Available online at www.sciencedirect.com
SCIENCE DIRECT
Theriogenology
ELSEVIER
Theriogenology 63 (2005) 2113–2125
www.journals.elsevierhealth.com/periodicals/the

Seasonal changes in fecal testosterone concentrations and their relationship to the reproductive behavior, antler cycle and grouping patterns in free-ranging male Pampas deer (*Ozotoceros bezoarticus bezoarticus*)

Ricardo José Garcia Pereira[a], José Maurício Barbanti Duarte[b,*], João Alberto Negrão[c]

[a]*Pós-graduação Departamento de Reprodução Animal, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP 14884-900, Brazil*
[b]*Departamento de Zootecnia, Faculdade de Ciências Agrárias e Veterinárias, Universidade Estadual Paulista, Jaboticabal, SP 14884-900, Brazil*
[c]*Departamento de Ciências Básicas, Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo, Pirassununga, SP 13635-900, Brazil*

Received 10 April 2004; accepted 5 August 2004

Abstract

The purpose of this study was to validate noninvasive endocrine monitoring techniques for Pampas deer and to evaluate seasonal changes in testicular steroidogenic activity and their correlation to reproductive behavior, antler cycle and group size. Thus, fecal samples, behavioral data and observations of antler status were collected at monthly intervals during 1 year from free-ranging Pampas deer stags (three radio-collared individuals and 15 random individuals) living in Emas National Park, Brazil (18°S latitude). Fecal steroids were extracted using 80% methanol and steroid concentrations were quantified by a commercial enzyme immunoassay (EIA). Fecal testosterone concentrations peaked in December–January (summer), March (early autumn) and in August–September (winter–spring), with minimal values from April–July. Reproductive behavior had two

* Corresponding author. Tel.: +55 16 3209 2678; fax: +55 16 3202 4275.
E-mail address: barbanti@fcav.unesp.br (J.M.B. Duarte).

doi:10.1016/j.theriogenology.2004.08.014

peaks, the first in December–January, characterized by predominately anogenital sniffing, flehmen, urine sniffing, chasing and mounting behavior, and the second peak in July–September (behavior primarily related to gland marking). There were significant correlations between fecal testosterone and reproductive behavior ($r = 0.490$), and between fecal testosterone and antler phases ($r = 0.239$). Antler casting and regrowth occurred under low testosterone concentrations, whereas velvet shedding was associated with high concentrations of testosterone. We inferred that Pampas deer stags exhibited a seasonal cycle that modulated sexual behavior and the antler cycle, and we concluded that fecal steroid analysis was a practical and reliable non-invasive method for the evaluation of the endocrine status of free-ranging Pampas deer.

Keywords: Pampas deer; Reproductive cycles; Testosterone; Feces; Antler cycle

1. Introduction

In most seasonally breeding mammals, several environmental cues (e.g., nutritional, physical and social factors) modulate the reproductive performance of an individual and act as indicators of the onset and cessation of sexual activity [1,2]. For the family Cervidae, almost all detailed reproductive knowledge in males has been derived from studies of temperate species that exhibit a distinct seasonal cycle of reproductive behavior and antler growth [3,4]. Photoperiod is responsible for the adaptation of these deer species to high latitudes, therefore breeding occurs during decreasing daylengths [3]. Although many species of deer live in tropical and subtropical regions (with comparatively minor annual changes in photoperiod), information about their reproductive biology and antler cycles is comparatively rare.

The Pampas deer (*Ozotoceros bezoarticus*) is a medium-sized cervid originally distributed throughout the grasslands of eastern South America (between 5° and 40°S latitude). Due to human activities, these ungulates are currently listed as endagered in Appendix 1 of the CITES and exist in small, isolated populations in Argentina, Bolivia, Brazil, Paraguay and Uruguay [5–7]. Information regarding Pampas deer reproduction has been reported mainly on females, which are polyoestrous, with estrous cycles approximately 21 days long and a 7 months gestation [7,8]. Studies in South America have shown that births in Ozotoceros are not strictly seasonal, but occurred more frequently from September to November in Argentina and Uruguay [6], and from August to November in Brazil [7]. In additional, Frädrich [9] reported that captive Pampas deer in the West Berlin zoo had no fixed rutting season. Nevertheless, males of this species exhibit a well-defined antler cycle, similar to temperate stags, with antler casting from April to May in the Brazilian Pantanal [10], June to August in Uruguay [6] and August to September in Argentina [11]. Therefore, studies involving assessment of hormones, behavior and antler cycle in stags are important to compare seasonal reproductive changes of this tropical species to temperate cervids.

The development of assisted reproductive techniques such as artificial insemination, in vitro fertilization and embryo transfer for non-domestic animals depend on the knowledge of their basic reproductive physiology, which normally requires the collection of repeated blood samples for hormonal evaluations. However, in many cervid species, these endocrine studies are not practical and even dangerous due to excessive stress caused by capture and

restraint [12,13]. Consequently, non-invasive monitoring of fecal and urinary steroid metabolites has proven valuable to determine many reproductive end points (e.g., ovarian cyclicity, gestation and seasonality) in both captive and free-ranging ungulates [14–21]. Notwithstanding the feasibility of the non-invasive techniques, few studies monitored androgenic status in males [13]. Urinary and fecal androgen analysis involving stags have been reported only in Eld's deer (*Cervus eldi thamin*) [22], Sika deer (*Cervus nippon*) [23] and Père David's deer (*Elaphurus davidianus*) [24]. Thus, the objectives of this work were to: (1) validate an enzyme immunoassay to quantify fecal testosterone in Pampas deer; (2) study annual changes in fecal testosterone of free-ranging stags and (3) correlate fecal concentrations of testosterone with reproductive behavior, antler cycle, group size and environmental factors.

2. Materials and methods

2.1. Study area

The Emas National Park (ENP) is a federal reserve located in the southwest of the state of Goiás (18°S/52°W) and is the largest protected area of savanna vegetation in Brazil (131,868 ha). The park contains almost all typical vegetation of the savanna, with approximately 60% of the total area covered by open grasslands. The population of the northern subspecies of Pampas deer (*O. b. bezoarticus*) is estimated to be 1300 individuals [7,25,26].

2.2. Animals

The Pampas deer monitored in this study were free-ranging stags from ENP, which were divided into two groups, marked ($n = 3$) and random males (n = approximately 15 per month). In order to attach plastic ear tags and radiocollars (150–151 MHz; Wildlife Materials Inc., Carbondale, IL, USA) three animals composing the marked group (aged 2–6 year, weighing 27–34 kg) were captured employing the ''fast-setting net'' method [27]. They were anesthetized with a mixture of intramuscular xylazine hydrochloride (Rompun/Bayer–São Paulo, Brazil; 1 mg/kg) and ketamine (Dopalen/Agribrands–Paulínia, Brazil; 5 mg/kg). The aleatory group consisted of Pampas deer stags randomly found along the roads in the ENP, which facilitated observation and fecal collection. External characteristics (i.e., antler features, pelage color and different body signs or marks) of all aleatory individuals collected were recorded to reduce the possibility of pseudo-replication of samples in the same month. Additionally, different areas were previously defined for visitation during the collection period of each month, ensuring no replicability. Experimental protocols for this study were approved by the Brazilian Institute of Forestry Development (IBAMA, license no. 055/2000).

2.3. Sample collection and observational data

From October 2000 to September 2001, fecal samples were collected monthly from both groups. The three marked deer were tracked using telemetry, while the aleatory stags

were encountered beside the roads during daily excursions in ENP. After the initial siting, males were followed until they defecated. Each sample, recovered within 15 min of voiding, was placed in individually labelled plastic bags. Fecal samples were placed on ice until they could be frozen (−20 °C), which was done within 4 h of collection. All fecal samples were stored at −20 °C until steroid analysis was performed.

Information about reproductive behavior, antler cycle and the group size were recorded when the observer was waiting for males to defecate. Antler status was divided into three categories: cast, velvet and hard antler. In this study, the appearance of any activity of the monitored stags, e.g., chasing hinds, flehmen, anogenital and urine sniffing, gland marking (forehead and antler rub), herding hinds, fighting, mounting and copulating, was considered a reproductive behavior. Animals were considered to belong to the same group when they remained within of 100 m of each other and showed a tendency to travel in the same direction [28]. Single individuals were also included in our analysis.

The visits to ENP for behavior observation and sample collection were concentrated between days 10 and 20 of each month, with a total of 21 and 193 fecal samples from the marked deer and the aleatory group, respectively. The aleatory group consisted of predominantly adult males with three-pronged antlers (89.1%), with the rest being young males with two-pronged antlers. Unfortunately, we had losses in the marked group during the year. The radiocollar of one male (Male ''A'', 5-year-old) malfunctioned and the signal was lost after January 2001. Nevertheless, this male was occasionally found during daily excursions in ENP and samples were collected twice (February and August 2001) after the radiocollar failed. Another adult marked deer (Male ''C'', 6-year-old) was found dead in February 2001; it had been attacked and killed by a cougar (Puma concolor).

2.4. Fecal steroid extraction and enzyme immunoassay

Fecal samples (total amount collected) were lyophilized for 24 h (Model LGA05, MLW, Leipzig, Germany). Dried feces were pulverized in the blender and all solid inert materials (e.g., seeds and rough dietary fiber) were removed. Steroids were extracted from feces according to a modification of the method described by Palme et al. [29]. A proportion of the resulting powder (0.50 ± 0.02 g) was weighed and extracted with 5 mL of 80% methanol. After vortexing for 30 s at high speed, the sample was shaken for 12 h on a mechanical shaker. After centrifugation (700 × *g* 15 min, 4 °C), the supernatant was decanted into a clean tube. An aliquot of the supernatant (100 μL) was diluted 1:16 with assay buffer (20 mmol trishydroxyaminomethan, 0.3 mol NaCl, 0.1% bovine serum albumin and 0.1% Tween 80; pH adjusted to 7.5 with 1 mol HCl) for EIA analysis (Multiskan MS, Labsystem, Helsinki, Finland). A total of 15 ng of testosterone (standard testosterone 10ng/mL, EIA DSL-10-4000, Diagnostic Systems Laboratories Inc., Webster, IA, USA) was added to fecal samples before extraction to monitor recovery. Extraction efficiency was 92.9 ± 11.8% ($n = 5$).

Testosterone concentrations were measured in duplicate 50 μL aliquots of fecal extracts using a commercial Testosterone Enzyme Immunoassay kit (EIA DSL-10-4000, Diagonstic Systems Laboratories Inc., Webster, IA, USA). The antiserum had the

following cross-reactivities (provided by the company): 100% testosterone, 6.6% 5α-dihydrotestosterone, 2.2% 5-androstane-3β,17β-diol, 1.8% 11-oxotestosterone, 0.9% androstenedione, 0.6% 5β-dihydrotestosterone, 0.5% 5β-androstane-3β,17β-diol, 0.4% estradiol-17β and 0.2% 5α-androstano-3α-ol-17-one. The testosterone assay was validated for fecal extracts from Pampas deer stags by demonstrating: (1) parallelism between serial dilutions of fecal extracts (1:2–1:64) and the standard curve (0.1–25 ng/mL); and (2) significant recovery of exogenous testosterone (0.1–10 ng/mL) added to fecal extracts ($y = 0.74x + 35.7$; $r^2 = 0.97$). Assay sensitivity was 0.04 ng/mL, and the intra- and inter-assay coefficients of variation for Pampas deer control samples were 10.8 and 16.8%, respectively. To assess the physiological relevance of immunoreactive fecal testosterone, samples were collected from free-ranging animals, which were divided in three groups: adult males with sexual activity (i.e., males presenting intensive behaviors such as chasing hinds, gland marking and copulating; $n = 5$); adult females ($n = 3$); and young males (about 1-year-old; $n = 5$). The concentrations of these three groups were 343.9 ± 211.4 ng/g, 47 ± 22.9 ng/ g and 34.1 ± 8.6 ng/g (the adult males were higher ($P < 0.05$) than the other two groups. All fecal data are expressed on a dry-weight basis.

2.5. Statistical analysis

Testosterone concentrations in feces are presented as mean ± standard deviation (S.D.). Differences among monthly testosterone concentrations and among fecal steroid concentrations in different group size were determined by a Kruskal–Wallis test, followed by a Wilcoxon test. The percentage of males showing reproductive behavior per month was calculated and the differences among months were determined by a χ^2-test. Group size data are presented as mean ± S.D. per month and differences among months were calculated by a Kruskal–Wallis test, followed by a Wilcoxon test. The percentage of mixed-sex groups per month was also calculated. Pearson correlation coefficients were determined between fecal testosterone and reproductive behavior, and between fecal testosterone and antler status. A difference of $P < 0.05$ was taken as significant for all statistical tests, which were performed using SAS System for Windows 6.12 (SAS Institute Incorporation, Cary, NC, USA).

3. Results

3.1. Monthly changes in antler cycle, group size and reproductive behavior

The antler cycle of males in the aleatory group is shown in Fig. 1(b). Almost all Pampas deer stags in this group exhibited hard antlers from October 2000 to February 2001, with the exception of three animals with deformed antlers covered with velvet. Antler casting occurred from March to May and antlers in velvet started appearing in April, with maximal frequency during June and July (100 and 93%, respectively). Velvet shedding was observed in August 2001, whereas the majority (95%) of males observed in September were in hard antler. In the marked group, Male ‘‘C’’ had hard antler until its death in February (Fig. 2), while Male ‘‘A’’ was in hard antler from October to February (its last appearance in this

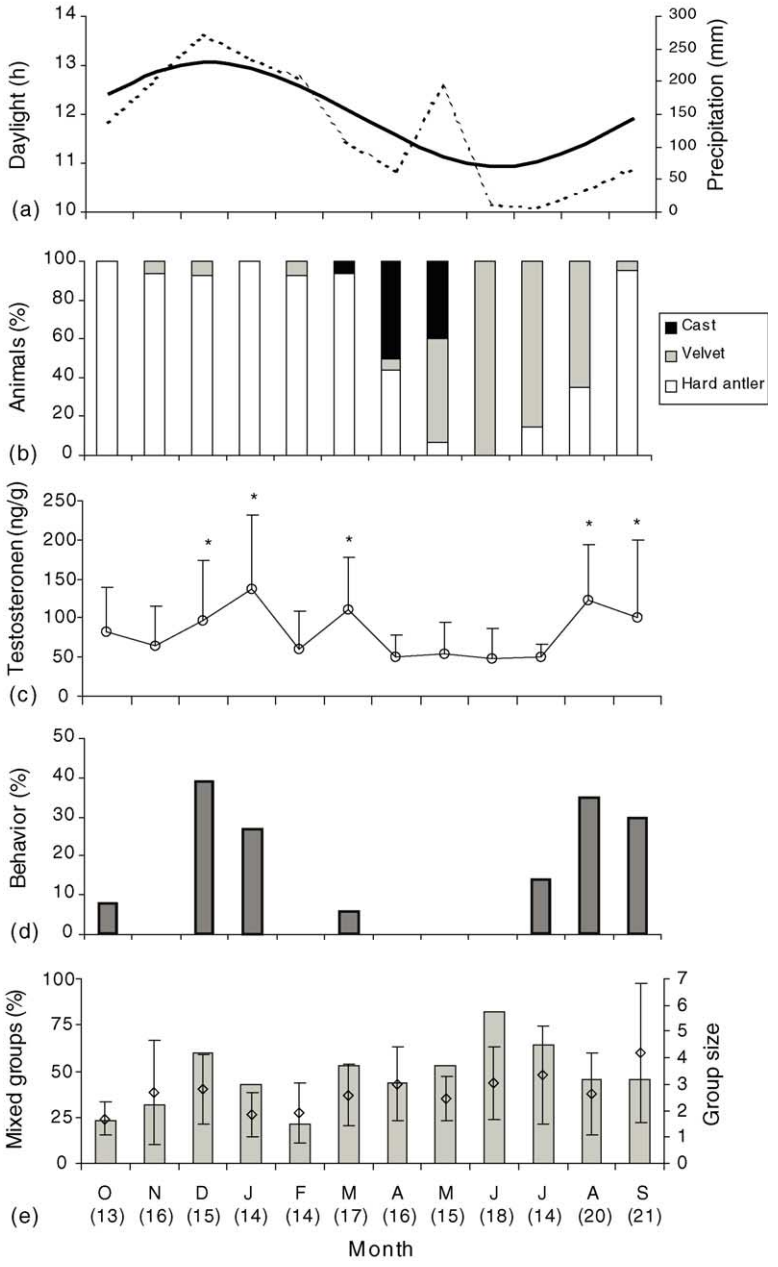

Fig. 1. (a) Hours of daylight (—) and precipitation (. . .) at the study area; (b) monthly percentage of animals in each antler category; (c) monthly mean (± S.D.) of fecal testosterone concentrations (asterisks indicate significant differences); (d) monthly percentage of animals with reproductive behavior and (e) Percentage of mixed groups (bars) and monthly mean (± S.D.) of group size (◇) from Pampas deer stags of the aleatory group sampled from October 2000 to September 2001 in Emas National Park. Number of samples are indicated in parentheses.

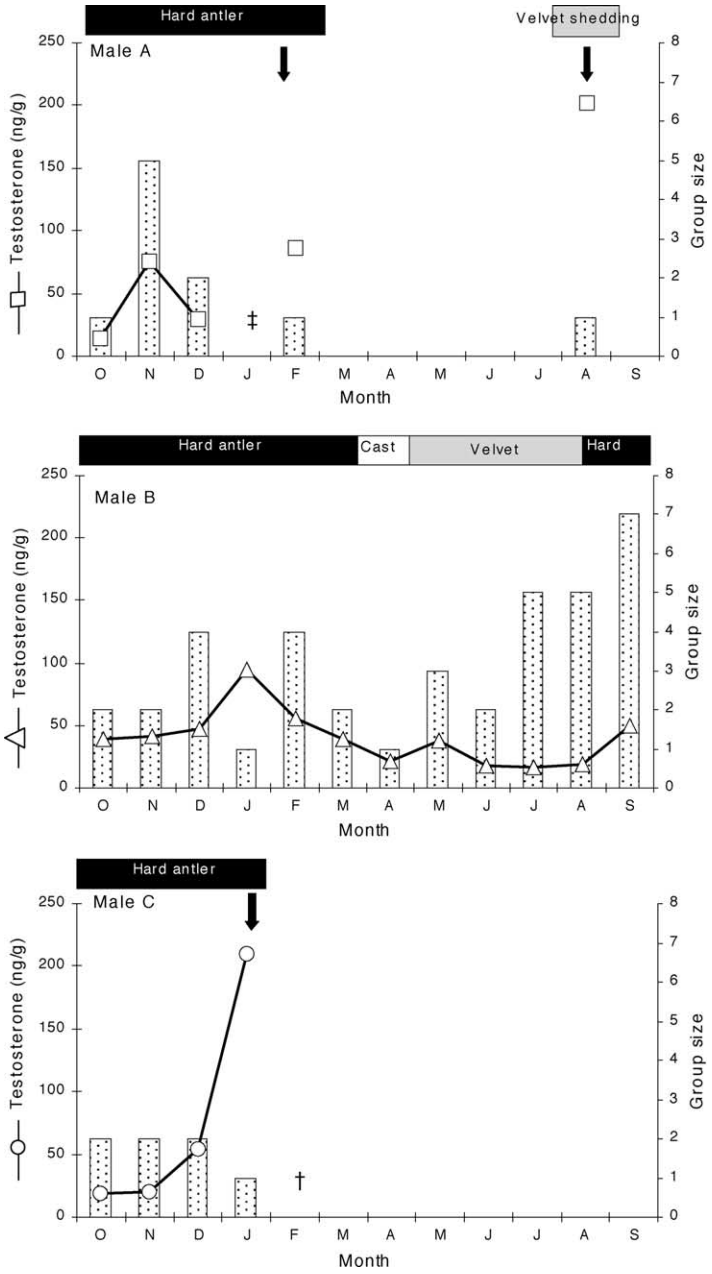

Fig. 2. Changes in antler cycle, group size, reproductive behavior and fecal testosterone concentrations of the three marked Pampas deer monitored from October 2000 to September 2001 in Emas National Park. Displaying of reproductive behavior (e.g., chasing hinds, flehmen, anogenital and urine sniffing, gland marking, herding hinds, fighting, mounting and copulating) is indicated with closed arrows (↓). (‡) Indicates the malfunction resulting in signal lost from radiocollar of Male "A" and (†) indicates the death of Male "C" (due to predation).

stage) and shedded velvet in August. Male ''B'' exhibited hard antlers from October to March, the old set of antlers were cast in April, with the development of new antlers from May to August, and velvet stripped by September.

The percentage of males in the aleatory group displaying reproductive behavior were different ($P < 0.05$) among months, with two major peaks, one in December–January (summer) and other in July–September (winter/spring; Fig. 1(d)). Anogenital sniffing, urine sniffing, flehmen, chasing, fighting and mounting were the prevailing behaviors during the first increase of reproductive activity, whereas gland marking was the most frequently observed behavior in the second peak. There were no observations of reproductive behavior from males collected in November, February and April–June, but sexual activity was noticed in <10% of the stags sampled in October and March. Data on reproductive behavior in the marked Pampas deer are shown in Fig. 2. Male ''A'' exhibited behaviors such as chasing hinds, flehmen and gland marking in February, and only gland marking behavior during August. Anogenital sniffing, urine sniffing and flehmen were recorded in January for Male ''C'' and no reproductive behavior was observed for Male ''B'' during the monitoring period.

The greater number of animals in the aleatory group was found isolated or in groups of two or three individuals (77.5%), with maximum group size of nine. Mean group size (Fig. 1(e)) was highest in September (4.20 ± 2.62 animals/group), with lowest means ($P < 0.05$) in October, November, January and February (mean range, 1.7–2.7 animals/group). The largest group size observed in all marked deer throughout the year was seven individuals (Fig. 2). The percentages of mixed-sex groups were higher during December, June and July (60, 82.3 and 64.2%, respectively), with minimum values in October (23.1%) and February (21.4%; Fig. 1(e)).

3.2. Fecal testosterone concentrations and their correlations to other parameters

For mean monthly fecal testosterone concentrations in aleatory stags, there were significant differences among sampling months (Fig. 1(c)). During early summer, testosterone concentrations increased to reach maximum concentrations ($P < 0.05$) in January (137.01 ± 95.39 ng/g). Thereafter, testosterone concentrations declined in February (60.94 ± 48.30 ng/g) and increased sharply in March (early autumn, 109.94 ± 68.05 ng/g; $P < 0.05$). Fecal testosterone values were uniformly low between mid-autumn and mid-winter (April–July; mean range 48.36– 54.9 ng/ g), but peaked again in August and September (mean range 100.98–123.01 ng/g; $P < 0.05$). In the marked group, Male ''A'' presented its highest fecal testosterone concentration during August (202.16 ng/g), whereas Males ''B'' and ''C'' reached a peak in January (94.56 and 209.92 ng/g, respectively; Fig. 2).

Differences in fecal testosterone concentrations among Pampas deer stags from varied group size were not significant (Table 1). There were significant correlations between fecal testosterone concentrations and the frequency of reproductive behaviors ($r = 0.490$, $P < 0.05$), and between fecal testosterone and antler status ($r = 0.239$, $P < 0.05$). Stags in hard antler had higher concentrations of fecal testosterone (101.86 ± 82.86 ng/g) ($P < 0.05$) when compared to males in antler casting or antlers in velvet (mean range 47.19–58.30 ng/g).

Table 1
Mean ± S.D. of fecal testosterone concentrations of Pampas deer stags from different group size

Group size	Fecal testosterone (ng/g dry feces)
1 Individual	94.05 ± 86.86 (49)
2 Individuals	68.25 ± 45.35 (48)
≥ 3 Individuals	83.84 ± 66.86 (96)

No significant differences. Values in parentheses represent numbers of samples analysed.

4. Discussion

The present study provided the first seasonal evaluation employing non-invasive techniques to monitor testicular steroidogenic activity in Pampas deer. Testosterone concentrations were measured in fecal samples using a simple extraction method and a commercial enzyme immunoassay. Our first objective was to validate a practical and appropriate procedure of monitoring fecal testosterone in Pampas deer stags. Therefore, EIA methodology was employed because such a system can be more economical and require less sophisticated laboratory equipment than RIA analysis. The results confirmed that this endocrine approach was applicable for Pampas deer, since fecal testosterone quantified by EIA were consistent with physiologic state (comparing adult males to immature males and females), and there was good correlation with reproductive behaviors.

Seasonal concentrations of fecal testosterone in Pampas deer showed two major peaks in the aleatory group, and only one peak in the single marked deer (Male ‘‘A’’) monitored throughout the year. Furthermore, high concentrations of fecal testosterone were found in adult Males ‘‘C’’ and ‘‘A’’ at similar periods (summer and winter to spring, respectively), regardless of the absence of their hormonal data in the remainder of the year. Annual biphasic elevation in testosterone concentration has been recorded regularly in males of roe deer and pudu [30–33], whereas a temporary spring increase in testosterone concentrations was occasionally observed in columbian black-tailed deer, red deer and fallow deer [34–36]. In the former cases, roe deer and pudu, one testosterone peak was related to rutting season, while the second increase was associated to the velvet shedding period [30,32,37,38]. The results reported here, although inconclusive, are similar to these findings, since stags had peak fecal testosterone concentrations during summer–autumn, considered the major rutting season for Pampas deer in Brazil and during winter–spring, a period of antler mineralization and velvet shedding [7,10,28]. Regarding the biannual activation of the reproductive system, it has been speculated that this model of secretion is a relic of the original reproductive pattern of earlier cervids, which developed in the semitropical regions [32]. This hypothesis could explain why males of some cervid species living in tropical and subtropical areas, including Pampas deer, have well-defined and synchronous antler cycle, even though sexual activity may happen throughout the year.

Another important aspect associated with testosterone secretion in cervid species is the display of sexual behavior. Bubenik et al. [39] stated that in white-tailed deer, maximal testosterone concentrations were not necessary for the process of spermatogenesis, but were essential for manifestation of reproductive behavior. Conversely, sexual and aggressive behaviors in Eld’s deer bucks were highest when testosterone concentrations and testis size started declining [40]. In Pampas deer, maximal percentages of reproductive

behavior occurred concurrent with fecal testosterone peaks, supporting the concept that androgens are critical for these behaviors. Furthermore, there was a significant correlation between fecal testosterone and sexual behaviors in the present study. However, it remains unclear if the high frequency of gland marking behavior recorded in August–September is more related to the cleaning of antler velvet or to sexual activity. Interestingly, Li et al. [24] noted that in Père David's deer, some reproductive behaviors (e.g., anogenital and urine sniffing, chasing stags, herding hinds, antler adorning, mounting and copulating) were highly correlated with fecal testosterone, although there were no significant correlations between fecal testosterone concentrations and other recorded behaviors such as fighting, flehmen, preorbital gland marking and chasing hinds.

The annual mean group size in the present study (2.74 individuals/group) was consistent with the mean values obtained in Argentina and Uruguay, which oscillated from 1.7 to 3.1 animals/group throughout the year [6]. These findings were in contrast to the assertion that most cervid species living in open habitats establish larger groups than those living in closed habitats [1,41]. Netto et al. [28] suggested that the low gregariousness described in Pampas deer may be related to group instability and low population density, characteristics also observed in this study and the study by Jackson and Langguth [6]. Nevertheless, our results demonstrated no significant differences in fecal testosterone concentrations among males from groups of varying sizes, contradicting the belief that the increase of male grouping in free-ranging Pampas deer may be influenced by low concentrations of testosterone [28]. In this species, food distribution and availability appear to be more important elements associated with grouping patterns, since larger aggregations were found on common feeding grounds such as burnt patches, both in the present study and as previously reported [6].

The antler cycle reported here was consistent with previous studies of Pampas deer in Brazil [10,28] and corresponded to seasonal testosterone variations similar to that described for other tropical and temperate species [32,37,38,40]. Moreover, the antler cycle of males was synchronous, contesting the assertion that this species does not have a synchronized antler cycle [7]. Other tropical species, such as rusa deer, Reeves' muntjac, Eld's deer and pudu also exhibited highly synchronous antler growth among adult bucks [32,40,42,43]. Conversely, Loudon and Curlewis [44] reported poor synchronization of the antler cycle among male chital deer; they inferred that photoperiod may not be involved in controlling the antler cycle in this tropical species. However, it should be mentioned that these tropical species were mostly studied in temperate or boreal latitudes and therefore, the results might not represent their seasonality and synchrony in their original habitat.

In seasonally breeding deer from temperate regions, changes in antler and reproductive cycles are determined by fluctuations in the photoperiod [1,2]. In contrast, it is widely accepted that reproductive activity in tropical deer may be not photoperiodically dependent, but more influenced by local climatic factors such as annual rainfall patterns [40,45,46]. In Pampas deer, there was an apparent synchrony in antler cycle, reproductive behavior and testosterone secretion among free-ranging stags, suggesting a degree of seasonality in this tropical species. Nevertheless, it remains unclear whether the photoperiod is a major environmental factor modulating testicular and antler cycles in Pampas deer, since captive individuals maintained in temperate latitudes presented no fixed rutting season [9]. Furthermore, in Emas National Park, Ozotoceros is not exposed to

extensive variation in photoperiod (maximal difference of 1.76 h of daylight), but undergoes a great change in precipitation (mean range 17.5–238.3 mm; Fig. 1(a)). Monfort et al. [40] suggested that Eld's deer may be responsive to low-photoperiod oscillations and that they differ from tempertade cervids only by responding 6 months out-of-phase to the same photoperiodic cues. Thus, further data are necessary to better understand the effects of photoperiod and rainfall patterns on reproduction of Pampas deer.

Acknowledgements

We thank the Brazilian Institute of Forestry Development (IBAMA) for permission to conduct our study in Emas National Park. Thanks to FAPESP for financial support.

References

[1] Putman R. The natural history of deer. New York: Cornell University Press; 1988.
[2] Bronson FH. In: Bronson FH, editor. Mammalian reproductive biology. Chicago: The University of Chicago Press; 1989. p. 7–28.
[3] Lincoln GA. Biology of antlers. J Zool Lond 1992;226:517–28.
[4] Bubenik GA, Schams D, White RJ, Rowell J, Blake J, Bartos L. Seasonal levels of reproductive hormones and their relationship to the antler cycle of male and female reindeer (*Rangifer tarandus*). Comp Biochem Physiol 1997;116B:269–77.
[5] Jackson JE. *Ozotoceros bezoarticus*. Mamm Spec 1987;295:1–5.
[6] Jackson JE, Langguth A. In: Wemmer C, editor. Biology and management of the cervidae. Washington DC: Smithsonian Inst. Press; 1987. p. 402–9.
[7] Redford KH. In: Wemmer C, editor. Biology and management of the cervidae. Washington DC: Smithsonian Inst. Press; 1987. p. 410–4.
[8] Merino ML, Gonzales S, Leeuwenberg F, Rodrigues FHG. Pinder L, Tómas WM. Biologia e Conservação de Cervídeos Sul-Americanos: Blastocerus, Ozotoceros e Mazama. In: Duarte JMBB, editor. Funep, Jaboticabal. 1997; 42–58.
[9] Frädrich H. Beobachtungen am Pampashirsch. ceitalicBlastoceros bezoarticus/ceitalic L 1758 Zool Garten 1981;51:7–32.
[10] Tomás WM. Seasonality of antler cycle of Pampas deer (*Ozotoceros bezoarticus* leucogaster) from the pantanal wetland. Brazil Studies Neotrop Fauna Environ 1995;30:221–7.
[11] Jackson JE. Antler cycle in Pampas deer (*Ozotoceros bezoarticus*) from San Luis. Argent J Mamm 1986;67:175–6.
[12] Peter AT, Kasputin N, Crister JK. Analysis of sex steroid metabolites excreted in feces and urine of nondomesticated animals. The Compedium 1996;18:781–91.
[13] Schwarzenberger F, Möstl E, Palme R, Bamberg E. Faecal steroid analysis for non-invasive monitoring of reproductive status in farm, wild and zoo animals. Anim Reprod Sci 1996;42:515–26.
[14] Monfort SL, Martinet C, Wildt DE. Urinary steroid metabolite profiles in female Père David deer (*Elaphurus davidianus*). J Zoo Wildl Med 1991;22:78–85.
[15] Knox WM, Miller KV, Collins DC, Bush PB, Kiser TE, Marchinton RL. Serum and urinary levels of reproductive hormones associated with estrous cycle in white-tailed deer (*Odocoileus virginianus*). Zoo Biol 1992;11:121–31.
[16] Ramsay EC, Moran F, Roser JF, Lasley BL. Urinary steroid evaluations to monitor ovarian function in exotic ungulates: X. Pregnancy diagnosis in Perissodactyla. Zoo Biol 1994;13:129–47.
[17] Blanvillain C, Berthier JL, Bomsel-Demontoy MC, Sempere AJ, Olbricht G, Schwarzenberg F. Analysis of reproductive data and measurement of fecal progesterone metabolites to monitor the ovarian function in Pudu, Pudu puda (Artiodactyla, Cervidae). Mammalia 1997;61:589–602.

[18] Hosack DA, Miller KV, Marchinton RL, Monfort SL. Ovarian activity in captive Eld's deer (*Cervus eldi thamin*). J Mamm 1997;7:669–74.
[19] Garott RA, Monfort SL, White PJ, Mashburn KL, Cook JG. One-sample pregnancy diagnosis in elk using fecal steroid metabolites. J Wildl Dis 1998;34:126–31.
[20] Kapke CA, Arcese P, Ziegler TE, Scheffler GR. Estradiol and progesterone metabolite concentration in white-tailed deer (*Odocoileus virginianus*) feces. J Zoo Wildl Med 1999;30:361–71.
[21] Stoops MA, Anderson GB, Lasley BL, Shideler SE. Use of fecal steroid metabolites to estimate pregnancy rate of a free-ranging herd of tule elk. J Wildl Manag 1999;63:561–9.
[22] Monfort SL, Harvey E, Geurts L, Padilla L, Simmons HA, Williamson LR, et al. Urinary 3α, 17β-androstanediol glucuronide is a measure of androgenic status in Eld's deer stags (*Cervus eldi thamin*). Biol Reprod 1995;53:700–6.
[23] Hamasaki S, Yamauchi K, Ohki T, Murakami M, Takahara Y, Takeuchi Y, et al. Comparison of various reproductive status in sika deer (*Cervus nippon*) using fecal steroid analysis. J Vet Med Sci 2001;63: 195–8.
[24] Li C, Jiang Z, Jiang G, Fang J. Seasonal changes of reproductive behavior and fecal steroid concentrations in Père David's deer. Horm Behav 2001;40:518–25.
[25] Bestelmeyer SV, Westbrook C. Maned wolf (*Chrysocyon brachyurus*) predation on Pampas deer (*Ozotoceros bezoarticus*) in Central Brazil. Mammalia 1998;62:591–5.
[26] Bezerra AMR, Rodrigues FHG, Carmignotto AP. Predation of rodents by yellow armadillo (*Euphractus sexcintus*) in cerrado of the Central Brazil. Mammalia 2001;62:86–8.
[27] Nunes ALV. Gasparini RL, Duarte JMB. Pinder L, Buschinelli MC. Biologia e Conservação de Cervídeos Sul-Americanos: Blastocerus, Ozotoceros e Mazama. In: Duarte JMBB, editor. Funep, Jaboticabal. 1997; 142–70.
[28] Netto NT, Coutinho-Netto CRM, Costa MJRP. Bon R. Grouping patterns of Pampas deer (*Ozotoceros bezoarticus*) in the Emas National Park. Brazil Rev Etiol 2000;2(2):85–94.
[29] Palme R, Robia C, Messmann S, Hofer J, Möstl E. Measurement of faecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function. Wien Tierärz Monats 1999;86:237–41.
[30] Sempéré AJ, Boissin J. Relationship between antler development and plasma androgen concentration in adult roe deer (*Capreolus capreolus*). J Reprod Fertil 1981;62:49–53.
[31] Schams D, Barth D. Annual profiles of reproductive hormones in peripheral plasma of the male roe deer (*Capreolus capreolus*). J Reprod Fertil 1982;66:463–8.
[32] Bubenik GA, Reyes E, Schams D, Lobos A, Bartos L. Seasonal levels of LH, FSH, testosterone and prolactin in adult male pudu (Pudu puda). Comp Biochem Physiol 1996;115B:417–20.
[33] Goeritz F, Quest M, Wagener A, Fassbender M, Broich A, Hildebrandt TB, et al. Seasonal timing of sperm production in roe deer: interrelationship among changes in ejaculate parameters, morphology and function of testis and acessory glands. Theriogenology 2003;59:1487–502.
[34] West NO, Nordan HC. Hormonal regulation of reproduction and the antler cycle in male columbian black-tailed deer (*Odocoileus hemionus* columbianus) Part 1 Seasonal changes in the histology of the reproductive organs, serum testosterone, sperm production, and antler cycle. Can J Zool 1976;54: 1617–36.
[35] Suttie JM, Kay RN... Influence of plane witner nutrition on plasma concentration of prolactin and testosterone, and their association with voluntary food intake in red deer stags (*Cervus elaphus*). Anim Reprod Sci 1985;8:247–58.
[36] Rolf HJ, Fischer K. Serum testosterone and 5-α-dihydrotestosterone in male fallow deer (*Dama dama* L.): seasonality and age dependence. Comp Biochem Physiol 1990;95A:445–52.
[37] Sempéré AJ, Mauget R, Bubenik GA. Influence of photoperiod on the seasonal pattern of secretion of luteinizing hormone and testosterone and on the antler cycle in roe deer (*Capreolus capreolus*). J Reprod Fertil 1992;95:693–700.
[38] Reyes E, Bubenik GA, Schams D, Lobos A, Enriquez R. Seasonal changes of testicular parameters in southern pudu (Pudu puda) in relationship to circannual variation of its reproductive hormones. Acta Theriogenology 1997;42:25–35.
[39] Bubenik GA, Morris JM, Schams D, Claus A. Photoperiodicity and circannual levels of LH, FSH and testosterone in normal and castrated male, white-tailed deer. Can J Physiol Pharm 1982;60:788–93.

[40] Monfort SL, Brown JL, Bush M, Wood TC, Wemmer C, Vargas A, et al. Circannual inter-relationships among reproductive hormones, gross morphometry, behaviour, ejaculate characteristics and testicular histology in Eld's deer stags (*Cervus eldi thamin*). J Reprod Fertil 1993;98:471–80.
[41] Barrrete C. The size of axis deer fluid groups in Wilpattu National Park Sri Lanka. Mammalia 1991;55:207–20.
[42] Chapman DI, Chapman NG. The antler cycle of adult Reeves' muntjac. Acta Theriogenol 1982;27:107–14.
[43] van Mourik S, Stelmasiak T, Outch KH. Seasonal variation in plasma testosterone, luteinizing hormone concentrations and LH–RH responsiveness in mature male rusa deer (*Cervus rusa timorensis*). Comp Biochem Physiol 1986;83A:347–51.
[44] Loudon ASI. Curlewis JD. Cycles of antler and testicular growth in an aseasonal tropical deer (Axis axis). J Reprod Fertil 1988;83:729–38.
[45] Mishra HJ, Wemmer C. In: Wemmer C, editor. Biology and management of the cervidae. Washington DC: Smithsonian Inst. Press; 1987. p. 259–71.
[46] Bubenik GA, Brown RD, Schams D. Antler cycle and endocrine parameters in male axis deer (Axis axis): seasonal levels of LH, FSH, testosterone and prolactin and results of GnRH and ACTH challenges tests. Comp Biochem Physiol 1991;99A:645–50.