



Evaluation of the breeding behavior of Brazilian Pony stallions



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ABSTRACT

The aim of this study was to evaluate the breeding behavior of Brazilian Pony stallions during semen collection to examine the effect of age category (AC) and month of evaluation on the characteristics of breeding behavior. Nine stallions were evaluated during the breeding and non-breeding season, 5–8 (AC1) and 9–13 years (AC2) of age. The variables evaluated were reaction time (RT), mount time one (MT1) and two (MT2), mount and ejaculation time (MEJT), mounting without erection (MWEr), mounting without ejaculation (MWEj), flehmen, kicks, bites, smells, vocalizations and serum testosterone concentration. Analyses of variance and correlation were performed. The average scores were RT: 56.2 ± 92.9 s, MT1: 115.0 ± 122.4 s, MT2: 196.4 ± 160.7 s, MEJT: 21.8 ± 3.2 s, MWEr: 0.8 ± 1.3 s, MWEj: 2.1 ± 3.5 s, flehmen: 3.8 ± 3.0 s, kicks: 0.3 ± 1.2 s, bites: 1.0 ± 2.0 s, smells: 5.7 ± 3.5 s, vocalizations: 6.4 ± 6.3 s and testosterone: 0.89 ± 0.52 ng/mL. The AC affected the RT ($P < 0.05$) but not the other traits. Month of evaluation did not affect most variables ($P > 0.05$). The correlations between age and RT and between age and MT1 and MT2 were significant. The correlation between testosterone and MEJT was moderate and negative. The breeding behavior of Brazilian Pony stallions was similar across different months of evaluation and different age categories. The month of evaluation and age category also did not affect testosterone concentrations, meaning that semen collection is feasible throughout the year.

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1. Introduction

In Brazil, miniature horse breeds, including the Brazilian Pony, have been popularized. The advent of these miniature horse breeds results from genetic selection that has been performed by humans for many years. The Brazilian Pony breed is intended for beginning child riders, ther-

apy programs and traction. They are docile and have body proportions similar to large draft horses.

The semen of stallions with high genetic value is frequently collected. Thus, the species' natural breeding behavior is manipulated to allow collection of semen for use in reproductive biotechnologies, such as artificial insemination (AI) and semen cryopreservation. However, restricting the natural behavior of these animals may negatively affect breeding behavior and libido (Noue et al., 2001).

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To use stallions most efficiently, the semen should be collected with a frequency that maintains proper breeding behavior and good libido. Therefore, it is recommended to use appropriate semen collection methods (Sieme et al., 2004).

Horses are considered a “long-day” reproductive species because the reproductive capacity is maximized in the summer when the number of hours of daylight increases, which increases the testicular volume, sperm production, libido and plasma concentrations of luteinizing hormone, follicular stimulating hormone, testosterone, estrogen, etc. All of these changes seem to be induced by changes in sunlight decreasing the amount of melatonin released by the pineal gland and increasing the production and release of GnRH and gonadotrophins (Roser, 2009). Thus, the reproductive activity of stallions is affected by the seasons and photoperiod. Another factor that can influence stallions' reproductive activity is age (McKinnon et al., 2011). However, there are few studies on the breeding behavior and serum testosterone in Brazilian Pony stallions.

Thus, the aim of this study was to evaluate the effects of age and month of evaluation on the breeding behavior of Brazilian Ponies during semen collection using an estrous mare.

2. Materials and methods

2.1. Animals and study design

This study was performed with the approval of the Comissão de Ética de Uso Animal (CEUA-UENF) in accordance with the Sociedade Brasileira de Ciência de Animais de Laboratório/Colégio Brasileiro de Experimentação Animal (SBCAL/COBEA) and assigned protocol number 245.

In this study, the months of December, March and April constitute the breeding season (BS), and May, August and September constitute the non-breeding season (nBS). In northern Rio de Janeiro State, Brazil, the BS occurs in the spring and summer from October to April, and the nBS occurs in the fall and winter from May to September.

The breeding behavior of nine ($n = 9$) Brazilian Pony stallions was evaluated between the ages of 5 and 13 years. Stallions were separated into two age categories (AC): between 5 and 8 years of age (AC1) and between 9 and 13 years of age (AC2). The stallions were evaluated once a month for 6 months (for a total of 54 observations). The stallions were bred on a stud farm in northern Rio de Janeiro State, Brazil (latitude: $-21^{\circ}45'15''$, longitude: $-41^{\circ}19'28''$, altitude: 13 m).

During the period in which the experiment was conducted, the stallions were kept in individual 12 m² boxes with one wooden door and 3-m-high concrete walls that were ventilated by two large windows. Stalls were bedded with straw. The stallions were fed pellets supplemented with minerals (12% crude protein, 15% fiber and 20 g/kg of fat) and hay three times daily and had ad libitum access to water. During the experiments, the stallions were not exercised.

Prior to the experiment, the stallions underwent a breeding soundness examination and all stallions used in the present study were considered suitable for breeding. All

stallions had been used for breeding in at least two breeding seasons prior to the experiment, and all were capable of mounting an estrous mare and ejaculating inside an artificial vagina (Botupharma® Botucatu, Brazil). Therefore, stallions were fit for semen collection when the evaluations started.

All mares used in this study were in estrus and this status was verified with ultrasonography (ultrasonic machine – DP-220Vet Mindray®, China).

2.2. Breeding behavior analysis

Each stallion was observed and filmed during semen collection with a camcorder (Sony® Cyber-shot digital camera, Tokyo, Japan). The stallions were allowed to interact with mares until penile erection occurred and attempts were made to mount. Evaluations ended if the stallions did not mount and ejaculate within 15 min.

The reaction time (RT) was evaluated, which is the time from when the stallion was presented to a mare in estrus until the penis was exposed and totally erect; mount time one (MT1), which is the time from when the animal was presented to a mare in estrus until copulation first started; mount time two or effective mount (MT2), which is the time from when the stallion was presented to a mare in estrus until the beginning of the bout of copulation that resulted in ejaculation (this applied only if the stallion had already performed other mounts without ejaculating); the mount and ejaculation time (MEjT), which is the time it took the stallion to mount, ejaculate and then dismount the mare; the number of mounts without erection (MWEr); the number of mounts without ejaculation (MWEj), which is defined as the stallion inserting the penis into the artificial vagina, not ejaculating and then dismounting the mare; flehmen; number of sniffing events; number of biting events; number of kicking events; and number of vocalizations.

All evaluations and semen collection were performed by the same veterinarian during all experiments.

2.3. Testosterone analysis

Testosterone concentrations were determined from four blood samples from each stallion, which were all collected in the morning using tubes (Vacutainer® BD Diagnostics, New Jersey, USA) without ethylenediaminetetraacetic acid (EDTA); two samples were collected during the BS, and the other two sample were collected during the nBS. The blood samples were transported to the laboratory in a Styrofoam® box (Sunrise, Florida, USA) after being placed on ice for 20 min. Testosterone concentrations were determined through use of a solid-phase radioimmunoassay (RIA) using a commercial diagnostic kit from Immunotech® (São Paulo, Brazil) Beckman Coulter Laboratories (Marseille, France).

2.4. Statistical analyses

The analyses of the breeding behavior variables were performed with the MIXED procedure of SAS (2009) to evaluate the effect of age category (AC) and month of

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