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Effects of testicle insulation on seminal traits in rams: Preliminary study

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ABSTRACT

To evaluate the effect of heat stress on sperm characteristics, as well as testicular biometrics and heat radiation, six breeds of rams: two Brazilian locally adapted breeds (Santa Ines and Bergamasca) and four exotic breeds (Dorper, Texel, Ile de France, and Hampshire Down) underwent scrotal insulation. The experiment consisted of pre-scrotal insulation (week −1), insulation (week 0), and post-insulation (week 1–11) phases. Insulation changed the scrotum and testicular measurements, the heat radiated from the gonads caused deleterious effects, both on the process of spermatogenesis and sperm maturation in the epididymis. However, all the characteristics studied showed reversibility at 11 weeks post-insulation, showing the ability of the seminiferous epithelium to react to the effects of environmental factors. The locally, adapted breeds were more resistant than exotic to testicular heat stress.

1. Introduction

The reproductive performance of small ruminants is influenced by their adaptation to the environment in which they are reared. Recently, Brazilian sheep production has seen an increase in the use of exotic wool breeds, mainly in crossbreeding programs (McManus et al., 2010), but little is known about ram adaptability to local climatic conditions. Most studies are carried out with hair breed dams (Castanheira et al., 2010) and their crossbred offspring (McManus et al., 2011; Correa et al., 2012, 2013; Paim et al., 2014). The Dorper breed, which was only introduced into Brazil in the mid 1990s is one of the most widely distributed breeds in the country (McManus et al., 2014), but as yet there is little data on its heat tolerance in Brazilian conditions. The expansion of sheep production in Brazil to areas that previously were not used for this type of animal production (Hermuche et al., 2012, 2013a,b) mean that studies on adaptability of exotic breeds are necessary to maintain high production indices in hostile environments. In the Brazilian, mid-west most sheep production systems use wool and hair rams for meat production in natural mating programs at pasture under intense ambient heat and low relative humidity, even in the rainy season.

Spermatogenesis is under the physiological control of the neuroendocrine system and under direct influence of scrotum–testicle thermoregulation (Courot and Ortavant, 1981; Byers and Glover, 1984). In mammals, with testes located permanently in the scrotum, thermoregulation occurs mainly by three mechanisms: the apocrine glands located in the scrotum allow sweating with subsequent testicular cooling; the *tunica dartos* and cremaster muscle that favor maintenance of the testicles in the abdominal inguinal region and the pampiniform plexus, consisting of testicular arteries and veins, which is responsible for the exchange of heat and consequent cooling of arterial blood (Kastelic et al., 1996). Testicular hyperthermia caused by increased subcutaneous scrotal temperature by direct solar radiation can alter ram reproduction by causing seminal degeneration (Marai et al., 2007), interfering directly in fertility.

Scrotal insulation is the most commonly used experimental model to determine the effects of increased testicular temperature on sperm production and semen quality. The technique consists of blocking or reducing the thermoregulatory capacity of the testes by coupling a thermal bag on the scrotum (Souza et al., 2004) and has often been used to study the dynamics of sperm defects in cattle spermograms, based on severity and duration of the thermal insult and the interval between the insulation and the semen collection.

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Few studies of testicle insulation have been conducted with rams. Protocols in general are performed intermittently for 8 or 16 h per day for extended periods of 160 or 144 days, respectively (Mieusset et al., 1992), for 16 h a day for 21 days and for 12 h a day for 28 days (Arman et al., 2006), four consecutive days (Ibrahim et al., 2001) and for 7 consecutive days (Moreira et al., 2001). Comparative studies between breeds of rams were not found. Most studies are also with animals that are adapted to the environment where the experiment is being carried out. Therefore, this experiment was conducted to study the effects of the testicular insulation (7 days) in rams of two Brazilian locally adapted breeds (Santa Ines and Bergamasca) and four exotic breeds (Dorper, Ile de France, Hampshire Down, and Texel) in a tropical environment.

2. Material and methods

Animal care procedures throughout the study followed protocols approved by the Ethics Committee for Animal Use (CEUA) at the University of Brasilia, number 33/2009.

2.1. Animal data

Purebred rams from six breeds (Bergamasca, Dorper, Ile de France, Hampshire Down, Santa Ines, and Texel) were used. Within breed there was no genetic relationship between animals for at least three generations. These were clinically healthy, sexually mature, with an average age of 6 years and average body weight of 77.43 kg, with good reproductive history and fertility, conditioned to semen collection by electro ejaculation. The number of rams per breed (3) was determined using the minimum number of replications formula in Kaps and Lamberson (2009) in accordance with CEUA regulations to detect differences between treatments at a level of 5% and 80% power of the test. The animals were managed in intensive system and fed with Tifton hay (*Cynodon* spp.), supplemented with a concentrate for sheep (22.00% crude protein, 2.30% ether extract, crude fiber 4.30%, 1.20% calcium, 0.38% phosphorus, and 71.50% TDN), mineral salt and water ad libitum.

2.2. Experimental phases and evaluations

The experiment consisted of three phases. The pre-scrotal insulation phase corresponded to the first week of the experiment (week −1) where the breeding soundness, testicle morphometric, and thermographic evaluations were performed. Semen was collected three times from each animal before scrotal insulation, at intervals of 3 days (Ibrahim et al., 2001). The first collection was performed to remove aged and degenerated sperm from the epididymis tail. Immediately after the last collection, the scrotum of each ram was insulated. Scrotal insulation phase (week 0) corresponded to the period of seven days in which the animals were kept with testicle thermal bags to cause severe stress on testicular thermoregulation. The post-insulation phase corresponded to the period after removal of the testicle thermal bags, where animals were examined again as before as well as morphometry and thermography for a period of 11 weeks (week 1–11), with intervals between collections of 7–15 days. The values obtained in the post-insulation were compared to the average of the pre-insulation values. Semen samples were obtained by electro ejaculation (Eletrovet® Premium) (Abdel-Rahman et al., 2000).

2.3. Scrotal insulation

Scrotal insulation was performed with two overlapping thermal bags (disposable diaper), each of which consists of double plastic layer separated by a layer of cotton, similar to that used in the study by Brito et al. (2004). This bag was fixed with masking tape, so that the sheep could not remove it. Care was taken so the bag did not cause interference in the scrotal circulation. Throughout the scrotal insulation (7 days), until the completion of data collection, the animals were observed twice daily for verification of possible diseases or discomfort due to the use of the scrotal insulation bag. No occurrences of diseases or changes in behavior of the animals were detected due to the use of scrotal thermal bag.

After the removal of thermal bags all animals had a swollen scrotum, preventing the individualization of the gonads (Fig. 1a) for two consecutive weeks (weeks 1 and 2 post-insulation), with measurement of testicular morphometry only possible from week 3 post-insulation. This figure also shows thermographs of injured (Fig. 1b) and healthy (Fig. 1c) scrotum at 1 and 11 weeks, respectively. In the injured testicle soon after removal of the scrotal bag, it is hard to see a well defined temperature gradient from the top to the bottom of the scrotum, which is much more evident in the healthy scrotum.

2.4. Data collection

Testicular measurements included scrotal circumference, testicle length, width, and thickness. Using caliper the measurements were made on the right testicle with in centimeters, excluding the epididymis tail. The data collections were performed at weeks −1 and every fortnight thereafter.

Skin temperature on the posterior surface of the scrotum was measured weekly by infrared thermography ThermaCAM® (FLIR Systems Inc., Wilsonville, OR, USA) at a distance of 1 m from the animal in 5 points: top, bottom, left, right, and central region.

For assessment of sperm concentration, semen was diluted in buffered formalin at a ratio of 1:400 and readings were taken at Neubauer chamber under an optical microscope at 200× magnification. A second aliquot, also fixed in buffered formalin was separated to study sperm morphology in microscopy of phase contrast, with 1000× magnification under immersion in wet preparation. The procedures were performed according to the CBRA (2013).

Computerized evaluation of sperm motility (CASA) was performed with Ivos-Ultimate 12 equipment (Hamilton Thorne Biosciences) using blade cell count (Leja® – 8 cameras) heated at 37 °C. From each ejaculate 1 μl fresh diluted semen (30–50 × 10^6 sperm/ml) in citrate–glucose was taken (Evans and Maxwell, 1990). Three fields of each sample, randomly chosen, were evaluated. The following motility parameters were analyzed: total motility (MOT%), average path velocity (VAP μm/s), progressive linear velocity (VSL μm/s), curvilinear velocity (VCL μm/s), amplitude shift lateral head (ALH μm), beat cross frequency (BCF Hz), straightness (STR%), and linearity (LIN%).

Membrane integrity was assessed using 6-carboxyfluorescein diacetate (CFDA) and propidium iodide (PI) (Molecular Probe®, Eugene, Oregon, USA) according to Harrison and Vickers (1990). A semen sample (10 μl) was added to the dye solution (40 μl) and incubated for 10 min in a microtube protected from light. A 10 μl aliquot of semen with the dye solution was placed on a slide covered with a coverslip, and observed in an epifluorescence microscope (Zeiss Axiophot: wavelength of 395/420 nm excitation/emission filter).

Two hundred sperm cells per slide were examined and classified according to the sperm membrane: intact membrane (presence of green coloration on the head); semi-injured membrane (presence of green and red coloration on the head); damaged membrane (presence of red coloration on the head). For purposes of analysis, the percentage of sperm with intact membrane and injured membrane were counted.

Acrosome integrity was evaluated using a combination of the fluorescein isothiocyanate – FITC (fluorescent probe) with peanut lecithin (peanut 57 aglutinin – PNA) and propidium iodide, accord-