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Evaluation of the sexual behavior and testosterone concentrations of Mangalarga Marchador stallions

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ABSTRACT

Assessing the sexual behavior of stallions is extremely important because sexual behavior is linked to reproductive efficiency. The objective of this study was to evaluate the sexual behavior, testosterone concentration and libido of Mangalarga Marchador stallions from northern Rio de Janeiro State, Brazil. The sexual behavior of 10 stallions was evaluated during semen collection while mounting a mare in estrus. Blood samples from each animal were collected for the evaluation of serum testosterone. The study was conducted during the breeding and non-breeding seasons with animals separated into two age categories: young (<4 years) and adult (>5 years). The libido of the stallions was evaluated using the observations of reaction time (RT), mount time (MT) and mount and ejaculation time (MEjT). Analysis of variance and analysis of data frequency were performed. The stallion behavior and testosterone concentrations were not affected by the breeding season or stallion age ($p > 0.05$). The overall mean of RT was 14.0 ± 2.4 s, MT was 32.7 ± 3.5 s and MEjT was 24.3 ± 0.8 s. We observed a few instances of a mount without an erection and a mount without ejaculation a maximum of 4 and 1 times, respectively. The percentage of stallions that did not perform Flehmen, biting, kicking, sniffing and vocalization were 72, 93, 93, 34 and 45% respectively. The average testosterone concentration was 0.64 ± 0.06 ng/mL. We concluded that the animals exhibited good libido overall, thereby indicating that they were well adapted to the environmental conditions of northern Rio de Janeiro state, Brazil.

1. Introduction

Equine production is an important source of income and jobs worldwide. In Brazil, the equine industry occupies a prominent position because the country has a large number of animals and excellent breeding stock; thus, Brazil has been exporting genetic material to several countries (Alvarenga, 2002).

The Mangalarga Marchador horse is a native Brazilian breed; there are large numbers of this breed in all of the Brazilian territories. The principal function of the Mangalarga Marchador is its gait, which is different from other horses worldwide. The gait of the Mangalarga Marchador is the most comfortable for riders. This breed has other important traits, such as its docile behavior, and can be ridden by people of all ages; they are agile, rustic and can adapt to different environments, making this breed one of the most important in the country.

The semen of stallions that have high breeding value is collected frequently during the breeding season. Therefore, the natural sexual behavior of stallions changes when semen is collected for use in reproductive biotechnologies, such as artificial insemination and cryopreservation. According to Noue et al. (2001), the sexual behavior of the animals during semen collection is different from that during natural breeding.

To maximize the reproduction efficiency of stallions, they should be handled frequently to maintain adequate sexual behavior and good libido. Thus, the use of suitable semen collection methods is recommended (Sieme et al., 2004).

Horses are considered to be "long-day breeders" because their reproductive capacity is maximized during the seasons in which the photoperiod is increased. The testicular weight and volume

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of stallions increase during spring and summer, which leads to an increase in sperm production, libido and plasma hormone concentrations of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, estrogen, inhibin and prolactin (McKinnon et al., 2011). All of these changes occur because of light-induced suppression of melatonin by the pineal gland, which allows for a considerable increase in the hypothalamic production of gonadotropin-releasing hormone (GnRH) and of gonadotropins and sex hormones (Roser, 2009).

The spermatogenesis and sexual behavior of stallions are dependent on the proper functioning of the hypothalamic-pituitary-gonadal axis (Garner and Hafez, 2004). GnRH is released in pulses by the hypothalamus and acts on the anterior pituitary to stimulate gonadotropin release (Garner and Hafez, 2004). One gonadotropin is LH, which binds to Leydig cells in the testicular parenchyma of adult stallions, thereby stimulating the production and release of testosterone. Testosterone is a sex steroid hormone that exerts regulatory effects on sexual behavior and spermatogenesis (Felix et al., 2001).

The reproductive activity of stallions is regulated by seasonality and photoperiods, according to Clay and Clay (1992) and Pickett et al. (1989). These two reports note that the libido and concentration of gonadotropin, estrogen and testosterone are significantly influenced by the breeding season (BS). Studies are required to assess stallion sexual behavior and libido because, according to Alvarenga and Papa (2009), problems related to sexual behavior are the second leading cause of reproductive disorders, which frequently affect the ability of stallions to ejaculate. Usually, the stallion achieves an erection and is able to mount, but is not able to ejaculate.

The aim of this study was to evaluate the sexual behavior and serum testosterone concentrations of Mangalarga Marchador stallions from northern Rio de Janeiro State, Brazil, during the BS and non-BS and according to age.

2. Materials and methods

2.1. Animals and study design

This animal experimentation was performed with the approval of the Comissão de Ética de Uso de Animais (CEUA – UENF) according to the Sociedade Brasileira de Ciência de Animais de Laboratório/Colégio Brasileiro de Experimentação animal (SBCAL/COBEA) with protocol number 245.

The study was conducted in the north of Rio de Janeiro State, Brazil (latitude 21°45′15′, longitude 41°19′28′, and 13 meters above sea level), in two reproduction seasons (RS): the BS and non-BS of 2013. The BS occurs in spring and summer, from September to April, and the non-BS occurs in fall and winter from, May to August. The peak BS is in December and January when the stallions show the highest semen output.

In this study, we used ten ($n = 10$) animals of two age categories with three collections during the BS and non-BS (a total of 30 observations/season). The young stallions were up to four years of age, and the adult stallions were older than four years of age. The animals were from a stud farm located in northern Rio de Janeiro, Brazil.

During the experimental period, each stallion was maintained in an individual pen that was 4 x 4 m with concrete walls that were three meters high and that was ventilated by two large windows and one wooden door. The stallions were not taken to exercise during the study. They were fed with hay and commercial feed (Equitec®, Brazil) with 12% crude protein, 15% fibrous fraction and 20 g/kg fat twice a day; water and mineral salt were available *ad libitum*.

Before the beginning of the study, all of the animals underwent a breeding soundness examination in accordance with the Brazilian College of Animal Reproduction (CBRA, 2013), and all of the animals were considered to be suitable for breeding. Prior to the evaluation of the sexual behavior of the stallions, the semen of all of the stallions was collected over three weeks with a 48 h interval between collections. The younger stallions had just started their performances in the last BS prior to the experiments, and the adult stallions participated in at least two BS prior to the experiments. All of the stallions were capable of mounting the mare and ejaculating inside the artificial vagina (Botupharma® Botucatu, Brazil); therefore, they were conditioned when the semen collection started.

The mares used in this study were evaluated with ultrasonography (ultrasound DP-220Vet Mindray®, Nanshan, China) to certify that they were in estrus. The sexual behavior of each stallion was observed and filmed (recording digital) using a camera (Sony® Cyber-shot DSC-WX50 digital camera, Tokyo, Japan) during semen collection.

The following variables were registered when analyzing the videos to assess the sexual behavior exhibited by each animal: reaction time (RT), which is the time from when the stallion is presented to the mare in estrous until the penis is exposed; mount time (MT), which is the time from when the animal is presented to the mare in estrous until the start of copulation; mount and ejaculation time (MEjT), which is the time when the stallion starts the mount, ejaculates and then dismounts the mare; the frequency of mounts without erection (MWEr); mounts without ejaculation (MWEj), which is when the stallion inserts the penis into the artificial vagina, does not ejaculate and then dismounts the mare; Flehmen responses; sniffing; biting; kicking; and vocalizations made by the stallions.

To classify the libido of the animals according to their sexual behaviors, it was suggested, that the RT, MT and MEjT were grouped into 1 – Poor libido (when the stallions displayed a RT greater than 30 seconds (s), MT greater than 41 s and MEjT greater than 32 s; 2 – Medium libido (when the stallions displayed a RT between 11 and 30 s, MT between 21 and 40 s and MEjT between 20 and 32 s) and 3 – Good libido (when they displayed a RT of less than 10 s, MT less than 20 s and MEjT less than 19 s).

The testosterone concentration was determined from two blood samples collected from each stallion in the morning using tubes (Vacutainer® BD Diagnostics, New Jersey, USA) without ethylenediaminetetraacetic acid (EDTA); one sample was collected during the BS, and another sample was collected during the non-BS. The blood was transported to the laboratory in a Styrofoam® box (USA) on ice for 40 minutes. Upon arrival at the laboratory, the tubes containing the blood samples were centrifuged at 1400 × *g* for 15 min. The serum was separated, and the samples were stored in a freezer at −20 °C until analysis. The testosterone concentrations were determined through a solid phase radioimmunoassay (RIA) technique using a commercial diagnostic kit from Immunotech® Beckman Coulter Laboratories (Marseille, France). The laboratory procedures for the sample extraction and assay were performed according to the manufacturer's instructions.

2.2. Statistical analyses

Statistical analyses were performed with MIXED procedure (SAS, 2009) to verify the age categories (AC) and reproductive season (RS) effects on RT, MT, MEjT and testosterone concentration. The AC and RS interactions were studied, but they were not significant ($p > 0.05$).

The means were compared using the Student–Newman–Keuls (SNK) test at 5% probability.

The final model used was : $Y_{ijl} = m + \mathrm{AC}_i + \mathrm{RS}_j + E_{ijl}$;

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