Braz. J. vet. Res.anim.Sci.,
São Paulo, v. 39, n. 2, p. 93-96, 2002.

Serial clinical, colpo-cytological and endocrinological evaluations of *Cerdocyon thous* bitches from the Rio de Janeiro zoo

Avaliação clínica, colpo-citológica e endocrinológica de fêmeas de *Cerdocyon thous* do zoológico do Rio de Janeiro

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SUMMARY

Serial clinical, colpo-cytological and endocrinological examinations of two five-year-old females of the crab-eating dog (*Cerdocyon thous*), from the RIOZOO Foundation in the State of Rio de Janeiro - Brazil, were carried out over a 10-month period. Clinically healthy animals were kept in sand substratum enclosures, located 500m apart from each other. They were each housed with two males. The colpo-cytological technique employed for *Cerdocyon thous* used methods similar to those developed for domestic bitches. Unlike domestic dogs, blood cells were absent in all phases of the estrus cycle, including the pro-estrus phase. Differentiation of each type of vaginal cells during the estrus cycle phases in this species follows the same patterns shown by domestic bitches. The estradiol and progesterone levels were similar to those occurring in domestic bitches. The progesterone levels reach their maximum (46 ng/ml) around the 10th day of pregnancy. The estradiol analysis demonstrated that, although levels of this hormone could be high at various times throughout the year, mating actually occurs in late winter and in spring. It was impossible to evaluate whether males and females kept in close proximity throughout the entire year would stimulate the production of estradiol, resulting in what would be considered a captivity artifice.

KEY-WORDS: Canidae. Cytology. Endocrinology. Reproduction.

INTRODUCTION

The crab-eating dog (*Cerdocyon thous*) is one of the most common wild carnivore species living in Brazil. Its generalist feeding habit allows the species to spread out from Central America to the southern part of South America, occupying several different ecological niches, such as forests, savannas and caatingas. In spite of its wild behavior, *Cerdocyon thous* can invade urban areas, searching for food when there is a decline of this element in its natural habitat [4, 10]. Despite of its relatively high population and the proximity of man, very few studies have been conducted about the basic physiology of this species. The knowledge about the reproductive characteristics of wild canines is based upon the domestic dog literature. Although certain studies have already addressed the reproductive behavior of those animals in their ecological niches or in captivity[1,2], only recently the hematological profiles for the species were determined[6]. Studies on the species can become a basic supporting groundwork for future scientific studies concerning the reproduction of endangered wild canines. The present study sought the establishment of clinical, colpo-cytological and endocrinological parameters, during the estrus cycle of the crab-eating dogs kept in captivity at Fundação RIOZÔO - Rio de Janeiro, Brazil. It is also an effort to improve the engagement of Brazilian veterinarians in the Zoo's growing program for preservation of wild animals in South America.

MATERIAL AND METHOD

Studies were conducted with two five-year-old crab-eating bitches, clinically examined and considered healthy (henceforward referred to by their registration numbers: 79 and 6003). They were housed in two sand substratum enclosures (20 m^2 each), located at extreme points in the Zoo, 500m apart from each other. Each female was kept in the company of two males. All animals were examined at the beginning of the experiment and fed once a day, according to the handling procedures of the Zoo, with a standardized diet (commercial dog food, eggs, fruits, vegetables, fish, and a mineral supplement) and water *ad libitum*. During a 10-month period, bitches were submitted, twice a week, to collecting of

material for colpo-cytology, respecting intervals of 3 to 4 days between collections. Females were hand-restrained using a net, and a pediatric speculum was introduced in their vagina. Vaginal swabs (cotton tips were humidified with sterile saline before the procedure) were scraped against the vaginal mucosa, and the material was immediately sent to the laboratory. Swabs were rolled against glass slides, which were fixed with methanol and stained[7] with Giemsa[a] . The hormonal determinations were done by collecting 2,0 ml of blood from the jugular, brachial or inguinal veins of each animal, at 15-day intervals. When the colpo-cytology pointed out to a morphological indication of the estrus phase, the interval between collections was reduced to 3 days. Collected blood was immediately taken to the laboratory for serum extraction, which was identified and stored at -20ºC, until analyzed by a radioimunoassay technique. Sexual steroid levels were determined with a commercial kitÒ for estradiol which works in the range between 20 and 3600 pg/ml, having a sensibility of 8 pg/ml and for progesterone which works in the range between 0,1 and 40 ng/ml, having a sensibility of 0,02 ng/ml.

RESULTS

Results from 119 colpo-cytological exams and 39 steroid assays from the *Cerdocyon thous* females identified as numbers 79 and 6003, can be seen in Fig. 1 and 2 and in Tab. 1 and 2.

A general summary of the vaginal cellular types observed in the females during the estrus cycle phase can be observed in Tab. 1. A summary of the pregnancy data for the two bitches is recorded in Tab. 2.

Table 1

General summary of mean percentage of vaginal cellular types observed in the studied females of *Cerdocyon thous* during the estrus cycle phases. Rio de Janeiro, 30/05/97.

Female number 79				
Cell type	**Anestrus**	**Pro-estrus**	**Estrus**	**Metaestrus**
Basal and Parabasal	90	15	00	50
Small Intermediary	05	25	05	25
Large Intermediary	05	30	10	20
Superficial	00	30	85	05
Female number 6003				
Cell type	**Anestrus**	**Pro-estrus**	**Estrus**	**Metaestrus**
Basal and Parabasal	80-90	20	20	50-60
Small Intermediary	10-15	30	10-15	15-20
Large Intermediary	05	30	20	15
Superficial	00	20	70	10

Table 2

Pregnancy data of two *Cerdocyon thous* females during a 10-month period. Rio de Janeiro, 30/05/97.

Female number	**Pregnancy period**	**Pregnancy (days)**	**Number of puppies+**
79	06/08/96 to 04/10/96	59	1/1/1
6003	17/08/96 to 11/10/96	55	0/0/3§
6003	20/11/96 to ??*	-	(??)*

+ males / females / undetermined;
§ Newborn parental predation;
* (??): There was no observation of parturition or abortion.

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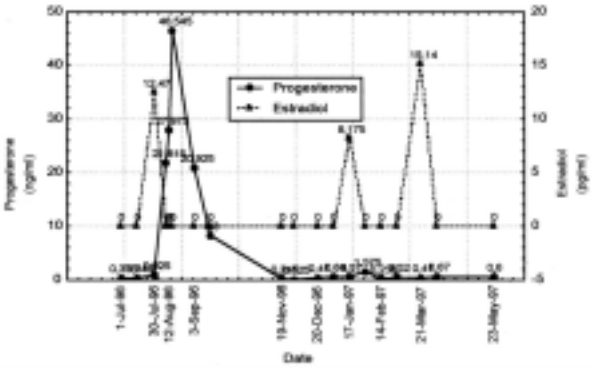

Figure 1
Progesterone (ng/ml) and Estradiol (pg/ml) serum levels of *Cerdocyon thous* bitch number 79 during a 10-month period. Estradiol y-axis had its zero scale shifted up for clearness. Rio de Janeiro, 30/05/97.

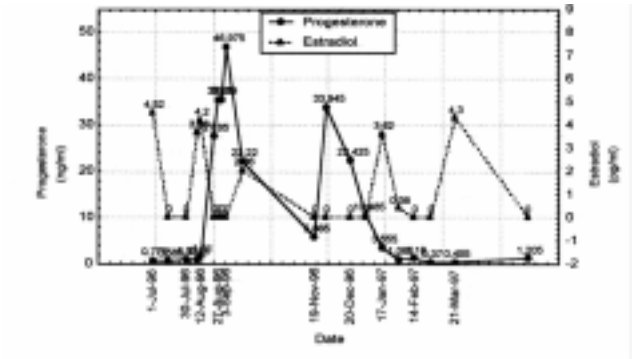

Figure 2
Progesterone (ng/ml) and Estradiol (pg/ml) serum levels of *Cerdocyon thous* bitch number 6003 during a 10-month period. Estradiol y-axis had its zero scale shifted up for clearness. Rio de Janeiro, 30/05/97.

DISCUSSION

Four different phases of the estrus cycle of two crab-eating dogs were determined during a period of 10 months. Comparative data were not found in the literature but a detailed colpo-cytological study of normal domestic bitches was used to compare and classify each different cellular type of the crab-eating dog females and thus to provide arguments for our discussion. The colpo-cytology technique was effective in determining the estrus cycle phases, but disease symptoms and other alterations were not considered if detected. The colpo-cytological evaluation was not different from that used for normal females of domestic dogs as described in the literature[3, 5, 9, 11].

Unlike what is observed for domestic female dogs, there was a total absence of red blood cells in all vaginal samples of the wild females in all phases of the estrus cycle, even in the pro-estrus[9, 11]. Despite the small size of our sample, this observation needs to be monitored for a larger number of animals in order to verify whether this is only a common factor for the two particular females or if it is indeed a trait in this species or even in all wild canines. If the pro-estrus vaginal bleeding is actually a phenomenon limited to domestic dogs, this could mean that this characteristic has developed after domestication, being advantageous to fit a more "protected" human environment.

In a wild species, the "smell of blood" exhaling from females could be undesirable, due to a potential attracting effect to predators; moreover, exhaling of just specific pheromones attracts the males of its own species but prevents stimulation of the other. When dogs develop association with humans, the females are: i) in a much more congested scent environment; ii) less exposed to predators; iii) apt to more opportunities of choosing a male, as male density is higher. These could be some factors that drove domestic females to bleed during pro-estrus, as a way of improving their capabilities to attract males. This would be evolutionarily advantageous, thanks to the possibility of a better genetic choice by the female.

The sexual steroid curve demonstrated a pattern similar to that seen for domestic female dogs[3, 8]. The estradiol analysis demonstrated that, although the wild females can exhibit estrogen peaks occurring during the entire year, and as high as during their reproductive season, mating actually occurs only in late winter and in spring. It was impossible to estimate in this study if males and females kept close together during the entire year could stimulate production of estradiol, thus configuring what could be considered a captivity artifice. This cannot happen in their natural habitat due to the fact that the couple frequently splits up after the end of the reproductive season. Nevertheless, the progesterone pattern during gestation was quite similar to that of domestic bitches[3], and the progesterone levels reached a maximum of 46ng/ml around the 10th day of pregnancy, decreasing as gestation progressed.

CONCLUSIONS

The results in this study allow the following conclusions to be drawn:

1. The colpo-cytology procedure demonstrated to be effective to determine the estrus cycle phases for *Cerdocyon thous* bitches.

2. The estradiol and progesterone levels in the *Cerdocyon thous* bitches examined revealed a pattern similar to that of the domestic female dog.

3. The annual variation of the sexual steroids estrogen and progesterone permitted to characterize *Cerdocyon thous* bitches as seasonal poliestric females, with the beginning of the reproductive season ocurring from mid to late winter through the spring.

ACKNOWLEDGMENTS

We wish to express thanks to DVM Elisabeth Criscuolo Urbinacci, from UNESP. Jaboticabal.SP who gently provided the equipment for the hormonal analysis. This work was supported by a grant from the Municipality of Rio de Janeiro. Secretaria Municipal do Meio Ambiente do Rio de Janeiro.

RESUMO

Foram realizadas avaliações clínicas, colpo-citológicas e endocrinológicas de duas fêmeas de cachorro-do-mato (*Cerdocyon thous*) pertencentes à Fundação RIOZOO, Rio de Janeiro, Brasil, durante um período de 10 meses. As fêmeas, consideradas clinicamente saudáveis, foram alojadas em recintos diferentes, cada uma com dois machos. Os recintos, de substrato arenoso distavam cerca de 500 m um do outro. A técnica colpo-citológica utilizada nas fêmeas de cachorro-do-mato segue os mesmos métodos para cachorros domésticos. É interessante ressaltar que não foi observado nenhum eritrócito nos esfregaços vaginais analisados, em qualquer fase do ciclo estral, em ambas as fêmeas. A diferenciação dos tipos celulares vaginais durante as diferentes fases do ciclo estral segue os mesmos padrões demonstrados por cadelas domésticas. Os níveis de estradiol e progesterona seguiram os mesmos padrões que ocorre nas fêmeas de cachorros domésticos. A progesterona atingiu níveis máximos (46 ng/ml) por volta do décimo dia de gestação. A variação do estradiol demonstrou que, ainda que as fêmeas possam apresentar picos deste hormônio, durante o ano, tão altos como durante a época reprodutiva, a reprodução ocorre, realmente, no final do inverno e primavera. É impossível inferir, neste trabalho, se a presença do macho próximo à fêmea durante o ano todo poderia induzir a produção de estradiol, redundando no que seria considerado um artifício de cativeiro.

PALAVRAS-CHAVE: Canidae. Citologia. Endocrinologia. Reprodução.

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Received: 01/06/2001
Accepted: 27/02/2002