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The relationships between scrotal surface temperature, age and sperm quality in stallions

Carlos Ramires Neto [a,*], Gabriel Augusto Monteiro [a], Diego José Zanzarini Delfiol [b], Marcel Cavalcanti Farras [a], José Antônio Dell'aqua Jr. [a], Frederico Ozanan Papa [a], Marco Antonio Alvarenga [a]

[a] Department of Animal Reproduction and Veterinary Radiology, College of Veterinary Medicine and Animal Science - Univ Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil
[b] Department of Veterinary Clinical Science, College of Veterinary Medicine and Animal Science - Univ Estadual Paulista (UNESP), Botucatu, São Paulo, Brazil

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ABSTRACT

In horses, spermatogenesis normally occurs at an average intratesticular temperature of 35 °C; therefore, mechanisms for testicular thermoregulation are essential. Measuring the scrotal surface temperature by thermography is one of the methodologies used to evaluate the effectiveness of testicular thermoregulation. The objective of this study was to determine the relationship between the control of scrotal surface temperature and sperm quality in horses of different ages. In total, 24 Quarter Horse stallions were divided into three groups: YS (young stallions), AS (adult stallions) and OS (old stallions). Initially, we calculated the testicular volume (TV) and evaluated various aspects of the semen (sperm kinetics, plasma membrane integrity and sperm morphology) for all the animals. We also evaluated rectal temperature (RT), body surface temperature (BST,) and average scrotal surface temperature in the testicular region (SST) before (M0) and after sun exposure (M1). Differences were observed ($p < 0.05$) between the RT and BST before and after sun exposure in all three groups. However, there were no differences ($p > 0.05$) in the SST values at these two time points, thus demonstrating the efficiency of the mechanisms for testicular thermoregulation. The SST was similar ($p > 0.05$) among all three groups. Based on these results, we conclude that fertile stallions of different age groups are able to maintain SST and measuring the heat radiating from the scrotum using a digital infrared thermographer. We can also conclude that measuring the heat radiating from the scrotum using a digital infrared thermographer is a practical and efficient tool for monitoring SST in horses.

1. Introduction

Testicular thermoregulation in domestic animals is dependent on the contraction and relaxation of the dartos and cremaster muscles, the activity of the sweat glands, heat irradiation of the scrotal surface and arteriovenous heat exchange via the pampiniform plexus counter-current mechanism (Ashdown and Hancock, 1980; Coulter and Kastelic, 1994; Setchell, 1991).

In horses, normal spermatogenesis occurs at an average intratesticular temperature of 35 °C. The majority of testicular problems in stallions are related to changes in the ability to control testicular temperature (Alvarenga and Papa, 2007).

In situations where there are fever events or increases in testicle temperature because of environmental or pathological conditions, such as infections, oedema, dermatitis, bleeding

* Corresponding author. Tel.: +55 14 3880 2142.
E-mail address: carlosramiresneto@hotmail.com (C. Ramires Neto).

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(Blanchard et al., 1996) and testicular torsion (Ball, 2008), the animal cannot maintain a scrotal temperature within ideal limits even though the processes of intratesticular temperature regulation are efficient (Moule and Waites, 1963). An elevated testicular temperature leads to an increase in cellular metabolism and, consequently, a greater tissue oxygen demand. Hypoxia can lead to cell death, thus initiating testicular degeneration (Blanchard et al., 1996). After evaluating the effect of thermal stress on bovine semen, Fernandes et al. (2008) observed a reduction in semen quality after scrotal insulation, whereas Mawyer et al. (2012) did not observe this result in miniature horses.

To evaluate the efficiency of testicular thermoregulation, testicular temperature measurements can be performed by introducing sensors into the gonads. However, this invasive procedure can pose risks to the animal (Coulter et al., 1988). Therefore, Coulter et al. (1988) evaluated ram testicular temperature using a non-invasive infrared thermography method and showed that there were no differences between these measurements and those obtained using the invasive sensors.

Despite the high incidence of reproductive problems related to testicular temperature control, there are only a few studies that have evaluated testicular temperature control in cattle (Barros et al., 2009; Coulter et al., 1997; Kastelic et al., 1996), goats (Coulter et al., 1988; Kastelic et al., 1999; Maloney and Mitchell, 1996), humans (Gold et al., 1977; Lafaye and Hermabessiere, 1980) and horses (Staempfli et al., 2006). Therefore, the objective of this study was to determine whether there is a correlation between the control of scrotal surface temperature (SST) and sperm quality in horses of different ages.

2. Materials and methods

We used 24 healthy Quarter Horse stallions without anatomical or functional alterations to their reproductive tracts as subjects and conducted the study from November to January 2010 (Latitude 22°53′09″S and Longitude 48°26′42″W). The animals were divided into three groups according to their ages: young stallions (YS) included stallions between 2 and 3 years old (2.6 ± 0.6, $n=9$), adult stallions (AS) included stallions between 5 and 15 years old (7.8 ± 3.4, $n=10$), and old stallions (OS) included stallions 17 years old or older (20.3 ± 2.5, $n=5$).

Rectal temperature (RT) measurements were obtained using a dry bulb thermometer, and the scrotal (SST) and body surface temperature (BST) were obtained using an infrared thermographer (Infra Cam™, FLIR Systems Inc.) on hot days (temperature >30 °C) in two stages: when the animals were previously conditioned in a shady environment (M0, 30.2 ± 0.7 °C) and after 1 h of sun exposure during intense daytime sunlight (M1, 37.5 ± 1.1 °C). The infrared thermographer was placed 1 m from the scrotum and neck. The images were stored and analyzed using ThermaCAM Quick Report™ software. The analysis of SST was performed using thermographic images of the testicular side of the scrotum; the temperature was measured at seven different testicular locations (two cranial, two caudal and three medial locations, (Fig. 2)) using the

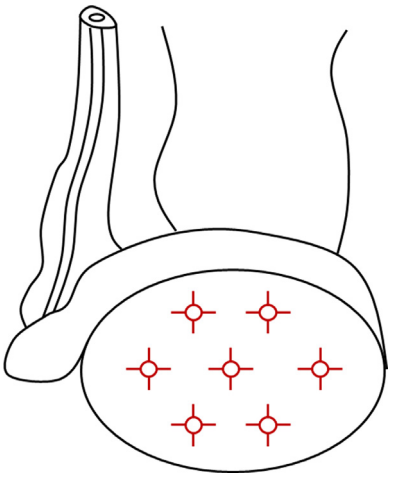

Fig. 2. Schematic design of the testicle with seven points analyzed at each side of the scrotum.

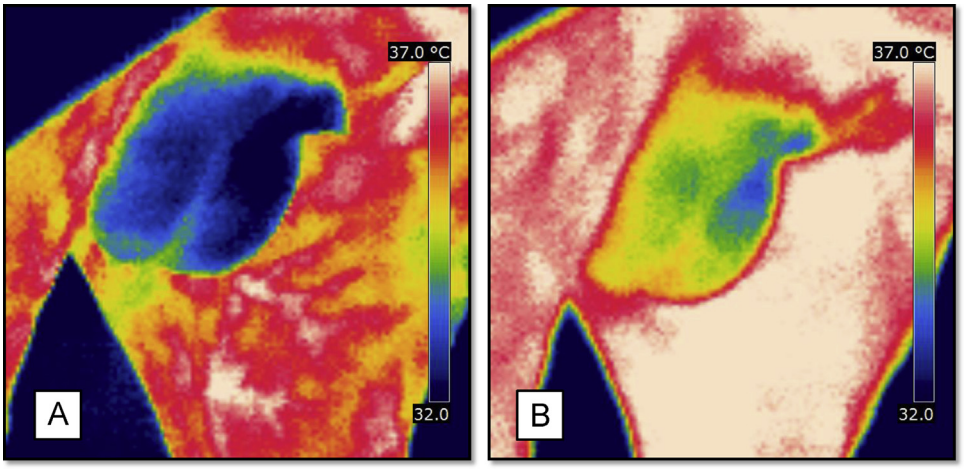

Fig. 1. Thermographic image A is a superficial scrotal thermograph from a stallion before exposure to the sun (M0), and thermographic image B is a superficial scrotal thermograph of the identical animal after one hour of sun exposure (M1).

SST software, and the average of both testicles was calculated. The BST analysis was performed using the average temperature of the right neck.

The stallions were previously submitted to three serial semen collections on alternate days during the three weeks prior to the experiment. Semen was collected from the stallions on hot days under the shade (temperature >30 °C) using an artificial vagina three days before the first mensuration of temperature, and the ejaculate was analyzed for the following parameters using a computer-assisted sperm analyser (CASA-system; HTM-IVOS vs. 12): total motility (TM, %), progressive motility (PM, %), path velocity (VAP, μm/s), straight velocity (VSL, μm/s), curvilinear velocity (VCL, μm/s) and rapid sperm cells (RAP, %). In addition, an aliquot was analyzed for plasma membrane integrity (PMI, %) using epifluorescence microscopy with the 6-carboxyfluorescein diacetate and propidium iodine fluorescent probes (Harrison and Vickers, 1990), and a complete semen sample was used to count the sperm cells in a Neubauer chamber. The percentage of sperm was evaluated by counting 200 cells in smears by microscopy using a modified Karras technique (Papa et al., 1988), and we evaluated the major morphological sperm defects (MD, %), minor morphological sperm defects (mD, %), defects in the sperm head (DSH, %), defects in the sperm mid-piece region (DMP, %), defects in the sperm tail (DST, %), sperm droplets (SD, %) and sperm teratological forms (TER, %). The major sperm defects included an underdeveloped sperm head, sperm head close the base, pyriform sperm head, isolated sperm head, abnormal contour of the sperm head, pouch formation, diadem defects, knobbed, teratological forms, oedema of the middle section, partial intermediate fractures, a strongly bent tail, a strongly curled tail, a tail wrapped around the head and a proximal droplet. The minor defects included a slender sperm head, giant sperm head, normally deployed but isolated sperm head and oblique, distal drops.

The height and anterior–posterior and medial–lateral axes (HGT, AP and ML, respectively) of both testes were measured. Testicular volume (TV, cm^3) was determined based on the formula $V=(4/3)\times\pi\times(AP/2)\times(ML/2)\times(HGT/2)$ as previously described (Blanchard and Varner, 1996). A combined TV was obtained considering the TV sum of both testes.

An analysis of variance (ANOVA) was used to compare all sperm parameters. The temperatures and TV were compared using unpaired Student's *t*-tests. The correlations between the parameters for sperm quality, TV, SST and total sperm per ejaculate (TSE) were performed using a Pearson's test. Correlations less than 0.3 were considered weak, between 0.3 and 0.7 were moderate and above 0.7 were strong. A value of $p<0.05$ was considered significant. We used the GraphPad InStat 3™ program.

3. Results

The kinetic sperm parameters (TM, VAP, VCL and RAP) for the YS were lower ($p<0.05$) than those of the AS and OS, as shown in Table 1. Sperm morphology did not differ between the groups ($p>0.05$) (Table 2).

Table 3
Mean values and standard deviations of the testicular volume (TV), the concentration of sperm per mL (CON) and the total sperm per ejaculate (TSE) for groups YS, AS and OS.

Group	TV (cm^3)	CON × 10^6 (sperm/mL)	TSE × 10^9
YS	152.7 ± 42.8	110.8 ± 60.1[a]	5.26 ± 0.19
AS	205.9 ± 66.9	105.4 ± 40.7[ab]	5.83 ± 1.25
OS	222.7 ± 42.8	198.4 ± 40.3[b]	9.35 ± 3.14

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). Lowercase letters in the same column indicate a significant difference ($p<0.05$)

Table 1
Mean values and standard deviations for the total motility (TM), progressive motility (PM), path velocity (VAP), straight velocity (VSL), curvilinear velocity (VCL), rapid sperm cells (RAP) and plasma membrane integrity (PMI) for groups YS, AS and OS.

Group	TM (%)	PM (%)	VAP (μm/s)	VSL (μm/s)	VCL (μm/s)	RAP (%)	PMI (%)
YS	52.5 ± 20.9[a]	28.0 ± 14.5	106.2 ± 11.4[a]	87.2 ± 9.5	193.8 ± 18.2[a]	42.4 ± 20.5[a]	47.8 ± 18.5
AS	78.1 ± 11.9[b]	43.5 ± 14.0	115.9 ± 16.6[b]	92.8 ± 8.2	207.0 ± 27.4[b]	67.6 ± 13.2[b]	51.6 ± 16.5
OS	83.1 ± 2.9[b]	38.8 ± 13.6	132.0 ± 19.8[b]	98.7 ± 8.9	238.8 ± 39.2[b]	74.6 ± 6.0[b]	76.5 ± 5.0

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). Lowercase letters in the same column indicate a significant difference ($p<0.05$).

Table 2
Mean values and standard deviations of major sperm morphological defects (MD), minor sperm morphological defects (mD), defects in the sperm head (DSH), defects in the sperm mid-piece parts (DMP), defects in the sperm tail (DST), sperm droplets (SD) and teratological sperm forms (TER) for groups YS, AS and OS.

Group	MD (%)	mD (%)	DSH (%)	DMP (%)	DST (%)	SD (%)	TER (%)
YS	30.7 ± 16.6	4.9 ± 2.6	13.3 ± 4.4	3.3 ± 3.9	13.9 ± 10.9	4.0 ± 6.6	1.7 ± 2.4
AS	19.9 ± 10.9	2.3 ± 1.8	9.6 ± 9.4	2.4 ± 2.7	8.6 ± 2.7	0.5 ± 0.8	0.6 ± 0.9
OS	18.0 ± 7.1	4.9 ± 6.2	10.4 ± 6.5	1.0 ± 2.2	7.8 ± 7.0	2.6 ± 2.8	0.2 ± 0.5

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). There were no differences between ($p>0.05$) G1, G2 and G3.

Table 4
Mean values and standard deviations of the scrotal surface temperatures (SST M0) and rectal temperatures (RT M0) prior to sun exposure for groups YS, AS and OS.

Group	SST M0 (°C)	RT M0 (°C)
YS	34.2 ± 0.6^{a}	37.8 ± 0.2^{b}
AS	34.1 ± 0.7^{a}	37.7 ± 0.3^{b}
OS	34.4 ± 0.8^{a}	37.5 ± 0.4^{b}

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). Lowercase letters in the same line indicate a significant difference ($p < 0.05$).

Table 5
Mean values and standard deviation of the scrotal surface temperature before (SST M0) sun exposure, total motility (TM), major defects (MD) and minor defects (mD) pathology sperm in animals with low ($n=9$, SST 0 < 34.0 °C) and high ($n=15$, SST 0 > 34.5 °C) SST.

Group	SST 0 (°C)	TM (%)	MD (%)	mD (%)
L SST	33.6 ± 0.3^{a}	72.3 ± 17.3	18.0 ± 3.9	3.8 ± 2.9
H SST	34.9 ± 0.3^{b}	68.0 ± 25.1	28.4 ± 17.1	3.8 ± 4.4

L SST (animals with SST 0 < 34.0 °C) and H SST (animals with SST 0 > 34.5 °C).

No difference was observed in TV or TSE ($p > 0.05$); however, the concentration of sperm per mL from the OS was greater than that of the YS ($p < 0.05$) (Table 3).

We noted a difference ($p < 0.05$) between the SST (0) and RT (0) (Table 4), in which the SST was an average of 3.4 °C lower than the RT. The stallion with the lowest SST was a YS in M0 (33.1 °C), and the stallion with the highest SST was an OS stud in M1 (36.0 °C).

An analysis of the data from stallions with low (SST < 34 °C) and high SST (SST > 34.5 °C) values showed no difference ($p > 0.05$) in TM, MD and mD between the two groups (Table 4).

There was no difference ($p > 0.05$) in RT between the groups; however, this parameter differed ($p < 0.05$) between the two time points (M0 and M1) in the three groups (Table 5).

There was also no difference in BST ($p > 0.05$) between the groups, and a comparison of the time points indicated higher values ($p < 0.05$) measured in M0 than in M1 in the YS, AS and OS groups (Table 6). Differences ($p < 0.05$) between the values for RT and BST of the animals before and after sun exposure were also observed in all three groups (Tables 5 and 6). However, no differences were detected between the SST for the two exposure time points ($p > 0.05$) (M0 and M1) in any of the three groups or between the different age groups ($p > 0.05$) (Table 7, Table 8 and Fig. 1).

There was a moderate positive correlation between SST and MD ($r=0.32$), changes in sperm head morphology ($r=0.33$) and weak positive correlation the presence of protoplasmic droplets ($r=0.29$). There was also a weak negative correlation ($r=-0.23$) between TV and testicular temperature and a moderate positive correlation ($r=0.58$) between TV and TSE.

Table 6
Mean values and standard deviations of the rectal temperatures before (RT M0) and after (RT M1) sun exposure for groups YS, AS and OS.

Group	RT M0 (°C)	RT M1 (°C)
YS	37.8 ± 0.2^{a}	38.6 ± 0.6^{b}
AS	37.7 ± 0.3^{a}	38.4 ± 0.5^{b}
OS	37.5 ± 0.4^{a}	38.7 ± 0.6^{b}

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). Lowercase letters in the same line indicate a significant difference ($p < 0.05$).

Table 7
Mean values and standard deviations of the body surface temperature (BST) before (BST M0) and after (BST M1) sun exposure for groups YS, AS and OS.

Group	BST M0 (°C)	BST M1 (°C)
YS	35.5 ± 0.9^{a}	37.6 ± 1.3^{b}
AS	36.7 ± 0.8^{a}	38.3 ± 0.8^{b}
OS	36.7 ± 0.8^{a}	37.7 ± 0.8^{b}

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). Lowercase letters in the same line indicate a significant difference ($p < 0.05$).

Table 8
Mean values and standard deviations of the scrotal surface temperature before (SST M0) and after (SST M1) sun exposure for groups YS, AS and OS.

Group	SST M0 (°C)	SST M1 (°C)
YS	34.2 ± 0.6	34.5 ± 0.6
AS	34.1 ± 0.7	34.7 ± 0.9
OS	34.4 ± 0.8	35.1 ± 0.7

YS (animals between 2 and 3 years of age), AS (animals between 4 and 16 years of age) and OS (animals 17 years of age or older). There were no differences between ($p > 0.05$) YS, AS and OS.

4. Discussion

Thermographic image evaluations of the scrotal surfaces were easily and quickly performed and shown to be a practical, non-invasive and risk-free technique, as was previously shown for other species (Coulter et al., 1988; Eddy et al., 2001; Yanmaz et al., 2007). This technique has great potential for use in the field as an additional tool during a stallion's andrological examination, as previously demonstrated in humans (Gazvani et al., 2000). This technique is a good indicator of intratesticular temperature, as reported by Coulter et al. (1988), who did not observe any differences between the testicular temperatures measured using an intratesticular sensor and the SST measured using thermography in sheep. In addition, the authors also discussed the invasiveness of using intratesticular sensors to measure temperature because the procedure involves the direct introduction of a needle into the testicles. Unfortunately, the needle insertion may lead to gonad inflammation. The present study is the first work in the literature to analyze the SST of stallions through digital infrared thermography.

In the present study, the testicular temperature was an average of 3.4 °C lower than the body temperature. Similar results were previously reported in bulls (Kastelic et al., 1996; Vogler et al., 1991). Efficient testicular thermoregulation is essential for physiologic spermatogenesis (Alvarenga and Papa, 2007; Blanchard et al., 1996). It is possible that increased sperm metabolism and higher testicular demand for oxygen occur when gonadal temperatures increase. The blood flow of the testicle is naturally low. Therefore, a local increase in the local temperature could result in a deficient oxygen supply, initiating a testicular degeneration process.

Exposure to 1 h of sunlight was sufficient to change the BST of the studied stallions without changing their SST. This increase in BST without significantly raising the temperature of the testes was also observed by Mawyer et al. (2012) using subcutaneous sensors in miniature horses before and after physical exercise. The ability to maintain a normal scrotal temperature while under environmental heat stress conditions demonstrates the effectiveness of testicular thermoregulation mechanisms (Ashdown and Hancock, 1980; Setchell, 1991). The ability to regulate testicular temperature is independent of age, which demonstrates that even older stallions have the ability to adequately achieve testicular thermoregulation. It is notable that in the present study, we only used animals that had a record of fertility.

In the present study, there was no effect of testicular temperature on sperm kinetics and sperm membrane integrity; however, there was a trend of an increased testicular temperature that may be related to reduced sperm quality because we observed a moderate positive correlation between SST and MD, additionally was observed moderate positive correlation between SST and alterations in the sperm head.

There was a weak positive correlation between SST and the presence of protoplasmic droplets ($r=0.29$), however this finding was not so significant as the study reported by Blanchard et al. (1996), in which the authors observed an increase in this pathology in stallions subjected to thermal stress for prolonged periods. In a study in bovines that evaluated the effect of thermal stress on sperm quality, Fernandes et al. (2008) observed an increase in morphological changes in sperm 14 days after testicular insulation. These data confirm the deleterious effect of temperature on sperm maturation.

The analysis of individual stallions showed that horses with higher testicular temperatures have a higher occurrence of changes in sperm morphology. This observation is consistent with the report of Vogler et al. (1991), in which the authors noted an increase in morphological changes of cattle sperm as the epididymal temperature increased after insulation. The largest percentage of morphological changes being observed in YS was an expected result. In cattle, Dowsett and Knott (1996) and Soderquist et al. (1996) also observed higher rates of sperm defects in young animals (25 months) compared to adult (5 years).

Dowsett and Knott (1996) observed that the TM, VAP, VCL and RAP in young animals were lower than the corresponding values in older animals, which can be explained by the sexual immaturity of these horses because stallions reach sexual maturity at 5 years old (Amann et al., 1979).

We observed a weak negative correlation between the SST before sun exposure and TV ($r=-0.23$). This correlation may be explained by the smaller area of the scrotum, which results in less area for heat exchange, and because the testes of horses with smaller scrotal are lower and closer to the abdomen.

Ball (2008) noted that TV is possibly related to the TSE of stallions. This observation was found in the present study, as a moderate positive correlation between TV and TSE ($r=0.58$). In humans, Hjollund et al. (2002) observed a negative correlation between an increase in testicular temperature and the total number of sperm. However, this correlation was not observed in our study, which is likely because we only used AS with a history of good fertility.

5. Conclusions

The results of this study demonstrate that normal stallions in different age groups can maintain a consistent testicular temperature even under environmental heat stress conditions for 1 h during days with ambient temperatures between 30 and 32 °C, thus demonstrating the efficiency of testicular thermoregulation mechanisms.

The use of a digital infrared thermographer to measure the heat radiated by the scrotum has been shown to be a important tool for characterising SST in stallion. This tool also has the potential for use as a complementary examination technique in andrological evaluations.

Future studies should be performed to verify the potential use of thermography in diagnosing testicular dysfunctions in stallions.

Conflict of interest

None.

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