Effect of Testicular Thermoregulation on the Quality of Buffalo Sperm

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ABSTRACT
The process of spermatic division and differentiation (spermatogenesis) occurs with intratesticular temperature lower than the corporal temperature and for that is essential that the testicular thermoregulation mechanism occurs properly. For evaluation of the scrotal surface temperature can be used the infrared thermography or testicular sensors, besides that, can be evaluated the blood flux in the spermatic cord through the Doppler ultrasonography. Thus, the aim of this study is to analyze the testicular thermoregulation in adult buffaloes through scrotal thermography and Doppler ultrasound of testicular artery and verify its effect on sperm quality. For that were used seven healthy buffaloes, with age of 3 and 4 years, of the Murrah breed. The animals were subjected to 3 semen collections using artificial vagina, with one day of interval. In addiction, the retal temperature measurement (RT) with dry bulb thermometer, the measurement of scrotal surface temperature (SST) and body surface temperature (BST) through infrared thermography and the pulsatility (PI) and resistivity (RI) index of testicular artery by Doppler ultrasonography, were performed using 2 distinct moments: animals previously placed to shade (M1) and animals subjected to 4 hours of sun (M2). All parameters were compared by T test and the correlations were performed by Pearson test using the In Stat Graph Pad 3® program. The significant level considered was 5%. There was an increase (p<0,05) of RT, SST, SNT and RI in M2. Increasing trend was observed (0,05>p>0,01) PI and RI between M1 and M2. There was a low correlation between SST and semen quality.

INTRODUCTION
The testicular thermoregulation in domestic animals depends on contraction and relaxation of dartos and cremaster muscles, sweat gland activity, heat radiation from the scrotal surface and arteriovenous thermal exchange through the countercurrent transfer system in the pampiniform venous plexus (Ashdown and Hancock, 1980; Setchell, 1991; Coulter and Kastelic, 1994).

Due to the fact of blood supply in the testes be naturally deficient in situations of increased intratesticular temperature, increased cellular metabolism occurs and consequently there is a higher need for oxygen. The low oxygen leads to cell death by triggering the process of testicular degeneration (Blanchard et al., 1996).

In order to evaluate the efficiency of testicular thermoregulation the testicular temperature can be measure by introducing sensors into the gonads, however, this procedure is considered invasive and can offer a danger to the animal. Therefore, Coulter et al. (1988) evaluated the testicular temperature using noninvasive method of infrared termography and showed no difference between this method in relation to sensors.

Furthermore, an indirect evaluation of testicular thermoregulation can be performed by Doppler ultrasound exam of the spermatic cord, once the testicular thermoregulation is directly

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related to blood flow in the testicular artery. Despite the high incidence of reproductive problems related to testicular temperature control, there are few studies evaluating this temperature in cattle (Coulter et al., 1997; Kastelic et al., 1996; Barros et al., 2009), in goats (Coulter et al., 1988; Maloney and Mitchell, 1996; Kastelic et al., 1999; Silva et al., 2006), in humans (Gold et al., 1977; Layfaye and Hermabessiere, 1980) and in horses (Ramires Neto et al., 2012).

Thus, the aim of this study is to analyze the testicular thermoregulation in adult buffaloes through scrotal thermography and Doppler ultrasound of testicular artery and verify its effect on sperm quality.

MATERIALS AND METHODS

Four Murrah buffaloes aged between 4 and 7 years old were used for the study. Initially, three ejaculates from each buffalo were collected with an interval of 2 days to eliminate possible damaged cells from epididymal cauda and to stabilize the sperm parameters.

After the stabilization the animals were subjected to 3 semen collections using artificial vagina, with one day of interval. The ejaculates were diluted with Tris-yolk extender at a concentration of 50x10^6 spermatozoids/ml. After that the kinetic parameters were analyzed through computerized system CASA and the morphological abnormalities by Differential Interference Contrast Microscopy (DIC).

The retal temperature measurement (RT) with dry bulb termometre, the measurement of scrotal surface temperature (SST) and body surface temperature (BST) through infrared thermography (Infra Cam™ by FLIR Systems Inc) and the pulsatility (PI) and resistivity (RI) index of testicular artery by Doppler ultrasonography (My Lab5 by Esaote), were performed in a day with high enviromental temperature (32,7 °C) using 2 distinct moments: animals previously placed to shade (M1) and animals subjected to 4 hours of sun (M2).

The thermography images were analyzed by ThetrofmaCAM Quick Report™ software and the ultrasonographs through Esaote software.

All parameters were compared by T test and the correlations were performed by Pearson test using the In Stat Graph Pad 3® program. To perform the correlation was considered only the value of variation of the parameters RT, SST, BST, PI and RI between M1 and M2.

The significant level considered was 5%.

RESULTS

There was an increase (p<0,05) of RT (37,5±0,34^a vs 39,0±0,43^b; M1 and M2 respectively), SST (31,0±0,55^a vs 35,2,0±1,37^b; M1 and M2 respectively) and BST (33,4±3,0^a vs 38,48±0,22^b; M1 e M2 respectively). Increasing trend was observed (0,05>p>0,01) in PI (0,99±0,45^a vs 1,2±0,26^b; M1 and M2 respectively) and in RI (0,64±0,15^a vs 0,71±0,07^b; M1 e M2 respectively) in M2.

It was observed strong negative correlation between the variation of SST and BST, between SST and TR and strong positive correlation between SST and RI. The correlation between the variation of SST and PI was considered negative average. There was a low correlation between SST and total sperm motility, between SST and progressive sperm motility, between SST and percentage of rapid sperm cells, between SST and major defects of the sperm morphology and between SST and minor defects of sperm morphology (Table 1).

DISCUSSIONS

In the present study was observed fewer 2.5 ° C in scrotal surface temperature than in body surface temperature, which is essential for spermatogenesis occurs normally (Setchell, 1991). These data corroborate with Ashdown and Hancock (1980) which found that in domestic animals, the testicular temperature is lower than the body temperature.

As observed in humans (EDDY et al., 2001), in sheep (Couter et al., 1988) and in horses (RAMIRES NETO et al., 2012), the thermographic analysis of the scrotum was demonstrated a practical and non-invasive method for measure the scrotal surface temperature of buffaloes. I was
demonstrated this method has a great potential to use in the field and an option to additional exam of the soundness of buffalo. Mainly because the measurement of scrotal surface temperature be a good indicator of intratesticular temperature as observed by Coulter et al. (1988).

In the present study there was a significant increase in rectal and body surface temperature after 4 hours of exposure of the buffaloes to the sun, indicating that the animals were in heat stress in M2. These data can be explained by the observation of Das et al. (1999) that because of having a lower density of sweat glands on the body surface the buffaloes have low ability to perform thermoregulation.

The increase in resistivity and pulsatility index of testicular artery in M2 demonstrate that in conditions of heat stress adult buffaloes activate its mechanisms of testicular thermoregulation. However, besides being observed increase in scrotal surface temperature in M1 in relation to M2, this increase was similar to the increase of body surface temperature, indicating that in the buffaloes the testicular thermoregulatory mechanisms are not efficient, which may be a consequence of its poor body thermoregulation, as described by Das et al. (1999).

It was observed a low correlation between testicular thermoregulation and sperm kinetic parameters of buffaloes. This find corroborate with Fernandes et al. (2008), which evaluated the effect of increased testicular temperature in bulls and have observed no changes in sperm kinetic parameters of these animals after insulation.

Another finding of this study was the low correlation between the ability to maintain the testicular temperature and the alterations in sperm morphology, disagreeing with Vogler (1991) and Fernandes et al. (2008) which observed an increase of sperm morphology alterations after testicular insulation in bovine and Blanchard et al. (1996) which observed an increase of alterations in sperm morphology in stallions subjected to heat stress for prolonged periods.

CONCLUSIONS

The results of this study allow us to conclude that adult buffaloes have low ability to perform body and testicular thermoregulation in situations of environmental heat stress. However, this low capacity of testicular temperature maintenance demonstrated no correlation with the sperm kinetic parameters and sperm morphological defects in buffalo spermatozoa.

REFERENCES


**Table 1:** Correlations between the variation of scrotal surface temperature (SST) and body surface temperature (BST), between rectal temperature (RT) and the pulsatility index (PI), between the resistivity index (RI) and total sperm motility (TM), between the progressive motility (PM) and percentage of rapid sperm cells (RAP), between the major defects of sperm morphology (DM) and minor defects of the sperm morphology (Dm).

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<th>BST</th>
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<th>PI</th>
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<td>0.62</td>
<td>0.73</td>
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