailed sperm concentration and morphology analyses, which were beyond the scope of the current study.

Interestingly, despite being a woolled breed with black skin and white fleece, originating from the Northern Hemisphere and thus potentially more susceptible to heat stress [25], Suffolk rams presented lower mean dorsal testicular temperatures

Table 2. Mean \pm standard deviation of testicular and body temperature evaluations across different sheep breeds.

Region	Poll Dorset	Dorper	Santa Inês	Suffolk	P-value
Right Testicle					
Dorsal (°C)	32.7 \pm 0.8	31.2 \pm 1.1	32.6 \pm 2.8	31.6 \pm 1.6	ns
Medial (°C)	31.4 \pm 1.3	30.4 \pm 1.1	31.8 \pm 2.1	31.3 \pm 1.7	ns
Ventral (°C)	29.3 \pm 1.6 ^{ab}	28.3 \pm 1.4 ^b	30.3 \pm 2.1 ^{ab}	30.5 \pm 1.6 ^a	0.039
Left Testicle					
Dorsal (°C)	33.3 \pm 1.0 ^a	31.4 \pm 1.7 ^{ab}	32.7 \pm 2.5 ^{ab}	30.1 \pm 2.6 ^b	0.006
Medial (°C)	32.1 \pm 1.2	30.8 \pm 1.2	31.9 \pm 2.5	31.2 \pm 1.5	Ns
Ventral (°C)	30.4 \pm 1.1	28.9 \pm 1.2	30.5 \pm 2.1	29.9 \pm 1.7	ns
Mean of Both Testicles					
Dorsal (°C)	33.0 \pm 0.8 ^a	31.3 \pm 1.2 ^b	32.7 \pm 2.6 ^b	30.8 \pm 2.0 ^b	0.025
Medial (°C)	31.8 \pm 1.0	30.6 \pm 1.0	31.9 \pm 2.2	31.3 \pm 1.5	ns
Ventral (°C)	29.9 \pm 1.3	28.6 \pm 1.1	30.4 \pm 2.0	30.2 \pm 1.6	ns
Body Temperatures					
Rectal Temp. (°C)	39.2 \pm 0.2	38.9 \pm 0.3	39.3 \pm 0.3	39.2 \pm 0.6	ns
Eye Temp. (°C)	36.6 \pm 1.4	35.0 \pm 1.6	36.9 \pm 2.3	35.9 \pm 1.9	ns
Nose Temp. (°C)	33.7 \pm 2.1 ^{ab}	30.8 \pm 3.3 ^{bc}	31.3 \pm 2.8 ^b	35.3 \pm 0.8 ^a	<0.001

^{a,b,c}: Means within a row with different superscripts differ significantly ($P < 0.05$). ns: non-significant.

compared to Poll Dorset, Santa Inês, and Dorper. This seemingly counterintuitive finding warrants further investigation. While darker pigmentation typically absorbs more heat, leading to higher body surface temperatures [26], the lower dorsal scrotal temperature in Suffolks might be influenced by specific anatomical configurations, such as a greater distance of the testicles from the animal's body or more efficient local blood flow [27] [28]. Conversely, when evaluating the mean ventral testicular temperature (Table 2), although not statistically significant across all breeds, Suffolk rams tended to exhibit higher values. This regional variation in temperature patterns highlights the complexity of scrotal thermoregulation and suggests that different areas of the scrotum might respond differently to heat challenges based on breed-specific traits.

The absence of wool and variations in coat color significantly influence sheep behavior and thermal stress response [2] [10]-[12]. Hair breeds and those with lighter coat colors generally possess a greater capacity for heat reflection and dissipation. This is corroborated by our finding of a significant difference in the ventral temperature of the right testicle between Dorper (hair, white-coated) and Suffolk rams, with Dorpers exhibiting lower temperatures. This suggests that Dorper rams' characteristics, such as their hair coat, confer an advantage in maintaining

lower testicular temperatures in tropical environments, potentially contributing to better reproductive resilience.

Finally, the study observed a significant difference in nose temperature among breeds, notably higher in Suffolk rams compared to Dorper (**Table 2**). While not directly linked to the reproductive system, this finding supports the general principle that darker skin/coat coloration leads to increased heat absorption [28]. The nose region, being uninsulated and exposed, often reflects peripheral heat exchange. This broader thermoregulatory response, even in areas not directly related to reproduction, reinforces the breed-specific differences in heat management and their potential implications for overall adaptation to hot climates.

5. Conclusion

Significant differences were identified among the studied sheep breeds. In terms of testicular morphology, only scrotal circumference showed significant variation. For thermographic evaluations, significant differences were found in the ventral temperature of the right testicle, dorsal temperature of the left testicle, mean dorsal temperature of both testicles, and nose temperature. These findings underscore the importance of breed-specific characteristics, particularly coat type and color, influencing testicular thermoregulation and overall heat dissipation in rams under tropical conditions, providing valuable insights for reproductive management and selection in these environments.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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