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Effect of increased testicular temperature on seminal plasma proteome of the ram

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ABSTRACT

The present study evaluated the effects of heat stress on the ram seminal plasma proteome. Six Morada Nova rams were scrotal insulated for 8 days. Scrotal circumference, sperm parameters, and seminal fluid proteins were evaluated before (Day 0) and twice during scrotal insulation (Days 4 and 8), and weekly until semen parameters returned to preinsulation values (normal). Seminal proteins were analyzed by two-dimensional SDS-PAGE and mass spectrometry. Scrotal circumference decreased from 30 ± 0.4 cm on Day 0 to 22.6 ± 0.6 cm on Day 36 ($P < 0.05$) and became equivalent to preinsulation values on Day 71. Motile sperm became nearly absent from Day 8 to Day 64 but returned to normal on Day 113. Percentage of normal sperm changed similarly and returned to normal on Day 106. Rams were azoospermic between Days 29 and 64, and sperm concentration came back to normal on Day 92. The number of spots/two-dimensional gel reduced from 256 ± 31 on Day 0 to 104 ± 14 on Day 29 (when rams were azoospermic) and then increased to 183 ± 9 on Day 113 ($P < 0.05$), similar to spot counts before insulation. The intensities of 24 spots, referring to 17 seminal plasma proteins, were affected by treatment ($P < 0.05$). After insulation, seminal plasma had greater expression of actin (two isoforms), albumin, heat shock protein 70 kDa, protein DJ-1, HRPE773-like, C-reactive protein precursor, bodhesin-2 (one isoform), spermadhesins. Most protein spots had the greatest intensity between Days 8 and 29, returning to preinsulation values on Day 113 (when many sperm criteria returned to normal). Proteins downregulated after scrotal insulation included dipeptidyl peptidase 3, isoforms of heat shock protein 90 kDa, RSVP22, MMP2 and of Bdh2. In this case, RSVP22 was reduced on Day 113 and all others, on Day 134. Expression of MMP2 and HSP90.1 was reduced throughout the study. Integrin β5, V-type H^+-ATPase subunit A, ZBTB 42-like protein, isoforms of Bdh2, PSP-I, and RSVP22 were upregulated after testis insulation. Intensities of these spots were maximum ($P < 0.05$) 8 days after insulation started or on Day 29. Expression of most of such proteins returned to normal on Day 113. In conclusion, scrotal insulation affected testis and sperm parameters of rams, indicating alterations in both spermatogenesis and sperm maturation. Changes of seminal plasma proteome were coincidental with variations in semen parameters. Proteins affected by heat challenge are potentially involved in sperm protection, maturation, and fertilization.

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1. Introduction

In the male, germ cells are produced in the testis, matured in the epididymis, and stored at a quiescent state in the cauda epididymis. On ejaculation, epididymal fluid and sperm are mixed with secretions of the accessory sex glands and placed in the female reproductive tract. As well known, sound fertility, from the male perspective, depends on hormonal and metabolic support to biochemical events occurring in the testis itself, epididymis, and also after ejaculation. Moreover, not only intrinsic factors of sperm origin but also external ones from the reproductive fluids can influence the male's capacity to produce sufficient number of functionally normal spermatozoa [1,2].

Normal testis function depends on temperatures 4 °C to 5 °C lower than body temperature [3], and disruption of this condition causes varied degrees of damages to the gonadal parenchyma and sperm production. Thus, exposure of animals to high temperatures potentially affects their reproductive efficiency, and this condition is of particular concern in tropical areas where air temperature is many times above the capacity of thermoregulation. In fact, altered mechanisms of testicular thermoregulation have been reported as one of the main causes of subfertility in herds raised in tropical regions [3]. Studies have been conducted using scrotal insulation as a model to evaluate the effects of thermal injury to the testes of domestic species, such as the ram [4]. Also, it has been well established that high testicular temperature affects sperm cell motility, morphology, acrosome status, and sperm chromatin protamination, as evaluated by phase-contrast microscopy, fluorescent dyes, Giemsa staining, and computerized assisted semen analyzer [5–7]. Elevated testis temperature affects pituitary and gonad hormonal secretion [8], testis echotexture [9], seminiferous epithelium structure [10], and it certainly also causes disruption of normal epididymal function. Considering the importance of information about the effects of high temperature on the testis and epididymis, it is surprising that knowledge about this type of stress on seminal plasma components is still scarce. As well reported, the seminal plasma is a mixture of components mainly secreted from the epididymis and accessory sex glands, and many of those components affect specific aspects of sperm function, such as motility [11], morphology [12], capacitation and acrosome reaction [13], interaction with the oviduct [14], and fertilization [15,16]. Also, proteins of the seminal plasma are also empirically related to sperm parameters and fertility indexes [17,18]. Thus, the present work was conducted to evaluate the effects of increased scrotal temperature, by means of scrotal insulation, on testis morphology, semen parameters, and seminal plasma proteome of adult, tropically adapted rams.

2. Materials and methods

2.1. General procedure

Six adult rams (57 ± 1.5 kg) of the Morada Nova breed, white variety, had their testes insulated for 8 days. Scrota of all rams were insulated with bags made with a double layer of plastic and an inner layer of cotton, with cords in the edge of the bag opening, as reported before [4]. Briefly, the bags were positioned surrounding all scrota and were fixated by lacing the cords at the neck of the scrotum. The bags remained at place during 8 days and were transiently removed only during measurements of scrotal circumference (SC) and scrotal surface temperature. Rectal and scrotal temperatures, SC, and semen parameters were evaluated 7 days before insulation, on the day of insulation (immediately before placing the insulation device), twice during scrotal insulation, and weekly until semen parameters became equivalent to preinsulation values. Thus, semen samples were collected during 141 days, including 7 days before insulation and 134 days after that.

Seminal plasma collected during the experiment was subjected to two-dimensional (2-D) electrophoresis, and protein spots differentially expressed as the result of scrotal insulation were identified by mass spectrometry (electrospray ionization-quadrupole-time of flight [ESI-Q-TOF]). All rams were managed in accordance to International Guiding Principles for Biomedical Research Involving Animals. Starting 1 month before insulation and throughout the experiment, the rams were kept in individual pens and fed Tifton hay (*Cynodon dactylon*) and concentrate, following recommendations of the National Research Council (NRC, 2007) for sheep. The rams also had free access to water and mineral supplement. The experiment was conducted in the Northeast of Brazil (3° 43′ 6″ S, 38° 32′ 34″ W), between July and December of 2012, when average air temperature, relative humidity, and the temperature–humidity index were 29.5 ± 0.1 °C, 57 ± 0.2%, and 78.0 ± 0.1, respectively. In that region, temperatures usually remain without major changes and days have the same length throughout the year. Thus, there are no variations in the photoperiod and no season effects on semen quality of rams [19].

2.2. Measurements of rectal and scrotal skin temperatures and testis size

In the mornings (8 AM) and immediately before every semen collection, we measured both the rectal temperature and the temperature of the scrotal skin of all animals. Rectal temperature was determined using a digital thermometer (Incoterm, São Paulo, Brazil). Temperature of the scrotal skin was taken using an infrared thermometer (Minipa MT-350, São Paulo, Brazil), pointing the device directly toward the caudal area of each testis, at a distance of 10 cm. Temperatures of the right and left testes were averaged and used for the statistical analysis. During the days of scrotal insulation, temperature of testis skin was obtained by quickly removing the insulation device. Scrotal circumference was measured during preinsulation, insulation, and after removal of the insulation device, at weekly intervals [4].

2.3. Collection and analysis of semen

Semen samples were collected weekly by electroejaculation (Torjet-65; Neovet, Minas Gerais, Brazil) and immediately placed in a water bath (37 °C; [20]). Only one ejaculate was collected per animal each week. Previous studies showed that elevated testis temperature reduces libido, especially when scrotal insulation is used as a model