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Abstract

The present study was performed with the objective to evaluate the effect of scrotal temperature elevation under condition of environmental discomfort (high temperature and humidity) in Santa Inês rams. This inedited study was conducted in Amazonian region during the rainiest period of the year, characterized by high temperature and humidity; condition related to thermal discomfort of the four rams used. The breeding soundness evaluations (BSE) (scrotal-testicular-epididymal biometry and seminal and libido evaluation) were realized before and after the scrotal insulation. The injury resulted in a decrease in the biometric values of scrotal circumference, length, width and testicular volume, between the period of pre-insulation and the one week after the damage ($P < 0.05$), and the reestablishment to normality occurred on the 13th week post-insulation. The seminal parameters (sperm aspect, sperm mass motility, motility, vigor, concentration and live spermatozooids percentage) showed significant reduction in the post-insulation firsts samplings, with the reinstatement of physiological standards on the 11th week post-insulation. In conclusion, the Amazonian region's climate (high temperature and humidity rate) indicated heat stress on animals, which had helped to intensify the deleterious effects caused by the scrotal insulation on spermatogenesis and spermatozooids maturation retarding the testicular regeneration process.

Keywords: Scrotal insulation, environmental heat stress, semen, scrotum-testicular biometry, ovine.

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Introduction

The elevation of the testicular temperature is one of the most common causes of animal subclinical infertility and sterility. This condition is responsible for testicular degeneration, spermatogenesis abnormalities and spermatid dysfunction resulting in sperm abnormalities (Saadi *et al.*, 2013). Moreover the spermatid cells during meiosis and the spermiogenesis are the most susceptible to the testicular temperature increase, probably because of the high chromatin reshuffling in these phases, leading to a spermatogenesis breakdown (Rahman *et al.*, 2011).

The scrotum-testicular thermoregulation is responsible to avoid a prejudicial temperature variation using heat counter-current exchange, blood stream regulation, scrotum strain and transpiration, maintaining the testicular temperature between 2°C and 6°C below the body temperature (Kastelic *et al.*, 1996; Oliveira and Silva, 2013). To investigate the effects of the testicular temperature increase over the sires behavior against the weather a several techniques were used such as the animal heat environment exposure, the insulation of scrotal neck or the insulation of the scrotum, testicular immersion in hot water, induced cryptorchidism and testicles exposure to radiation (Gabaldi and Wolf, 2002; Setchell, 2006).

The Amazon area is located near to the Equator line and so, not showing a photoperiod variation, making the small ruminants capable to reproduce all over the year, is a region characterized by an equatorial weather a combination of high temperature and humidity, causing in farm animals, including ovine and caprine, a great discomfort (Costa *et al.*, 2010) interfering in their reproductive and productive abilities. Considering the influence of the environment temperature on animal reproduction, in this peculiar case on the Amazonian ovine production, this study aimed to verify the andrological characteristics changes in Santa Inês rams subjected to a scrotal insulation in the Amazonian climate and determine the recovery time from the induced injuries. There was no similar study on the same climate condition (ie. thermal discomfort). Considering the high rates of

temperature and humidity of the Amazon region these results will allow a simulation of the behavior in a natural situation of heat stress.

Materials and Methods

The present experiment was performed under the allowance of Ethical and Biosafety committee of the Institute of Production and Animal Health (ISPA) at Federal Rural University of Amazonia (UFRA) and conducted over ethical norms.

For the trial a total of four adult rams of Santa Inês breed with ages between 18 and 24 weeks old, at the beginning, and with an average weight ($\pm$ SD) of 42.7 ± 5.3 Kg. The animals were kept in a semi-intensive system in the facilities of the UFRA, Belém city, Pará State (1° 28' south latitude and 48° 30' west longitude). The region has a predominant weather of Afi type, Köppen classification.

The study was fulfilled during the rainiest period of the year, characterized by high temperatures and humidity. The monthly climatic data (temperature maximum, minimum and mean, relative humidity and pluviometric precipitation) of the experimental period are summarized in Table 1. The temperature and humidity rate (THR) were calculated according to Thom (1958); $THR = 0.8 \cdot T + (RH/100) \cdot (T - 14.4) + 46.4$, wherein: T = environment temperature (°C); RH = relative humidity. The values of THR were utilized to classify the level of thermic comfort of the animals as: absence of stress (THR < 70); alert (THR between 70 and 72); critical (THR between 73 and 78); danger (THR between 79 and 82) and emergence (THR $\geq$ 83; Hahn, 1985).

During the pre-experimental period, six weeks, breeding soundness evaluation (BSE) were performed (once a week) to set the normal patterns. After this period the males were submitted to the scrotal insulation process, a double layer plastic bag, intercalated with a cotton layer, was utilized for seven days. During this process was checked daily the rectal temperature and the temperature among the scrotum skin and the bag; means values were $36.8 \pm 0.3^\circ\text{C}$ and $35.8 \pm 0.2^\circ\text{C}$, respectively. After the scrotum insulation bag removal, the BSE were performed once a week during 105 days, a total of 16 evaluations post-insulation. During the

BSE the external reproductive system were inspected (scrotum, testicles, epididymis and the structures of the pampiniform plexus), a caliper rule and a tape measure were used to assess scrotal circumference (SC), testicular length (TL) and width (TW), epididymis tail length (ETL) and scrotum skin thickness (SST). The testicular and

epididymis measures were obtained from right and left testicles and then the means were calculated. The testicular volume (TV, cm³) was obtained according to Fields *et al.*, (1979), by the formula: volume = $2 \{(\text{radius}^2) \times \pi \times \text{TL}\}$, wherein radius = TL/2 and $\pi = 3.14$.

Table 1: Mean monthly temperature values (maximum - Tmax; minimum - Tmin and average - Taverage), humidity, rainfall and temperature-humidity rate (THR¹; maximum - THRmax and average - THRaverage) of Belém, Pará (Meteorological data provided by the Agrometeorologic Station of Eastern Amazonia EMBRAPA; PACHÊCO and BASTOS, 2006; 2007).

Month	Tmax (°C)	Tmin (°C)	Taverage (°C)	Humidity (%)	Rainfall (mm ³)	THR max	THR Average
November	33.4	23.8	28.6	78.0	113.7	87.9	80.4
December	32.4	24.0	28.2	82.0	347.0	87.1	80.3
January	32.1	24.0	26.9	84.0	365.7	86.9	78.4
February	31.5	23.8	26.6	83.0	240.7	85.8	77.8
March	31.4	23.9	26.5	89.0	593.2	86.7	78.4
Mean	32.2	23.9	27.4	83.2	332.1	86.9	79.0

¹THR=0.8*T+ (humidity/100)*(T-14.4) + 46, 4; wherein T = temperature, according to Thom (1958).

The testicular consistency was obtained by palpation and then classified on a scale from 1 to 5 according to Chemineau *et al.*, (1991): 1 = flaccid; 2 = decreased firmness; 3 = firm and elastic; 4 – firm and 5 = hardened (fibrosis). The semen collection was obtained by short artificial vagina; the sexual stimulation was achieved using a female in an artificially-induced estrus by the administration of 10 mg of estradiol benzoate, twice a week. The ram's libido was evaluated by their behavioral sexual characteristics and ejaculation ability and time between the female identification and semen collection.

The following semen parameters were assessed: volume (mL), appearance (1 = translucent, 2 = opalescent, 3 = milky and 4 = creamy), sperm mass motility (scale of 0-5), sperm progressive motility (%) e spermatid vigor (0-5, CBRA, 1998). The last three characteristics were assessed under a microscope with magnifications of 40X, 200X and 200X, respectively. Sperm concentration was obtained by spectrophotometry. For this, 0.02 mL of semen was diluted into 8 mL of a 3% sodium citrate solution. Results were determined by reading the absorbance value at a wave length of 535nm. The value obtained was

converted to a final value according to: $y=0.4539+11.6062x$, in terms of billions of spermatozoa/ml of semen ($r=0.9979$, $P<0.05$). The supravital staining technique (eosin-nigrosin) was used to determine the percentage of live spermatozoa. The semen morphology evaluation was obtained by the humid preparation method, fresh semen was diluted in tamponed formol saline solution and observed by the phase contrast microscope, following CBRA (1998) morphological classification (major, minor and total defects). Data were analyzed by the SASTM software (SAS Institute, Cary, NC, USA, 2004). The results were expressed in means, standard deviation and analyzed by ANOVA for repeated measures and the means compared by Kruskal-Wallis test at 5% statistical significance.

Results and Discussion

The high temperature and relative humidity of the region caused a great thermic discomfort to the animal during the entire period of trial (average THR and maximum THR classified as danger and emergency, respectively; Table 1). Thus, besides the increase in scrotal temperature induced, the animals of the present study suffer effect of climate

(ie. heat stress; condition common in equatorial regions). The exposure of sheep to elevated ambient temperature negatively affects the biological functions which are reflected in the impairment of their production and reproduction traits such as scrotal skin temperature, which is directly associated to semen damage (Marai *et al.*, 2007). The Santa Inês rams been considered as very sensitive to heat stress, which caused temporary interruption of sperm production and impaired sperm motility (Pinto *et al.*, 2001). There were no changes in the animal's libido, sexual interest, mounting and ejaculation ability during the experimental period. The time between the identification of the female and ejaculation was always below one minute, characteristic of an excellent libido, according to Freitas and Nunes (1992). The correlation between the scrotal-skin temperature and serum testosterone as well as with libido seems controversial. Significant negative

correlation between these variables has been reported by El-Daraway (1999). However, in the present study, the elevation of scrotal temperature caused by scrotal insulation associated to the high environmental temperatures do not seem to have interfered in the testosterone production by the Leydig cells, in accordance with the findings of Coelho *et al.*, (2008). The scrotum-testicular characteristics as shape, symmetry, mobility, positioning and sensibility shown to be normal and in accordance for the specie to all animals during the experiment period (CBRA, 1998; Oliveira and Silva, 2013); however, the thermic injury affected the scrotum-testicular biometry (Table 2). The values of scrotal circumference and width, length and volume of testicles showed a decrease from the pre-insulation period to the first week after injury ($P<0.05$), characterizing reduction of testicular parenchyma by the negative effect of thermic increase.

Table 2: Mean values ($\pm$ standard deviation) of scrotum-testicular-epididymis [scrotal circumference (SC), testicular length (TL), testicular width (TW), epididymis length (EL), scrotum skin thickness (SST) and volume (TV)] during the periods of scrotal pre- and post-insulation of Santa Inês rams. *Weekly interval.

TIME*	SC (cm)	SL (cm)	TW(cm)	EL (cm)	SST (cm)	TV (cm ³)
Pre-insulation	29.3 $\pm$ 2.6 ^{bc}	8.8 $\pm$ 0.8 ^{ab}	5.9 $\pm$ 0.6 ^a	2.5 $\pm$ 0.3 ^{ab}	6.6 $\pm$ 0.5 ^a	495.1 $\pm$ 151.1 ^a
1 st Post-	24.6 $\pm$ 2.9 ^d	6.8 $\pm$ 1.4 ^{fgh}	4.4 $\pm$ 0.8 ^{cd}	2.8 $\pm$ 0.1 ^{ab}	5.0 $\pm$ 0.8 ^{abc}	228.7 $\pm$ 115.1 ^{de}
2 nd Post-	24.2 $\pm$ 2.4 ^d	6.9 $\pm$ 1.2 ^{fgh}	4.5 $\pm$ 0.6 ^{cd}	2.9 $\pm$ 0.4 ^{ab}	5.1 $\pm$ 0.6 ^{ab}	226.3 $\pm$ 111.1 ^{de}
3 rd Post-	24.9 $\pm$ 1.7 ^d	5.9 $\pm$ 0.6 ^{hi}	4.3 $\pm$ 0.4 ^d	2.8 $\pm$ 0.3 ^{ab}	6.7 $\pm$ 1.9 ^a	177.9 $\pm$ 51.6 ^e
4 th Post-	24.0 $\pm$ 2.1 ^d	5.3 $\pm$ 0.4 ⁱ	4.2 $\pm$ 0.2 ^d	2.6 $\pm$ 0.5 ^{ab}	4.7 $\pm$ 1.7 ^{abc}	147.3 $\pm$ 19.0 ^e
5 th Post-	24.9 $\pm$ 1.7 ^d	6.1 $\pm$ 0.6 ^{ghi}	4.5 $\pm$ 0.4 ^{bcd}	2.5 $\pm$ 0.4 ^{ab}	5.0 $\pm$ 0.8 ^{abc}	201.8 $\pm$ 54.2 ^{de}
6 th Post-	24.6 $\pm$ 2.0 ^d	6.6 $\pm$ 0.8 ^{fghi}	4.6 $\pm$ 0.3 ^{bcd}	2.4 $\pm$ 0.4 ^b	2.5 $\pm$ 0.7 ^c	222.6 $\pm$ 49.5 ^{de}
7 th Post-	25.6 $\pm$ 1.7 ^d	7.0 $\pm$ 1.1 ^{efgh}	4.9 $\pm$ 0.6 ^{bcd}	2.5 $\pm$ 0.3 ^b	4.2 $\pm$ 0.3 ^{abc}	274.1 $\pm$ 94.5 ^{bcd}
8 th Post-	28.1 $\pm$ 2.9 ^c	6.8 $\pm$ 0.3 ^{fgh}	4.1 $\pm$ 0.4 ^d	2.5 $\pm$ 0.4 ^{ab}	5.0 $\pm$ 0.8 ^{abc}	182.1 $\pm$ 43.4 ^e
9 th Post-	29.5 $\pm$ 2.6 ^{abc}	7.2 $\pm$ 0.7 ^{efg}	4.3 $\pm$ 0.4 ^d	2.3 $\pm$ 0.3 ^b	5.2 $\pm$ 0.9 ^{ab}	213.0 $\pm$ 61.2 ^{de}
10 th Post-	29.4 $\pm$ 2.0 ^{ab}	7.6 $\pm$ 0.4 ^{bcd}	4.4 $\pm$ 0.6 ^d	2.6 $\pm$ 0.4 ^{ab}	4.2 $\pm$ 0.9 ^{abc}	235.5 $\pm$ 73.7 ^{de}
11 th Post-	30.1 $\pm$ 2.7 ^{ab}	7.5 $\pm$ 0.5 ^{cdef}	4.7 $\pm$ 0.5 ^{bcd}	2.6 $\pm$ 0.3 ^{ab}	5.2 $\pm$ 0.9 ^{ab}	262.3 $\pm$ 57.4 ^{cde}
12 th Post-	30.1 $\pm$ 2.3 ^{ab}	9.0 $\pm$ 0.8 ^a	4.9 $\pm$ 0.3 ^{bcd}	2.9 $\pm$ 0.3 ^{ab}	4.5 $\pm$ 0.6 ^{abc}	342.9 $\pm$ 66.3 ^{abcd}
13 th Post-	31.1 $\pm$ 2.5 ^a	8.7 $\pm$ 0.6 ^{abc}	5.4 $\pm$ 0.6 ^{abc}	2.9 $\pm$ 0.4 ^{ab}	3.7 $\pm$ 0.5 ^{bc}	409.7 $\pm$ 119.8 ^{abc}
14 th Post-	31.0 $\pm$ 2.2 ^{ab}	8.3 $\pm$ 0.8 ^{abcde}	4.4 $\pm$ 0.5 ^{cd}	3.0 $\pm$ 0.2 ^{ab}	4.0 $\pm$ 0.8 ^{bc}	263.0 $\pm$ 79.7 ^{cde}
15 th Post-	30.0 $\pm$ 2.0 ^{ab}	8.7 $\pm$ 0.2 ^{abc}	5.5 $\pm$ 0.4 ^{ab}	3.1 $\pm$ 0.2 ^a	4.5 $\pm$ 0.6 ^{abc}	420.1 $\pm$ 51.1 ^{ab}
16 th Post-	30.9 $\pm$ 2.0 ^{ab}	8.4 $\pm$ 0.3 ^{abcd}	4.6 $\pm$ 0.4 ^{bcd}	3.0 $\pm$ 0.3 ^{ab}	3.7 $\pm$ 0.5 ^{bc}	288.7 $\pm$ 59.6 ^{bcd}

* Letters in the same column are different from each other ($P<0.05$).

These biometrical changes remained for several weeks, and the reestablishment to normality for all

characteristics were occurred at the 13th week (91 days) post-insulation. Comparing these finds with

the ones reported by Moreira *et al.*, (2001) in rams (50 days) and by Santos and Simplício (2000) in bucks (59 days), which evaluated the scrotal insulation in hot and dry weather, is possible to observe a longer time to testicular recovery in the present study, suggesting an additional influence of Amazonian weather on rams and, consequently, on scrotum-testicular measurements. Differently of scrotum-testicular biometry from the animals subjected to insulation, the epididymis length and skin thickness variables did not show any alterations that could be correlated with these effects. The scrotum-testicular biometry showed to be an important measure that can be utilized for reproductive evaluation. Moreover, the testicular measures observed can reflect as modifications on gonadal physiology, primarily related to the germ cells in the seminiferous tubules. Additionally, it is known that the biometric measures are directly associated with daily semen production, spermatids number per Sertoli cells and seminiferous tubules area (Moreira *et al.*, 2001) and the consequent alteration, on then, promotes a decrease on the

animal's reproductive ability. The testicular consistency decreased from 3.0 ± 0.4 (firm and elastic) in the period pre-insulation to 2.0 ± 0.0 (decreased firmness) in the period post-insulation ($P > 0.05$). The decrease in testicular consistency adds evidence of degenerative process on the testicular parenchyma. The score of consistency went back to normality in the last three evaluations. Data regarding to the seminal characteristics during the period of trial are summarized in Table 3. Mean semen volume was 1.0 ± 0.3 mL during the experimental period and there was no change between periods of sampling ($P > 0.05$), similar values were described by Oliveira *et al.*, (2012; 1.2 ± 0.2 mL) in Santa Inês rams raised in the Amazon region. It is important to consider that semen volume is constituted by a great part of secretions from the male accessory glands and probably those glands were not affected by the induced insulation of this study, the secretions from the epididymis and testicles contribute with less than 5% of the ejaculate (Santos and Simplício, 2000).

Table 3: Mean values ($\pm$ standard deviation) of semen characteristics (volume, appearance, sperm mass motility (SMM), progressive motility, vigor, concentration and percentage of live spermatozooids (Live %)) during the period of scrotal pre- and post-insulation of Santa Inês rams. *Weekly intervals.

TIME*	Volume (mL)	Appearance (1-4)	SMM (0-5)	Motility (%)	Vigor (0-5)	Concentration ($\times 10^9$ spztz/mL)	Live %
Pre-insulation	1.1 ± 0.1	3.7 ± 0.5^a	4.4 ± 0.6^a	79.0 ± 6.5^{ab}	4.4 ± 0.5^{ab}	4.9 ± 0.7^{abc}	89.0 ± 0.8^a
1 st Post-	0.7 ± 0.3	3.0 ± 1.4^{ab}	2.5 ± 3.7^{abc}	0.0 ± 0.0^d	0.0 ± 0.0^d	5.7 ± 4.1^{ab}	12.5 ± 12.1^b
2 nd Post-	1.4 ± 0.8	1.5 ± 0.6^c	1.0 ± 0.0^{bc}	0.0 ± 0.0^d	0.0 ± 0.0^d	2.2 ± 1.4^d	0.0 ± 0.0^b
3 rd Post-	1.0 ± 0.1	1.0 ± 0.0^c	0.2 ± 0.5^c	0.0 ± 0.0^d	0.0 ± 0.0^d	0.7 ± 0.1^d	0.0 ± 0.0^b
4 th Post-	0.9 ± 0.4	1.0 ± 0.0^c	0.2 ± 0.5^c	0.0 ± 0.0^d	0.0 ± 0.0^d	0.7 ± 0.3^d	0.0 ± 0.0^b
5 th Post-	0.8 ± 0.3	1.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^d	0.0 ± 0.0^d	0.6 ± 0.1^d	0.0 ± 0.0^b
6 th Post-	0.6 ± 0.3	1.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^d	0.0 ± 0.0^d	0.5 ± 0.0^d	0.0 ± 0.0^b
7 th Post-	0.7 ± 0.2	1.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^d	0.0 ± 0.0^d	0.5 ± 0.1^d	0.0 ± 0.0^b
8 th Post-	0.7 ± 0.2	1.0 ± 0.0^c	0.0 ± 0.0^c	0.0 ± 0.0^d	0.0 ± 0.0^d	0.5 ± 0.4^d	0.0 ± 0.0^b
9 th Post-	0.9 ± 0.3	1.5 ± 0.6^c	0.5 ± 0.6^c	0.0 ± 0.0^d	0.2 ± 0.5^d	0.6 ± 0.0^{dc}	80.5 ± 4.9^a
10 th Post-	0.9 ± 0.1	2.0 ± 0.0^{bc}	1.2 ± 0.5^{bc}	0.1 ± 0.0^d	0.2 ± 0.5^d	1.1 ± 0.4^{ab}	82.2 ± 7.5^a
11 th Post-	0.8 ± 0.2	3.5 ± 1.0^a	4.0 ± 1.4^a	56.2 ± 28.7^{bc}	3.2 ± 1.7^{bc}	5.2 ± 2.6^a	88.7 ± 5.0^a
12 th Post-	0.8 ± 0.1	3.7 ± 0.5^a	3.2 ± 0.5^{ab}	41.2 ± 21.0^c	2.5 ± 1.0^c	7.8 ± 3.0^{ab}	84.5 ± 7.1^a
13 th Post-	0.8 ± 0.2	4.0 ± 0.0^a	4.2 ± 0.5^a	60.0 ± 7.1^{bc}	3.2 ± 0.5^{bc}	5.6 ± 2.1^{ab}	86.2 ± 6.5^a
14 th Post-	0.8 ± 0.3	4.0 ± 0.0^a	5.0 ± 0.0^a	86.2 ± 8.5^a	4.7 ± 0.5^a	5.8 ± 0.8^{ab}	81.5 ± 10.7^a
15 th Post-	1.0 ± 0.3	3.7 ± 0.5^a	5.0 ± 0.0^a	70.0 ± 9.1^{ab}	4.5 ± 0.6^{ab}	6.7 ± 1.6^a	93.2 ± 3.6^a

16 th Post-	1.0±0.3	3.7±0.5 ^a	5.0±0.0 ^a	77.5±6.4 ^{ab}	4.7±0.5 ^a	6.7±2.4 ^a	88.0±7.4 ^a
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* Letters in the same column are different from each other (P<0,05).

The seminal appearance, sperm mass motility, progressive motility, spermatoc vigor, concentration and percentage of live spermatozooids showed similar behavior of the scrotum-testicular biometry values, exhibiting a significant reduction in the post-insulation samplings when compared with samplings before the injuries. The results showed that the increase on the testicular temperature promoted a negative expressive effect on semen quality. The high temperatures on spermatogenesis involve complex interactions, such as, changes in intratesticular tension, blood and lymph circulations, testicular fluids and metabolic changes, resulting in testicular degeneration (Fonseca and Chow, 1995).

The lowest values recorded, for these seminal parameters, were obtained between the 3rd and 9th weeks, characterizing a degenerative process of the testicles. The reestablishment of physiological values occurred on 11th week (77 days after the scrotal insulation process). This period once again proved to be longer compared with that reported by Moreira *et al.*, (2001; eight weeks) in Santa Inês rams raised in Brazil's Northeast, suggesting that the edaphoclimatic characteristics of Amazonia (high temperatures associated with high relative humidity) can create a situation of heat stress on the animals and consequently a great impact on the reproductive characteristics. It is known that the degenerative process may be due to high environmental temperatures (Skinner and Louw, 1966) and the long period until the reestablishment to normal patterns observed in this present study suggests that the testicular injury associated to heat stress of the animals affects the first stages of spermatogenesis, probably, on the phases of spermatogonia multiplication (spermatocytogenesis) and in the beginning phase of meiosis. In rams and buck (Blanchard *et al.*, 1996) as well as in bovine (Rahman *et al.*, 2011) has been reported that the spermatoc cells on meiosis and spermiogenesis are the most susceptible to the testicular temperature increase. The abrupt reduction of sperm motility

and vigor to zero, immediately after scrotal insulation (1st week after injury), makes evidence over the direct effect of elevated temperature on the epididymis. Because, according to Pezzini *et al.*, (2006), the spermatozooids launched in the ejaculated in the first week post-insulation had been produced in the seminiferous tubules and were stored in the epididymis before the insulation.

The morphological evaluation of spermatoc cells realized before the scrotal insulation showed 96.3±0.8% of spermatozooids with normal characteristics and 3.7±1.5% of pathologic cells (total defects). The percentage proves the quality of the animals on trial, according to CBRA (1998). The increase of major and minor defect was observed in the first BSE post-insulation (Fig. 1), reaching a total of 78.5±18.9% of pathologic cells, which conforms to the prejudicial effect of insulation over the semen quality. Among the observed pathologies the most common was normal detached head, reaching the maximum percentage of 82.5%. Moreover, other pathologies classified as minor defects were detected in high rates such as, simple bent/coiled tail. The major defects found were proximal droplets, strong coiled tail and pathologic detached head. The presence of sperm abnormalities is explained by Saadi *et al.*, (2013), elucidating that the stress caused by heat affects the spermiogenic cells, creating ultrastructure modifications, producing defective spermatozooids considered prejudicial to the animal's fertilizing ability. Animals subjected to heat stress and/or scrotal insulation has increased blood levels of cortisol, which interferes in synthesis of LH and testosterone, compromising the spermatoc process (Barth and Bowman, 1994). Marai *et al.*, (2007) found that the exposure of sheep to heat stress evokes a series of drastic changes in the biological functions, which include a decrease in feed intake efficiency and utilization, disturbances in water, protein, energy and mineral balances, enzymatic reactions, hormonal secretions and blood metabolites.

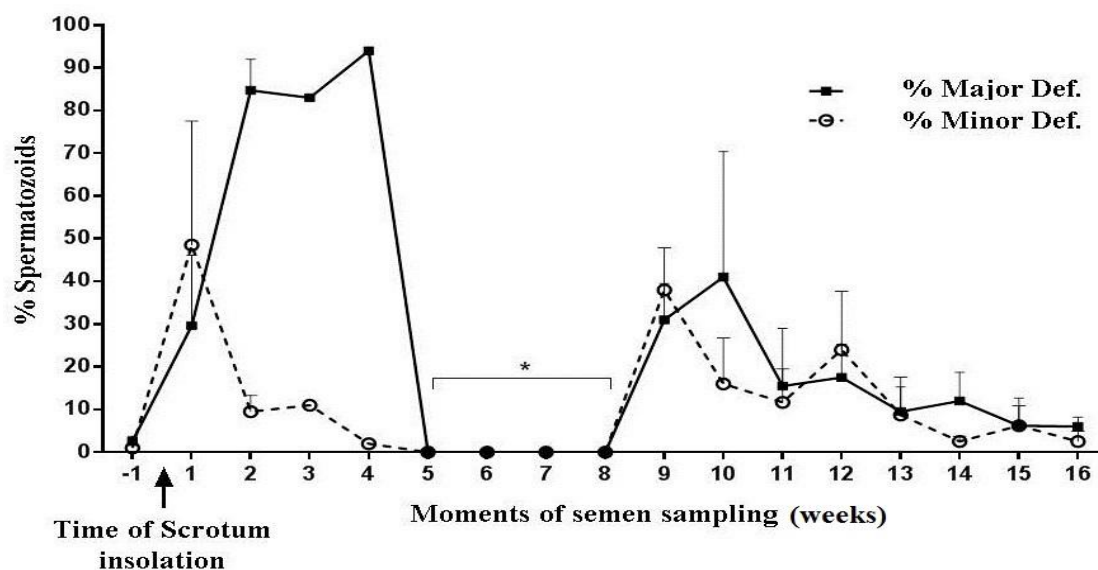


Fig. 1: Graphic representation of spermatic pathologic cells percentage, classified as major defects and minor defects, according to the moment of ovine semen sampling (weekly intervals) in relation to the time of scrotal insulation process. *Number of spermatozooids insufficient to perform the morphologic evaluation (semen abnormalities).

These changes are reflected in the impairment of their production and reproduction traits. The ejaculates collected between the 5th and 8th weeks showed reduced values of sperm concentration, precluding morphological assessments due to insufficient number of spermatozooids to evaluate the semen morphology by the humid preparation method; this fact did not compromised the process to set the period of normal pattern return. The total percentage of abnormalities decreased to $27.2 \pm 20.1\%$ on 11th week, similar to the other semen parameters (semen appearance, sperm mass motility, sperm progressive motility, spermatic vigor, concentration and percentage of live spermatozooids), confirming that the association of scrotal-insulation and heat stress delayed the reestablishment to physiological patterns, compared with animals in another bioclimatic situation.

It was possible to confirm the efficiency of heat-dissipating properties of the scrotum, mentioned by Malama *et al.*, (2013), when in the present study this function was limited by placing a double layer plastic bag, intercalated with a cotton layer, in the scrotum of rams. However, it was also possible to note the delay in reestablishment normality of andrological characteristics, a fact possibly explained by the additional effect of

whole-body exposure to thermal stress. The testicular degeneration induced by scrotal insulation is dependent on the duration and intensity of the causative agent as well as by climate region, thus based on the results of this study, it is recommended to take management practices suitable for regions with climatic conditions such as Amazon, which combines high temperatures and humidity. Thus, it is important to prepare shading areas, with wide availability of fresh food and water. This fact implies to farmers an effective control of climatic conditions of the region, thereby favoring a reproductive management appropriate.

Conclusions

The Amazonian climate promoted heat stress in animals (due to high temperature and humidity rate), which contributed to intensify the deleterious effects caused by the scrotal insulation on the spermatogenesis (testicular parenchyma and seminiferous tubules) and on the spermatozoa maturation process (epididymis). The injuries did not affect the seminiferous epithelium irreversibly, insofar as the reestablishment to normality of scrotum-testicular biometry and semen characteristics. Nevertheless, the heat stress of

animals associated to scrotal insulation retard the testicular regeneration process.

References

- Barth AD, Bowman PA (1994). The sequential appearance of sperm abnormalities after scrotal insulation or dexametasone treatment in bulls. *Can. Vet. J.*, 35: 93-102.
- Blanchard TL, Josgensen JB, Varner DD, Forrest DW, Evans JW (1996). Clinical observations on changes in concentrations of hormones in plasma of two stallions with thermally induced testicular degeneration. *J. Equine Vet. Sci.*, 16: 195-201.
- Colégio Brasileiro de Reprodução Animal (1998). Manual Técnico 2th ed. Belo Horizonte, 66p.
- Chemineau P, Cognié Y, Guérin Y, Leboeuf B, Orgeur P, Vallet JC (1991). Training manual on artificial insemination in sheep and goats. FAO, 222p.
- Coelho LA, Sasa A, Bicudo SD, Balieiro JCC (2008). Concentrações plasmáticas de testosterona, triiodotironina (T3) e tiroxina (T4) em bodes submetidos ao estresse calórico. *Arq. Bras. Med. Vet. Zootec.*, 60: 1338-1345.
- Costa EPS, Takeda FRPC, Lima RS (2010). Avaliação da Adaptabilidade de Ovinos Santa Inês ao Clima Amazônico. *Rev. Electrón. Vet.*, 11: 1-8.
- El-Darawany AA (1999). Tunica dartos thermoregulatory index in bull and ram in Egypt. *Ind. J. Anim. Sci.*, 69(8): 560-563.
- Fields MJ, Burns WC, Warwick AC (1979). Age, season and breed effect of testicular volume and semen traits in young beef bull. *J. Anim. Sci.*, 48: 1299-1304.
- Fonseca VO, Chow LA (1995). Características seminais de touros zebus com degeneração testicular transitória. *Arq. Bras. Med. Vet. Zootec.*, 47: 707-716.
- Freitas VJF, Nunes JF (1992). Parâmetros andrológicos e seminais de carneiros deslanados criados na região litorânea do Nordeste Brasileiro em estação seca e chuvosa. *Rev. Bras. Reprod. Anim.*, 16: 95-104.
- Gabaldi SH, Wolf A (2002). A importância da termorregulação testicular na qualidade do sêmen em touros. *Ciências Agrárias Saúde*, 2: 66-70.
- Hahn GL (1985). Management and housing of farm animals in hot environments. In: Yousef, M.K. (Ed.). *Stress physiology in livestock*, V.II, Boca Raton: CRC Press, 151-174.
- Kastelic JP, Cook RB, Coulter GH, Saacke RG (1996). Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology*, 45: 935-942.
- Malama E, Bollwein H, Taitzoglou IA, Theodosiou T, Boscos CM, Kiossis E (2013) Chromatin integrity of ram spermatozoa. Relationships to annual fluctuations of scrotal surface temperature and temperature-humidity index. *Theriogenology*, 80: 533-541.
- Marai IFM, El-Darawany AA, Fadiel A, Abdel-Hafez MAM (2007). Physiological traits as affected by heat stress in sheep - A Rev. *Small Rum. Res.*, 71: 1-12.
- Moreira EP, Moura AAA, Araujo AA (2001). Efeitos da insulação escrotal sobre a biometria testicular e parâmetros seminais em carneiros da raça Santa Inês criados no Estado do Ceará. *Rev. Bras. Zootec.*, 30: 1704-1711.
- Oliveira MEF, Silva JCB (2013) Andrologia. In: Oliveira MEF, Teixeira PPM, Vicente WRR (2013). (Eds). *Biotécnica reprodutivas em ovinos e caprinos*. 1ª Ed. São Paulo: Editora Med. Vet., 35-60. p.
- Oliveira MEF, Almeida OM, Pinho RO, Vicente WRR, Rodrigues LFS (2012). Behavioural and seminal characteristics of Santa Ines rams subjected to successive semen collections in the Amazonian region. *Ital. J. Anim. Sci.*, 11(67): 368-373.
- Pezzini TG, Sartori R, Silva TASN, McManus C, Mariante AS (2006). Características seminais de touros Curraleiros e Holandeses submetidos à insulação escrotal. *Pesq. Agropec. Bras.*, 41: 863-868.
- Pinto ME, Alencar-Araripe MA, Araujo AA (2001). Effects of scrotal insulation on testis size and semen criteria in Santa Ines hairy sheep raised in the State of Ceara, Northeast of Brazil. *Rev. Bras. Zootec.*, 30(6): 1704-1711.
- Rahan MB, Vandaele L, Rijsselaere T, Maes D, Hoogewijs M, Frijters A, Noordman J, Granados A, Dernelle E, Shansuddin M, Parrish JJ, Soon AV (2011). Scrotal insulation and its relationship to abnormal morphology, chromatin protamination and nuclear shape of spermatozoa in Holstein-Friesian and Belgian Blue bulls. *Theriogenology*, 76: 1246-1257.
- Saadi HAS, Riemsdijk EV, Dance AL, Rajamanickam GD, Kastelic JP and Thundathil JC (2013). Proteins associated with critical sperm functions and sperm head shape are differentially expressed in morphologically abnormal bovine sperm induced by scrotal insulation. *J. Proteomics*, 82: 64-80.
- Santos DO, Simplício AA (2000). Parâmetro escroto-testiculares e de sêmen em caprinos adultos submetidos à insulação escrotal. *Pesq. Agro. Bras.*, 35: 1835-1841.
- SAS Institute Inc. (1999). SAS, Version 8, Cary, NC: SAS Institute Inc.
- Setchell BP (2006). The parkes lecture heat and the testis. *J. Reprod. Fertil.*, 114: 179-194.
- Skinner JD, Louw GN (1966). Heat stress and spermatogenesis in *Bos indicus* and *Bos Taurus* cattle. *J. Appl. Physiol.*, 21: 1784-1790.
- Thom EC (1958). Cooling degree-days: air conditioning, weating and ventilating. *Transactions of the ASAE*, 55: 65-72.