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Serological evaluation of ovarian steroids of red-rumped agouti (*Dasyprocta leporina)* during the estrous cycle phases

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ABSTRACT

The objective of this study was to evaluate serum progesterone (P4) and 17β estradiol (E2) concentrations throughout the estrous cycle in the red-rumped agouti (*Dasyprocta leporina*). A total of eight multiparous, captive-bred females were bled throughout their estrous cycle via saphenous venipuncture, with E2 and P4 concentrations being measured via ELISA of the serum collected. Serum E_2 ranged from 1212 to 3500 pg/ml with a peak value coinciding with observed estrus. However, two additional peak values for E_2 were also recorded, one each in metestrus and proestrus respectively. P4 concentrations reached a maximum of 4.23 ng/ml, and increases in P4 immediately followed the second E2 peak in metestrus. The highest concentrations of P4 were recorded in mid diestrus; between days 23–25 of the 31-day cycle. This phase was the longest in the agouti, consisting of approximately 19 days and accounted for 61% of the cycle. This study increased the basal scientific reproductive knowledge of this potentially valuable neo-tropical species.

1. Introduction

Managing wildlife species is dependent on an extensive knowledge of their reproductive physiology, among other biological factors. This knowledge can contribute to greater use of reproductive technologies including: artificial insemination and embryo transfer Andrabi and Maxwell (2007); (Fickel et al., 2007), for wildlife conservation and production (Pukazhenthi et al., 2005)

In 1991, the National Research Council (U.S.A) identified the Neotropical rodents *Hydrochoerus hydrochaeris* (Capybara), *Cuniculus paca* (paca) and *Dasyprocta* spp. (agouti) with potential for micro livestock enterprises as they provide a source of protein to many people. In the Amazon, paca and agouti are among the most consumed rodents (Valsecchi et al., 2014; van Vliet et al., 2014). In Trinidad and Tobago, agouti is considered the most hunted game species, highly prized for its meat, costing approximately US$50 for a normal sized adult. Concerning this issue, effective captive breeding programs may provide an alternate sustainable system for conservation and production of the agouti, as well as a means of decreasing hunting pressure

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on the wild populations. However, for this to be feasible, an extensive knowledge of reproductive physiology of the agouti is necessary.

There are few reports on the endocrinology of *Dasyprocta* spp. in captivity (Guimarães et al., 2011). Females have been reported as polyestrous spontaneous ovulators demonstrating non-seasonal breeding, with an estrous cycle of 31 days (Roth-Kolar, 1957; Smythe, 1970; Weir, 1971; Meritt, 1983; Guimarães et al., 1997; Campos et al., 2015) and a period of sexual receptivity of approximately 24 h (Guimarães et al., 2011). Gestation length is 104 ± 1.3 days, with an average litter of two precocious young (Roth-Kolar, 1957; Guimarães et al., 1997; Langer, 2003; Brown-Uddenberg et al., 2004; Brito et al., 2010).

In order to develop effective management tools and to conserve the agouti, a deeper understanding of its reproductive endocrinology is important. The aim of this work was to deepen the understanding of the estrous cycle of the red-rumped agouti (*Dasyprocta leporina)* raised in captivity, by analysis of serum progesterone (P4) and 17β estradiol (E_2).

2. Materials and methods

2.1. Animals

The protocol of this experiment was carried out in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals (NRC, 1996). The study was conducted in Trinidad & Tobago which has an average humidity of 80% and a daily average temperature range from 22–31 °C. A total of eight multiparous, captive-bred females of red-rumped agouti were individually housed indoors in wire mesh cages (0.6 m wide × 0.6 m deep × 0.9 m high) under natural lighting and an ambient temperature of 29 °C. They were fed fresh locally available fruits and roots (*Mangifera indica, Musa spp., Curcubita, Anacardium occidentale, Cocos nucifera, Manihot esculenta, Ipomea batatas, Leucaena leucocephala, and Trichanthera gigantean*) and water *ad lib*. Females were isolated from the males by approximately 1 m. The females were subjected to a 30 days adaptation period from February to June. The mean (±SD) age of females was 2 (±0.3) years, with a weight of 3.2 (±1.7) kg.

2.2. Experimental design

The estrous cycles were individually monitored daily by vaginal cytology until estrus. A medicine dropper containing a 10% saline solution was inserted approximately 4 cm deep into the vagina and aspirated three times. A single drop of the final wash was placed on a standard microscope slide, allowed to air dry and stained with Diff-Quick® stain (Med Vet International, Illinois, USA).

The vaginal cytology were classified according to Guimarães et al. (1997), Thrall and Olson, (1999) and Mayor et al. (2003), as seen in Table 1.

The proportions of these cells were used to determine the stage of the estrous cycle of the female agouti. Slides were assessed by three independent experienced examiners. Once the onset of estrus was identified by vaginal cytology, it was labeled as Day 0 (zero), and blood sampling commenced to coincide with cytological collection.

Table 1
Vaginal Cytology Classifications of estrous phases.

1 – proestrus	high frequency of nucleated superficial cells, which are the largest and oldest epithelial cells, and intermediate cells usually polygonal, rounded or oval
2 – estrus	large numbers of nucleated with fewer enucleated superficial cells
3 – metestrus	appearance of leucocytes and intermediate cells, which are rounded or polygonal
4 – diestrus	mainly basal (small nucleated) and parabasal (round small nucleated) cells

Blood samples (1 ml) were collected from un-sedated females every two days by saphenous venipuncture for serum E_2 and P4 identification. Serum was collected after centrifugation at 2500 rcf for 10 min, within two hours of collection and stored at −20 °C until assay. E_2 and P4 concentrations were measured by 17β estradiol (pg/ml) and progesterone (ng/ml) enzyme-linked immunosorbent assay (ELISA; Sigma-Aldrich. MO, USA), respectively. Manufacturer supplied quality controls were used to ensure the reliability of the assay. The assays were validated by pooling samples with high estradiol and progesterone. Intra-assay and inter-assay coefficient of variations were both 10% for both assays. Assay standards were confirmed by parallel immunoreactivity of serial dilutions of pooled samples. Dilutions of known concentration samples and purified progesterone and estradiol standards (Sigma-Aldrich. MO, USA) were used to test linearity and antibody specificity according to manufacturer's specifications.

2.3. Statistical analysis

Statistical Package for Social Sciences 16 (SPSS), software for Windows (IBM, NY, USA), was used for statistical analyses. In eight animals, P4 and E2 concentrations were measured for four estrous cycles each. These values were used to calculate the mean per animal at each time-point. One way ANOVA ($p < 0.05$) was used to compare the mean P4 and E2 concentrations (±SE) of those means for the eight animals and this was plotted over time. Peak values of P4 and E2 were also compared using one way ANOVA and Tukey's post hoc test to identify any significant differences in peak hormonal levels during the four phases of the estrous cycle of the female agouti.

3. Results

Recovery was 93.6% ± 0.88% for P4 and 87.5% ± 2.6% for estradiol-17b. Cross-reactivity of the Progesterone kit was 100% with P4 and 0.66% ± 2.2% for E2. The estradiol kit had 100% cross-reactivity with E2 but went undetected with P4. Sensitivity was 42 pg/ml for P4 and 7 pg/ml for estradiol.

The average length of the estrous cycle for captive female agouti was 31(±1.3) days, with the follicular phase (proestrus and estrus) ranging from 6 to 9 days and the luteal phase from 19 to 23 days (N = 8). Representative changes in serological concentrations of ovarian steroids (P4 and E2) for the stages of the four cycles monitored for