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Recovery of normal testicular temperature after scrotal heat stress in rams assessed by infrared thermography and its effects on seminal characteristics and testosterone blood serum concentration

Maíra Bianchi Rodrigues Alves [a], André Furugen Cesar de Andrade [b], Rubens Paes de Arruda [c], Leonardo Batissaco [a], Shirley Andrea Florez-Rodriguez [a], Bruna Marcele Martins de Oliveira [a], Mariana Andrade Torres [b], Renata Lançoni [c], Gisele Mouro Ravagnani [b], Roberto Romano do Prado Filho [a], Vinícius Silva Vellone [a], João Diego de Agostini Losano [d], Celso Rodrigues Franci [e], Marcílio Nichi [d], Eneiva Carla Carvalho Celeghini [a,*]

[a] *Laboratory of Teaching and Research in Pathology of Reproduction, Center of Biotechnology in Animal Reproduction, Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, SP, Brazil*
[b] *Laboratory of Andrology and Embryo Technology, Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, SP, Brazil*
[c] *Laboratory of Semen Biotechnology and Andrology, Center of Biotechnology in Animal Reproduction, Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, SP, Brazil*
[d] *Laboratory of Andrology, Department of Animal Reproduction, School of Veterinary Medicine and Animal Science, University of São Paulo, Sao Paulo, SP, Brazil*
[e] *Laboratory of Neuroendocrinology and Reproduction, Department of Physiology, Faculty of Medicine, University of São Paulo, Ribeirao Preto, SP, Brazil*

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ABSTRACT

Reestablishment of testicular normal temperature after testicular heat stress is unknown and its effect varies widely. The aim of this study was to investigate the impact of scrotal insulation (IN) on testicular temperature and its relation to semen quality and testosterone blood serum concentration. For this, 33 rams were used; 17 submitted to IN for 72 hours (using bags involving the testes) and 16 not submitted to IN (control group). The experiment was performed between August and December 2013 in Pirassununga, Brazil (21°56′13″ South/47°28′24″ West). Seminal characteristics, testosterone blood serum concentration, rectal temperature (RT), respiratory frequency, scrotal superficies mean temperature (SSMT), and eye area mean temperature (EAMT) were analyzed 7 days before IN and 21, 35, 49, 63, and 90 days afterward. Scrotal superficies mean temperature and EAMT were measured by thermography camera FLIR T620. Testosterone was evaluated by radioimmunoassay. Analysis of variance was used to determine the main effects of treatment, time, and treatment-by-time interaction using PROC MIXED of SAS software adding command REPEAT. Pearson correlation test was used to verify correlation between SSMT, EAMT, RT, and respiratory frequency. Significant difference was considered when $P \leq 0.05$. At the end of IN, SSMT was higher ($P < 0.05$) in insulated group ($32.26 \pm 0.19^{o}C$) than in

* Corresponding author. Tel.: +55(19) 3565-4242; fax: +55(19) 3565-4060.
E-mail address: celeghin@usp.br (E.C.C. Celeghini).

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control group (30.58 ± 0.18°C), and the difference between rectal and testicular (deduced from SSMT) temperatures was 1.12 °C; in the other times of the evaluation this difference was between 2.91 and 4.25 °C in IN group. Scrotal superficies mean temperature was reestablished 24 hours after IN. Rectal temperature and EAMT presented correlation ($r = 0.59$; $P < 0.0001$). There was time-by-treatment interaction for total sperm ($P = 0.0038$) and progressive motility ($P = 0.01$), abnormal spermatozoa ($P < 0.0001$), membranes integrity ($P < 0.0001$), induced thiobarbituric acid reactive substances (TBARSs; $P = 0.05$), and DNA integrity ($P = 0.0004$). These semen characteristics were negatively affected 21 days after IN, and excluding induced TBARSs and abnormalities, recovered 35 days afterward; induced TBARSs just were affected after 49 days of IN; sperm abnormalities just recovered after 63 days. Testosterone blood serum concentration was lesser in insulated rams ($P = 0.03$). Thus, the difference of 1.12 °C between RT and testicular temperature impacts semen quality and testosterone blood serum concentration. Moreover, this study shows that rams can recover testes temperature efficiently toward IN and that infrared thermography is an efficient tool to identify differences on SSMT.

1. Introduction

Testicular thermoregulation consists of the maintenance of testicular temperature from 2 to 6 °C lower than body temperature enabling spermatogenesis to occur normally [1,2]. Pampiniform plexus, tunica dartos, cremaster muscle, scrotal superficies, and scrotal sweat glands are fundamental to testicular thermoregulation. The disruption of these mechanisms by factors such as high environmental temperature, fever, cryptorchidism, and inflammation can affect thermoregulation characterizing a testicular degeneration process [3].

There are many hypotheses about spermatogenesis dependency of testicular temperature lower than body temperature. Some research affirms that these lower temperatures are necessary to decrease DNA mutation rates and improve sperm quality [4,5]. In fact, higher temperatures increase cell metabolism [3]. However, blood flow does not increase in the same proportion; like this, cellular hypoxia is established [3]. Thus, cells suffer an imbalance in reactive oxygen species and present oxidative stress, DNA damage, and apoptosis [3,6–10]. Spermatocytes and spermatids are more susceptible to heat stress than spermatogonia [11]. With that in mind, if spermatogonia are not affected by heat, it is possible for spermatogenesis recovery. Leydig and Sertoli cells, somatic cells that are fundamental in spermatogenesis, are also more resistant to heat stress [3]. In rams, spermatogenesis takes about 47 days [11].

Beyond the injury in the seminiferous tubule cells, the spermatozoa quality is dramatically affected in the testicular degeneration process [8,9]. Actually, testicular degeneration is a very important process in male reproduction because it negatively affects sperm motility and increases sperm defects, sperm oxidative stress, and sperm DNA fragmentation [6,8–10,12]. In some cases, testicular degeneration can also alter testosterone concentration levels [13,14]. The impact on semen quality and seminiferous tubule cells varies depending on exposure time to heat stress, which is directly related to the recovery of sperm function and reestablishment of spermatogenesis [7,15,16].

Testicular degeneration can be experimentally induced by scrotal insulation (IN). This technique is very applicable to rams [10,15,17,18], bulls [12,19,20], and stallions [21]. Scrotal IN prevents heat loss by scrotal superficies, causing an increase in testicular temperature [3]. According Coulter et al. [22], scrotal temperature correlates with internal testicular temperature. In this study, they established that the difference between scrotal temperature and internal testicular temperature is 4.8 °C, when environmental temperature varies from 24° to 26.6 °C. Although testicular temperature correlates with scrotal temperature, the normal and altered scrotal temperature of rams is still unknown.

Although testicular temperature evaluation is a very invasive procedure [22], it is important to know for early detection of the animals that have testicular thermoregulation disruption and potentially will have sperm injuries affecting fertility rates. Thermography, a noninvasive technique, is a technology that enables measurement of surface temperature and the precision of new cameras is impressive [23]. This technique was used successfully to access scrotal temperature in humans [24], bulls [25,26], and rams [27]. However, its importance is doubtful in breeding soundness [28] because it still requires the establishment of a pattern and also presents many variables that must be controlled, such as environmental temperature, distance of the superficies from the camera, presence of substances, and dirt and/or hair on evaluated superficies [23]. Moreover, although there are studies with scrotal thermography in IN, they did not investigate the recovery of normal scrotal temperature.

Considering that scrotal temperature measurement is efficient to infer internal testicular temperature and that recovery of normal scrotal temperature after IN is still unknown, this study investigates the effects of scrotal IN on scrotal and rectal temperatures (RTs) as well as on semen characteristics and testosterone blood serum concentration. The objective of this study is to investigate the impact of scrotal IN on testicular temperature and its impact on semen quality and testosterone blood serum concentration. Moreover, this study intends to show the importance of thermography in scrotal heat stress detection.

2. Material and methods

2.1. Animals and experimental design

Thirty-three healthy White Dorper rams with an average age of 17.6 ± 2.8 months and body weight of