



Scrotum bipartite in sheep as a parameter indicative of adaptation to semi-arid climates: A thermographic and reproductive study

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ARTICLE INFO

Article history:

Received 24 January 2018

Received in revised form

20 July 2018

Accepted 26 July 2018

Available online 29 July 2018

Keywords:

Reproduction

Morphology

Temperature

Ruminants

Semi-arid

ABSTRACT

With the objective of assessing the influence of scrotum bipartition on scrotum-testicular thermoregulation and semen quality in sheep native to a semiarid region, 14 adult crossbred rams were placed into groups, GI (7 with bipartition in the scrotum) and GII (7 without bipartition). Images were taken of the caudal scrotum surface using a Fluke (Ti25[®]) thermographic camera, for temperature analysis. Two semen collections were made, at an eight-day interval, by electroejaculation, to analyze macroscopic and microscopic parameters. It was observed that the surface temperatures of the proximal, medial and distal regions of the testicle and the epididymis tail did not present significant statistical difference ($p > 0.05$) between the groups. The GI showed a great ability to regulate the temperature in the tail region of the epididymis ($p = 0.062$), location of the bipartition, and the difference in temperature between the body surface and the epididymis tail was 0.54°C much lower than the G2. Although no significant statistical difference ($p > 0.05$) was observed, the animals with bipartition presented higher means for body surface temperature, showing greater efficiency in heat dissipation and indicating that these animals used peripheral vasodilation on a larger scale to eliminate excess heat and thus had a lower energy expenditure. The semen parameters studied in both groups were within the desirable values for the species, with no differences between the groups ($p > 0.05$). Higher scrotum testicular values were observed (scrotum circumference GI = 30.40 ± 0.53 and GII = 28.42 ± 1.13 cm, testicle length, right and left, respectively GI = 8.14 ± 0.90 cm, 8.00 ± 0.00 cm and GII = 7.28 ± 0.04 cm, 7.28 ± 0.48 cm) and bodyweight (GI = 44.57 ± 5.25 Kg and GII = 39.85 ± 1.57 Kg) in rams with scrotum bipartition ($p > 0.05$). It is concluded that scrotum bipartite in rams was shown to be an evolutionary indicator showing that animals with this characteristic dissipate heat more efficiently, have bigger scrotum-testicle biometrical parameters and heavier body weight. However, as the rams with scrotum bipartite presented division of less than 50% of the scrotum length, this degree did not influence the scrotum surface temperature and semen quality, as has been observed in goats with the same characteristic.

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1. Introduction

Goat and sheep rearings of major social and economic

importance in many countries because these animals are highly adaptable to adverse conditions in different regions, especially semi-arid regions, with irregular rainy seasons and periodic droughts. However, these factors can limit the animals reproduction process. When management practices cannot in part correct these imbalances, natural selection processes will favor those animals better adapted to the adverse conditions of the environment.

Goats from the arid and semi-arid regions of East Africa present morphological alterations in the reproductive organs, such as the

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appearance of scrotum bipartite [1]. This characteristic has been observed frequently in goats reared in the Northeast of Brazil, and is known as “bipartite scrotal sack” [2]. Studies have shown that this anatomy considerably amplifies the surface of each testicle exposed to environmental temperature, providing better heat dissipation, with consequent increase in the testicle biometrical parameters, sperm quality and reproductive efficiency of these animals compared to those that have a simple scrotum [2–4].

To analyze the effect of morphological alterations in the reproductive organs on reproductive efficiency, it is important to correlate them with other factors that affect animal reproduction, especially those regarding environmental temperature and relative humidity [5].

Scrotal conformation and environment temperature exercise considerable influence on the testicle temperature. When the environmental temperature increases, the thermoregulation mechanism is damaged and testicular degeneration is more likely. This increase in temperature has serious consequences for semen quality and subsequently for embryo fertilization and survival and it interferes directly in the fertility results at birth [6–10].

To meet the demands of semen production and quality, the scrotum temperature should be 2 to 6 °C lower than that of the abdomen, so that verifying the scrotum/testicular temperature contributes to a better understanding and assessment of the reproductive function of the animal [11].

With advances in technology, new equipment is always available that facilitates in loco analysis and provides more significant and precise data. Infrared thermography is important because it is a non-invasive method to assess skin surface temperature, that captures charges of infrared radiation and expresses the heat gradient in a pattern of colors [12].

Variation in skin temperature results from changes in the tissue perfusion and blood flow [13] and the use of a thermographic camera that detects, with high sensitivity, radiation emitted by the bodies so that minimal changes in temperature on the surface can be monitored rigorously.

In this context, the objective of the present research was to assess the influence of scrotum bipartite on thermoregulation and semen quality in rams native to a semi-arid region.

2. Material and methods

2.1. Ethics committee

The methodology protocols of the present study followed the ethical concepts in animal experimentation, approved by the Ethics Committee for Animal Use-CEUA, protocol No 277–2015, of the Committee for Ethics in Research at the Federal University of

Campina Grande- UFCG, Patos-PB, Brazil.

2.2. Animals, research location and formation of experimental groups

The study was carried out in June and July on the farm Fazenda Águas da Tamanduá, (6°47'46.95"S and 38° 8'51.78"W), located in the irrigated perimeter of Várzeas de Sousa, municipality of Sousa, Paraíba, Brazil. The climate in the region is the Aw' type by the Köppen classification [14], with 780 mm mean annual rainfall [15], concentrated from January to May and 27 °C mean annual temperature.

Fourteen rams of no specific defined breeding (with predominance of Santa Inês breed) were used, 12–18 months old, identified by inspecting the dental arch [16], with homogeneous body score (44.57 kg mean weight). They were divided into two groups considering the scrotal conformation, as proposed by Almeida [17] for goats, making an analogy to the classification by Nunes et al. [2]. Group I (GI) contained animals that had scrotum bipartite, on average, up to 16% of the scrotum length, and group II (GII) consisted of seven animals with simple scrotum (Fig. 1).

The animals were kept under semi-intensive management, released in the morning to graze in a 60 m × 362 m paddock with native pasture and were corralled at the end of the afternoon, where they had access to corn and soybean-based concentrate (80:20) and mineral salt.

2.3. Environmental variables

The environmental variables air temperature (T°Ar) and relative air humidity (UR%) and black globe temperature (TGN) were obtained using a HOBO (Temp/RH/2-U12-013) type datalogger, the external cable was attached to the black globe and installed in the animals shelter.

The datalogger was programmed, using its software, to record the data set every hour, for 24 h before and after collecting the thermographic data and during the experiment days, in order to obtain the means of all the variables for each day of the experiment.

The environmental data obtained was used to calculate the indices of black globe temperature and humidity (ITGU), according to the formula: $Tgn + 0.36 \cdot Tpo + 41.5$ [18], where Tgn is the temperature of the black globe and Tpo: Temperature of the dew point.

2.4. Physiological parameters

Rectal temperature (TR) and respiratory frequency (FR) were recorded at two times, 8 a.m. and 3 p.m. The TR was determined



Fig. 1. Photographs of ram scrotum. A) Animal with scrotum bipartite (GI), B) animal without scrotum bipartite (GII).

using a veterinary clinical thermometer with scale to 44 °C, placed directly in the animal's rectum. The respiratory frequency was measured by heart sound auscultation using a flexible stethoscope placed on the thoracic region, and the number of respiratory movements per minute was obtained.

2.5. Thermography

Thermographic images were obtained of each animal, one of the whole animal and the other of the scrotum caudal surface. The thermograms were obtained in the morning (08:00–09:00 h) and in the afternoon (2 p.m.–3 p.m.), with the animals stationary in the shade and with a minimum of handling, and one collection was made per week for four weeks.

The images were obtained using a model Ti25® (Fluke) thermal imager approximately 1 m from the animal. A 0.98 emission was used with 0.1 °C precision. The thermographic images generated had 160 × 120 pixel resolution, and each pixel represented a temperature point.

The thermograms were assessed by the SmartView 3.2 software (Fluke). To analyze the temperatures, lines were drawn in the proximal, mid and distal regions of the testicle and in the epididymis caudal region, presenting the maximum, mean and minimum temperature of each delimited area (Fig. 2).

2.6. Semen analysis

Two semen collections were made at an eight-day interval, by electroejaculation, using an automatic device (Boijektor-2001). The semen samples were kept on a heated table at 37 °C for immediate

analysis of progressive sperm motility, sperm vigor and gross motility. The sperm collection and analysis procedures followed the standards of the Andrology Manual of the Brazilian College of Animal Reproduction (CBRA) [19].

2.7. Scrotum-testicle biometrics and body weight

The animals were weighed and the scrotum perimeter and testicular length measurements confirmed. The body weight was obtained on scales appropriate for small ruminants (Acb Baltec). The scrotal perimeter was measured with a tape measure, at the widest part of the scrotum, and the testicular length, the distance between the head and tail of the organ, was measured with a 0.05 mm precision pachymeter.

2.8. Statistical analysis

A 2 × 7 complete randomized design (two treatments and seven replications) was used. The results of the thermographic analysis were submitted to analysis of variance and the mean values compared by the F test. The means of the data of the physiological variables, semen analysis, biometric parameters and body weight were compared by the Student T test and the level of significance adopted was 5%. The analyses were made with the SAEG, v. 9.1 program.

3. Results

Table 1 shows the means of the environmental variables and an increase was observed in the ITGU in the afternoon period and an increase of approximately 10° in the environmental temperature (TA) from the morning to the afternoon period. The relative humidity (UR) presented values below the ideal for rearing domestic animals in the afternoon period.

Higher means were observed in the afternoon period for all the physiological variables assessed (FR, TR and TSC) (Table 2), and no significant statistical difference was observed between the groups ($p > 0.05$).

Although there was no significant statistical difference between groups ($p > 0.05$), the results of the thermographic analysis showed that, regardless of the scrotal morphology and the period of the day, the scrotum surface temperatures decreased gradually from the

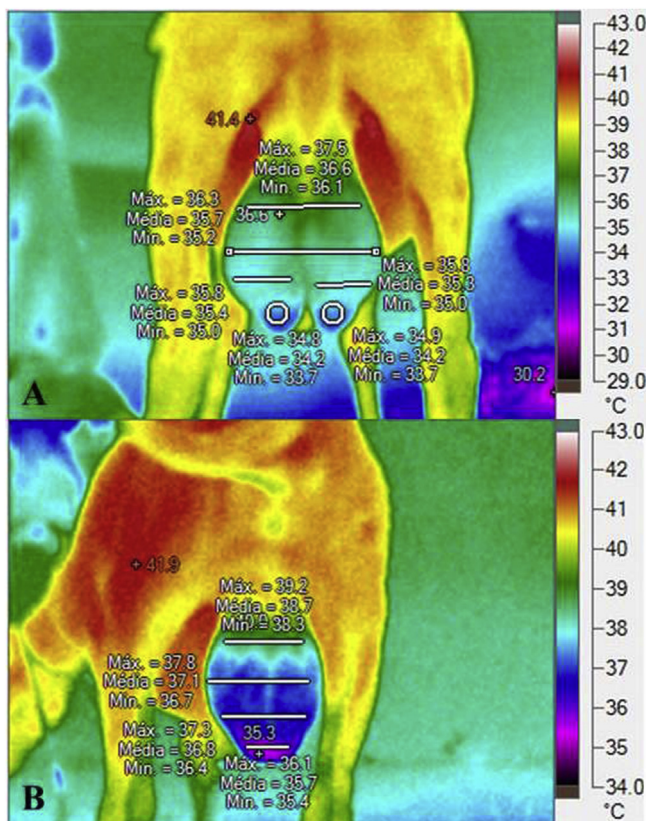


Fig. 2. Infrared thermographic image demonstrating location of the markers to assess ram scrotum surface temperature. A) ram with scrotum bipartite (GI), B) ram without scrotum bipartite (GII).

Table 1

Means of the environmental variables, environmental temperature (TA), black globe temperature (TGN), relative humidity (UR) and the black globe temperature at the morning and afternoon data collection times. Sousa, PB, Brazil, 2017.

Data collection time	Variables			
	TA (°C)	TGN (°C)	UR (%)	ITGU
Morning	22.37	22.50	58.25	69.04
Afternoon	32.25	32.87	39.87	80.49

Table 2

Means of the physiological variables respiratory frequency (FR) and rectal temperature (TR) and body surface temperature (TSC) in rams at the morning and afternoon data collection times. Sousa, PB, Brazil, 2017.

Data collection time	Group	FR	TR	TSC
Morning	GI	49.60 ± 10.69	38.36 ± 0.62	37.41 ± 1.16
	GII	55.85 ± 19.30	38.14 ± 0.59	36.96 ± 1.87
Afternoon	GI	60.60 ± 21.91	38.86 ± 0.35	39.29 ± 0.89
	GII	67.96 ± 17.88	38.68 ± 0.47	38.91 ± 1.44

GI: Group I (Bipartite); GII: Group II (Non-bipartite). Values with different superscripts in the same column differ ($P < 0.05$).

Table 3
Mean temperature (°C) and standard deviation of the proximal (TP), medial (TM) and distal (TD) regions of the scrotum the epididymis tail (TCE) of the groups of rams studied. Sousa, PB, Brazil, 2017.

Data collection time	Group	TP	TM	TD	TCE
Morning	GI	36.17 ± 0.56	35.54 ± 0.59	35.04 ± 0.88	33.86 ± 0.88
	GII	36.25 ± 0.82	35.53 ± 0.88	34.84 ± 0.95	34.06 ± 1.17
Afternoon	GI	36.74 ± 0.70	36.14 ± 0.64	35.81 ± 0.70	34.87 ± 0.88
	GII	36.47 ± 1.11	35.77 ± 0.95	35.43 ± 0.86	34.90 ± 0.89

GI: Group I (Bipartite); GII: Group II (Non-bipartite). Values with different superscripts in the same column differ ($P < 0.05$).

proximal to the distal third of the organ (Table 3).

Even though the GI presented the higher body surface temperature, it showed larger capacity to regulate the temperature in the epididymis caudal region ($p = 0.062$), location of the bipartition, and the difference in temperature between the body surface and the epididymis tail was 0.54 °C lower than in GII.

The period of the day also showed influence regarding the scrotum temperature in the rams (Table 3), and the afternoon period presented higher scrotum temperature than in the morning period ($p > 0.05$).

The mean values for the macroscopic parameters (volume) and microscopic parameters (turbulence, progressive motility and vigor) of the ram semen in the two experimental groups were not significantly different ($p > 0.05$) and are shown in Table 4.

The bodyweight, scrotum circumference measurements and testicular length differed statistically ($p > 0.05$) between the two groups studied, as shown in Table 5.

4. Discussion

The increase in the ITGU environmental temperature indicates a risk situation for the animals in the study in this period, based on data from the National Weather Service USA [20]. As a consequence of this increase in temperature, the animals may develop heat stress, causing damage to food intake and digestion [21–23], alteration in the metabolic rate [24], that negatively affects performance [25,26] and the reproductive function [27,28].

According to data in the literature, the heat comfort zone (ZCT) for hairless rams is between 20 and 30 °C, and temperature is critical over 35 °C [29], therefore, in the morning period the animals in the present study were within the ZCT, but in the afternoon period the temperatures were above the values determined, but did not reach the higher critical temperature.

Regarding the influence of relative humidity (UR) on domestic animal rearing, the ideal is between 50 and 70% [29], and values

were observed below this in the afternoon period in our study.

Thus, when the TA was high and the UR was outside the ZCT limits, the ability of the animals to dissipate heat may have been impaired, making it necessary to use other forms of heat loss [30] a fact that explains the reason for the increase in the ram FR in the afternoon period, that was higher in GII, thus keeping the TR within the acceptable limits, according to the literature [31]. However, it was observed that the TSC (Body Surface Temperature) presented higher means in the GI rams, indicating that these animals used peripheral vasodilation on a larger scale to eliminate excess heat, demonstrated by the increase in the TSC and respiratory thermolysis on a small scale compared to the rams in GII, especially in the afternoon period, and for having maintained the rectal temperature within the physiological levels, indicating that this mechanism was efficient and prevented hyperthermia. This may indicate that the animals in GI were more adapted to high temperature environments, as they use better the sensitive way of heat loss, in which there is no energy expenditure, in contrast the animals in GII that used evaporative mechanism (sudoresis and/or respiratory frequency) to lose heat, so that high FR may be an efficient way for heat loss for short periods, but continuous accelerated respiration may interfere in food intake and rumination and add endogenous heat from muscle activity and energy deviation, that could be used in other metabolic and productive process [32].

It must be considered that, because the animals in GI presented the genetic characteristic of scrotum bipartite, a morphological adaptation reported mainly in animals reared close to the equator, in regions with a hot climate [1,2], these animals may express their adaptation potential, not only with the scrotum bipartite characteristic, but also with other variables that could be analyzed as, for example, forms of heat loss.

Regarding the thermographic analysis of the caudal scrotum surface, the fact that the scrotum surface temperatures of the rams in the present study decreased gradually from the proximal to the distal third of the organ was due to the heat loss contraflow mechanism that, in rams fall reaches fall of approximately 4 °C from the internal inguinal canal to the testicle surface [33].

Other factors may interfere in the testicle thermoregulation mechanism, such as difference in the morphological characteristics of the spermatic chord at different ages [34]. It was also observed that goats with scrotum bipartite had bigger spermatic chord diameter and length and that the segment of the testicle artery contained in the chord was bigger than in the animals without this characteristic, which provides more extended contact between the testicle artery and veins that favors heat exchange and contributes

Table 4
Semen analysis of the groups of rams studied (mean ± standard deviation). Sousa, PB, Brazil, 2017.

Group	Volume	Gross motility	Motility	Vigor
GI	0.35 ± 0.49	3.85 ± 0.86	73.57 ± 11.50	3.78 ± 0.80
GII	0.57 ± 0.75	4.14 ± 0.66	76.42 ± 8.41	3.92 ± 0.82

GI: Group I (Bipartite); GII: Group II (Non bipartite). Values with different superscripts in the same column differ ($P < 0.05$).

Table 5
Mean values and standard deviation for bodyweight, scrotal perimeter and testicle length in the two groups of rams studied. Sousa, PB, Brazil, 2017.

Group	Body weight (Kg)	Scrotal circumference (cm)	Testicular length (cm)	
			Dir.	Esq.
GI	44.57 ± 5.25 ^a	30.40 ± 0.53 ^a	8.14 ± 0.90 ^a	8.00 ± 0.00 ^a
GII	39.85 ± 1.57 ^b	28.42 ± 1.13 ^b	7.28 ± 0.04 ^b	7.28 ± 0.48 ^b

GI: Group I (Bipartite); GII: Group II (Non bipartite). Values with different superscripts in the same column differ ($P < 0.05$).

to testicle thermoregulation [35], and further bipartition provides better heat dissipation because it increases the skin surface in contact with the environment, as observed in goats [36].

The semen volume of the sheep in GI and GII was within the range of normality for the species, for which volumes are described in the literature that range from 0.5 [37] to 1.1 mL [33]. The results of the present study are in line with those observed by Nunes et al. [1], Viera et al. [38] and Almeida et al. [4], who studied goats with different scrotal conformations and did not observe differences in semen volume between the groups studied.

The other parameters studied, turbulence, motility, vigor, sperm concentration and sperm morphology, were within the desirable values in both groups [33,37], although the literature indicates that high temperatures affect more the parameters of motility, vigor, sperm concentration and morphology [39,40]. On the other hand, studies have shown that the hairless breeds are well adapted to regions with semiarid climate and the semen quality is not influenced by climatic factors [41–43].

In this way, as there was no statistical difference between the two groups of rams studied regarding the semen parameters, there is indication that the animals demonstrated adaptability to the climatic conditions of the region. However, it is pointed out that in goats better semen quality was shown when the animals presented bipartition longer than 50% of the scrotal length [4,44] whereas the animals in the present study presented bipartition of less than 50% of the scrotum length.

Thus, similar to in our study of rams, it has also been reported for goats that animals with scrotum bipartition also present heavier bodyweight and longer testicle length [4].

It has already been shown that several factors interfere in the scrotal biometry parameters such as the relation of these parameters in function of bodyweight and period of the year [45]. Another factor is environmental temperature, that also considerably influences the scrotum circumference, as demonstrated in Moxotó goats and crossbred Moxotó-Pardo Alpina goats submitted to scrotum insulation, in which as the scrotum temperature increased, the scrotum perimeter values decreased by approximately 4.15 cm eight weeks after starting insulation [46].

5. Conclusion

It was concluded that scrotum bipartite in rams was shown to be an evolutionary indicator, as the animals with this characteristic presented more efficient heat dissipation, bigger biometrical scrotum-testicle parameters and heavier bodyweight. However, as the rams presented scrotum bipartite less than 50% of the scrotum length, this degree did not influence the scrotum surface temperature and semen quality, as observed in goats with the same characteristic.

Acknowledgments

The authors thank the Comissão de Aperfeiçoamento de Pessoal do Nível Superior (CAPES) (No. 2015403010) for the Master of science grant. They also thanks the owner of the Fazenda Águas da Tamanduá, Pierre Landolt, for agreeing to carry out the study on his property.

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