



## Ultrasound monitoring of the uterus and ovaries of dominant and subordinate females of yellow-breasted capuchin (*Sapajus xanthosternos*) and robust tufted capuchin (*Sapajus robustus*) in captive colonies during the ovarian cycle and anestrus periods<sup>1</sup>

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**ABSTRACT.**-Pissinatti T.A., Ribas J.A.S., Maróstica E., Pissinatti A. & Ferreira A.M.R. 2019. **Ultrasound monitoring of the uterus and ovaries of dominant and subordinate females of yellow-breasted capuchin (*Sapajus xanthosternos*) in robust tufted capuchin (*Sapajus robustus*) in captive colonies during the ovarian cycle and anestrus periods.** *Pesquisa Veterinária Brasileira* 39(12):989-996. Departamento de Fisiologia e Farmacologia, Instituto Biomédico, Universidade Federal Fluminense, Rua Professor Hernani Melo 101, São Domingos, Niterói, RJ 24210-130, Brazil. E-mail: [ribasjas@vm.uff.br](mailto:ribasjas@vm.uff.br)

The yellow-breasted capuchin (*Sapajus xanthosternos*) and robust tufted capuchin (*Sapajus robustus*) are endangered species due to destruction of their natural habitat and predatory chase. However, it is still necessary to elucidate some details of their reproductive physiology in order to obtain better indices in the assisted reproduction of these species. This study aimed to evaluate the ovarian cycle of 13 dominant and subordinate females of *S. xanthosternos* (n=8) and *S. robustus* (n=5) using sagittal and transversally scanned ultrasound of their uterus and ovaries. Sonograms were performed every seven days for two months. The ovarian cycle phase and anestrus condition were confirmed by colpocytology. Our results showed different uterine parameters (craniocaudal diameter, dorso-ventral diameter, and transverse diameter) ( $P<0.05$ ) between anestrus subordinate females and other ovarian cycle phases and social classes. The mean of uterine volume was higher in dominant females than subordinate females in all cycle phases ( $P<0.05$ ), except in follicular phase. During anestrus, endometrial width was smaller in subordinate females than in dominant females ( $P<0.05$ ). Subordinate females showed differences in endometrial measures ( $P<0.05$ ) between anestrus period and follicular and luteal periods. Ovarian measures in dominant females were higher than in subordinate females only during anestrus ( $P<0.05$ ). In the subordinate females, ovarian parameters were different ( $P<0.05$ ) between anestrus and follicular and luteal phases. Dominant females showed higher volume of right ovary compared to volume of the left ovary during anestrus and follicular phase ( $P<0.05$ ). Follicles and corpus luteum were distinguished by ultrasonography in most exams (86.11%). During anestrus, measurable ovarian structures were not observed in both ovaries in dominant and subordinate females. In conclusion, the methodology used in this study allowed to evaluate the ovarian cycle in *S. xanthosternos* e *S. robustus* females and that cycle phase/anestrus and social class of the female influenced the size of the uterus and ovaries.

**INDEX TERMS:** Ultrasound, monitoring, uterus, ovaries, dominant female, subordinate female, yellow-breasted capuchin, *Sapajus xanthosternos*, robust tufted capuchin, *Sapajus robustus*, captive colonies, ovarian cycle, anestrus period, neotropical primates, reproductive system, female, reproduction, wildlife animals.

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**RESUMO.- [Acompanhamento ultrassonográfico do útero e ovários de fêmeas dominantes e subordinadas de macacos-prego-do-peito-amarelo (*Sapajus xanthosternos*) e macacos-prego-de-crista (*Sapajus robustus*) em colônias cativas, durante o ciclo ovariano e períodos de anestro.]**

O macaco-prego-do-peito-amarelo (*Sapajus xanthosternos*) e o macaco-prego-de-crista (*Sapajus robustus*) encontram-se em risco de extinção devido a destruição do seu habitat e a caça predatória. Porém, ainda necessita-se elucidar alguns detalhes de sua fisiologia reprodutiva, para obterem-se melhores índices por meio de reprodução assistida. Sendo assim, o objetivo deste trabalho foi acompanhar o ciclo ovariano de 13 fêmeas dominantes e subordinadas de *S. xanthosternos* (n=8) e *S. robustus* (n=5) por meio de cortes ultrassonográficos sagitais e transversais do útero e dos ovários. Estas fêmeas foram examinadas uma vez por semana durante dois meses. A fase do ciclo ovariano/anestro foi confirmada pela colpocitologia. Os resultados da pesquisa demonstrou diferença significativa ( $P<0,05$ ) relacionada ao diâmetro crânio-caudal, diâmetro dorso-ventral e diâmetro transversal entre as fêmeas subordinadas em anestro com todas as outras fases do ciclo ovariano e classes sociais. A média do volume uterino foi maior nas fêmeas dominantes que nas subordinadas em todas as fases do ciclo ( $P<0,05$ ) a exceção da fase folicular. A largura endometrial, durante o período de anestro, foi menor nas fêmeas subordinadas quando comparada às dominantes ( $P<0,05$ ). Nas fêmeas subordinadas, houve diferenças ( $P<0,05$ ) nas mensurações endometriais entre o período de anestro e das fases folicular e lútea. Quanto aos ovários, as médias das medidas observadas nas fêmeas dominantes foram superiores as das subordinadas durante o anestro ( $P<0,05$ ). As médias das medidas das variáveis ovarianas das fêmeas subordinadas apresentaram diferenças entre o anestro e as fases folicular e lútea ( $P<0,05$ ). Nas fêmeas dominantes o volume do ovário direito foi maior que o do ovário esquerdo durante o anestro e na fase folicular ( $P<0,05$ ). Foliculos e corpos lúteos foram diferenciados pela ultrassonografia na maioria das coletas (86,11%). Durante o anestro não foram observadas estruturas ovarianas mensuráveis nos ovários em ambas as classes sociais. Concluiu-se que a metodologia empregada neste estudo permitiu o acompanhamento do ciclo ovariano das fêmeas *S. xanthosternos* e *S. robustus* e que as fases do ciclo/anestro e a classe social das fêmeas influenciaram as medidas do útero e ovários.

**TERMOS DE INDEXAÇÃO:** Ultrassonografia, útero, ovários, fêmeas dominantes, fêmeas subordinadas, macacos-prego-do-peito-amarelo, *Sapajus xanthosternos*, macacos-prego-de-crista, *Sapajus robustus*, colônias cativas, ciclo ovariano, anestro, primatas neotropicais, sistema reprodutivo, ultrassom, reprodução, animais selvagens.

## INTRODUCTION

Brazil has the most abundant diversity of apes in the world (Mittermeier et al. 1994). A thorough understanding of these primates, both *in situ* and *ex situ*, is of vital importance to conservationist projects aimed at multiplying endangered specimens and preserving them of its genetic variety (Domingues et al. 2007). These include several species of capuchin monkeys (*Cebus* spp.) and hooded capuchin monkeys (*Sapajus* spp.) (Alfaro et al. 2012), such as the *Sapajus xanthosternos* and *Sapajus robustus*, which have their habitats very restricted due to deforestation caused by the need for

expansion of cities or farms, leading them almost to extinction (Aurichio 1995, Domingues et al. 2004). The genus *Sapajus* is characterized by living in groups of six to eight individuals usually formed by a dominant couple (alpha) and some of their infant females, which normally do not reproduce, are characterized as subordinate (Linn et al. 1995). The ovarian cycle of the genus *Sapajus* can vary from 16 to 24 days. Ovulation occurs from day 8 to 12 of the cycle, and in case of fertilization, the gestation lasts between 150 and 162 days, usually producing a baby (Nagle & Denari 1983, Nagle et al. 1994). The interval between births is 576 to 613 days, which leads to low annual population growth (Domingues & Caldas-Bussiere 2002, Domingues et al. 2005).

The correct understanding of the reproductive physiology of these primates is of vital importance for new conservation proposals (Monteiro et al. 2009), having a geometric growth from the moment when non-invasive diagnostic aid methods were started as instruments for research on this conservation purpose (Ortiz et al. 2005, Domingues et al. 2007, Guimarães 2007). Ultrasonography stands out because it allows a good evaluation of the reproductive organs of both females and males, and the monitoring of reproductive cycles, pregnancies, and detection of pathologies that would lead to reduced fertility or serious health damage (Yeager & Concannon 1996, Seier et al. 2000, Monteiro et al. 2006, Domingues et al. 2007).

Ultrasound follow-up of the uterus and ovaries should be performed by longitudinal and transverse scans, assessing the echogenicity, dimensions, contours, position, and shapes (Sauerbrei et al. 2000, Alves et al. 2007). For the uterus, echogenicity should be homogeneous, i.e., the parenchyma should not have texture variations (Ortiz et al. 2005). Uterine dimensions should be measured in longitudinal and sagittal planes. There is no considerable variation in contour, position, and shape of the uterus in animals of the same species. In the ovaries, echogenicity, size, and contour vary according to the phase of the cycle and the ovarian structures present at the time of examination. The position may change according to the fullness of the bladder and bowel loops, so the uterus is used as a reference for ovarian identification (Monteiro et al. 2009).

Given the importance of a better understanding of the reproductive physiology of endangered primates, this research aimed to ultrasonically evaluate the uterus and ovaries of dominant and subordinate females of captive colonies of *S. xanthosternos* and *S. robustus* in different phases of the world ovarian cycle and periods of anestrus.

## MATERIALS AND METHODS

This study was approved by "Comitê de Ética de Pesquisas em Animais (Animal Research Ethics Committee)" (CEPA) of "Universidade Federal Fluminense" under protocol number 051207.

Initially, all females used in the study were weighed and underwent a clinical examination. Soon after, those who were healthy had their sexual organs examined by trans-abdominal ultrasound, excluding pregnant females. Thirteen non-pregnant *Sapajus* females (eight *Sapajus xanthosternal*, five *Sapajus robustus*) of reproductive age (from two to seven years old), kept in captivity at the "Centro de Primatologia do Rio de Janeiro" (CPRJ), geographically located between the parallels 22°27' and 22°32' of latitude S and the meridians 42°50' and 42°56' of longitude WGr, in an almost central position of the state territory of Rio de Janeiro. Its perimeter comprises an estimated area of 259.54 hectares in the municipality of Guapimirim, Rio de Janeiro, Brazil, with the climate of the AW by Köppen-Geiger Climate

classification region. All females were required to be in direct contact with the alpha male in their groups. Their distributions regarding occupation and social classes were: Nursery 1 - a dominant female and three subordinates; Nursery 2 - two subordinate females (the dominant one did not enter the research because she was pregnant); Nurseries 3, 4 and 5 - one dominant female in each; Nursery 6 - one dominant and three subordinate females. When we divided the animals by social class, there were five dominant females and eight subordinate females.

**Capture and immobilization methods.** In an attempt to reduce the stress of the animals and prevent accidents and escapes, they were sedated in the enclosures and taken to the Veterinary Hospital of the CPRJ, where all necessary research procedures were performed. Sedation was performed with 5.0% ketamine hydrochloride at a dosage of 11.00mg/kg associated with diazepam at a dosage of 0.25mg/kg and atropine sulfate 0.04mg/kg.

**Gynecological ultrasound examinations and vaginal cytology collection.** Ovarian cycles were followed once a week for two months (which would correspond to three or four ovarian cycles) by ultrasound and vaginal cytology, and data were recorded on individual records. During the study period, females remained in the nurseries with their respective family groups.

Transabdominal ultrasound examination was performed using Carbogel® and a Toshiba Just Vision 200 B-mode device with a 5.0 to 8.0MHz adjustable linear transducer. Soon after chemical containment, the animals were positioned on the examination table in dorsal decubitus, and the uterus was examined as suggested by Monteiro et al. (2009), taking into account the contour, shape, echogenicity, and dimensions. In order to evaluate the dimensions, sagittal and transverse ultrasound sections were obtained. Through the sagittal section was obtained the length or craniocaudal diameter of the uterus (CCD), which represents the distance from the bottom of the uterus to the cervix and dorsal-ventral diameter (DVD) that indicates the depth of the uterus and is obtained perpendicular to the CCD. The transverse section obtained the transverse diameter (TD), which represents the width of the uterus (Monteiro et al. 2009), as well as the endometrial width (EW) (Ortiz et al. 2005). Uterus volume (UV) was calculated by the formula  $(\pi/6 [DCC \times DDV \times DCC])$  (Domingues et al. 2007).

Using the uterus as a reference point, rotate it or transducer to the transverse plane to locate and identify the right and left ovaries. These were subject to taxation as to contours, shape, echogenicity, dimensions, and presence or absence of ovarian structures (follicles

and corpus luteum). The sagittal section obtained the length and height variables, and the width was obtained by the transverse plane - these were always measured at the maximum ovarian parenchyma diameter. When detected, ovarian structures were measured using their largest internal diameters (Domingues et al. 2007).

Ovarian volume (OV) was calculated using the formula  $(\pi/6 [length \times width \times height])$ , as described by Domingues et al. (2007). A single observer measured all dimensions.

For diagnostic complement of the ovarian cycle of the monkeys, colpocytology was performed, and the slides stained by the Panotic® technique following the manufacturer's recommendations. Samples were analyzed, as indicated by Gompel & Koss (1997), based on the morphological characteristics of vaginal epithelial cells. After identification of the cells, the phase of the cycle in which the female was found was determined using the classification of Nagle & Denari (1983).

**Statistical analysis.** The results of ultrasound measurements of the uterus, endometrium, and ovaries were recorded on individual files, and after all, collections were recorded in charts. The basic parameters of descriptive statistics (mean, variance, and standard deviation) were calculated for all variables analyzed. After a preliminary analysis of data variance, no differences in uterine and ovarian measurements were observed between *S. xanthosternos* and *S. robustus* species. It was then decided to work with a single group, formed by the two species. The results were submitted to ANOVA (analysis of variance) to compare the characteristics submitted. The Tukey's test compared individual averages. The results were analyzed considering the anestrus, cycle phases, and social class, as well as the interaction between these variables. The paired Student's t-test was used to compare the means of measurements between right and left ovary at each phase of the ovarian and anestrus cycle, within the same social classes. P values <0.05 were considered statistically significant. GraphPad Prisma 5.0 software (GraphPad Software., California, USA) was used to perform statistical analysis.

## RESULTS

A transabdominal ultrasound examination was efficient in evaluating and measuring the uterus and ovaries throughout the study (Fig.1). However, the measurement of the endometrium could not be performed in 70.0% of the evaluations of female anestrus, due to its small size. The means ( $\pm$  standard

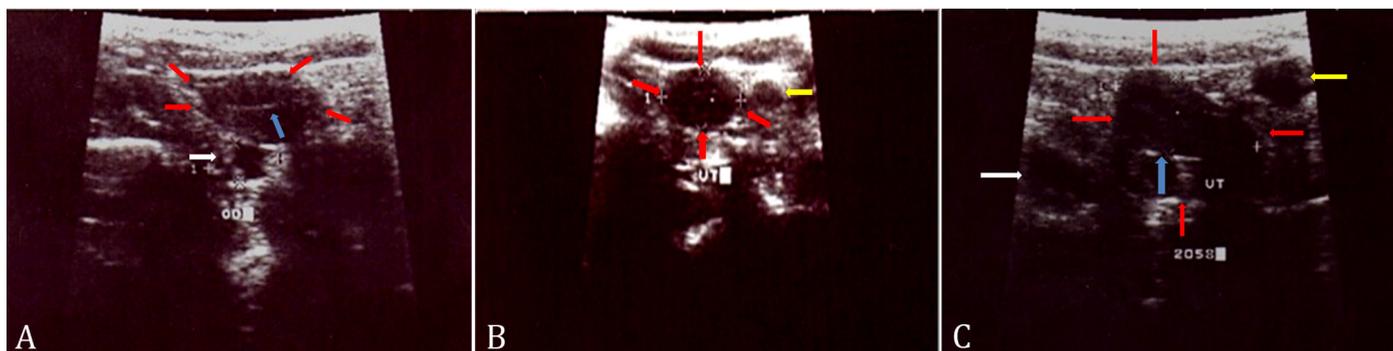


Fig.1. Representative ultrasound images of the uterus, endometrium, and ovary (s) of female *Sapajus xanthosternos* and *Sapajus robustus*. (A) Sagittal section of uterus (between red arrows), endometrium (blue arrow), right ovary (white arrow) of a cyclic subordinate female. (B) Cross-section of the uterus (between the red arrows) and left ovary (yellow arrow) of a subordinate subordinate female in the anestrus. Note the absence of endometrium and ovarian structures. (C) Sagittal section of uterus (between red arrows), endometrium (blue arrow), right ovary (white arrow), and left ovary (yellow arrow) of a cyclic dominant female.

deviations of the mean) of the uterine and ovarian variables at each phase of the cycle and in the anestrus are shown in Table 1 and 2, respectively.

We observed significant differences ( $P < 0.05$ ) related to all uterine variables (craniocaudal diameter-CCD), dorsal-ventral diameter-DVD, transverse diameter-TD, and uterine volume-UV) between the anestrus subordinate females with all other phases of the ovarian cycle and social classes. However, there was no variation in CCD, DVD, and TD between the phases of the ovarian cycle (follicular, luteal and menstrual), nor between social classes (dominant and subordinate) ( $P > 0.05$ ) (Table 1). The mean uterine volume was higher in dominant females than in the subordinate females in all phases of the cycle ( $P < 0.05$ ), except for the follicular phase (Table 1).

The smallest and largest uterine measurements in their respective diameters (cm) and volume ( $\text{cm}^3$ ) were: CCD=0.49 and 2.75, DVD=0.31 and 2.01, TD=0.23 and 1.81 and UV=0.11 and 1.12. The smallest measurements were observed in the anestrus of the subordinate females and the largest in the follicular phase of the dominant females. In dominant females, the smallest diameters (cm) and volume ( $\text{cm}^3$ ) were: CCD=0.77, DVD=0.49, TD=0.61, and UV=0.31, they are observed in the anestrus. In the subordinate females, the largest diameters (cm) and volume ( $\text{cm}^3$ ) were measured in

the follicular phase, being respectively CCD=2.18, DVD=1.98, TD=1.17, and UV=1.25.

Endometrial width during the anestrus period was smaller in subordinate females when compared to dominant females ( $P < 0.05$ ) (Table 1). Subordinate females revealed differences in endometrial measurements between the anestrus period and the follicular and luteal phases ( $P < 0.05$ ). Regarding the width of the endometrium, the maximum measured was 1.35cm in a dominant female in the early luteal phase and the smallest of 0.20cm in an anestrus subordinate female. It is recalled that, as already mentioned, the width of the endometrium could not be measured on some occasions, especially in the subordinate females, due to the small width. In dominant females, the smallest measurement was 0.38cm in the anestrus. In the subordinate females, the largest measured was 1.03cm in the follicular phase.

Regarding the ovaries, the average measurements observed in the dominant females were higher than those of the subordinate only during the anestrus ( $P < 0.05$ ). The average measurements of the ovarian variables of the subordinate females showed differences between the anestrus and the follicular and luteal phases in both ovaries ( $P < 0.05$ ) (Table 2). Dominant females had higher volume of the right ovary compared to the left ovary during the anestrus and in the follicular phase ( $P < 0.05$ ) (Table 2).

**Table 1. Mean  $\pm$  standard deviation of uterine variables of dominant ( $\alpha$ ) and subordinate ( $\beta$ ) non-pregnant *Sapajus xanthosternos* and *Sapajus robustus* females, obtained by transabdominal ultrasound examination during the ovarian and anestrus phases**

	Anestrus		Menstrual phase		Follicular phase		Luteal phase	
	$\alpha$	$\beta$	$\alpha$	$\beta$	$\alpha$	$\beta$	$\alpha$	$\beta$
CCD (cm)	1.42 $\pm$ 0.07	0.91 $\pm$ 0.18*	1.66 $\pm$ 0.39	1.50 $\pm$ 0.38	1.74 $\pm$ 0.31	1.70 $\pm$ 0.52	1.77 $\pm$ 0.35	1.61 $\pm$ 0.35
DVD (cm)	0.86 $\pm$ 0.28	0.56 $\pm$ 0.19*	1.02 $\pm$ 0.24	0.74 $\pm$ 0.27	1.10 $\pm$ 0.17	1.01 $\pm$ 0.33	1.06 $\pm$ 0.26	0.92 $\pm$ 0.28
TD (cm)	0.95 $\pm$ 0.33	0.54 $\pm$ 0.25*	1.05 $\pm$ 0.23	0.81 $\pm$ 0.31	1.05 $\pm$ 0.16	1.04 $\pm$ 0.27	1.04 $\pm$ 0.25	0.88 $\pm$ 0.23
UV ( $\text{cm}^3$ )	0.63 $\pm$ 0.35 <sup>a</sup>	0.27 $\pm$ 0.11 <sup>b*</sup>	0.94 $\pm$ 0.22 <sup>a</sup>	0.49 $\pm$ 0.28 <sup>b</sup>	1.06 $\pm$ 0.43 <sup>a</sup>	0.97 $\pm$ 0.55 <sup>a</sup>	1.02 $\pm$ 0.56 <sup>a</sup>	0.68 $\pm$ 0.55 <sup>b</sup>
EW (cm)	0.70 $\pm$ 0.25	0.41 $\pm$ 0.05 <sup>A**</sup>	0.73 $\pm$ 0.18	0.56 $\pm$ 0.20 <sup>AB</sup>	1.00 $\pm$ 0.49	0.70 $\pm$ 0.21 <sup>B</sup>	0.76 $\pm$ 0.23	0.71 $\pm$ 0.14 <sup>B</sup>

CCD = craniocaudal diameter; DVD = dorsal-ventral diameter; TD = transverse diameter; UV = uterus volume; EW = endometrial width; \* values differ ( $P < 0.05$ , ANOVA) from other values in the same row, \*\* values differ ( $P < 0.05$ , ANOVA) from the value of dominant females in the anestrus; values with different letters indicate significant differences ( $P < 0.05$ ) by the variance test (ANOVA): <sup>a,b</sup> lower case letters on the same line indicate differences between social classes at each phase of the ovarian cycle and in the anestrus, <sup>A,B</sup> capital letters indicate differences between the subordinate females at different stages of the cycle and in the anestrus.

**Table 2. Mean  $\pm$  standard deviation of ovarian variables of non-pregnant, dominant ( $\alpha$ ) and subordinate ( $\beta$ ) non-pregnant *Sapajus xanthosternos* and *Sapajus robustus* females, obtained by transabdominal ultrasound examination during the ovarian cycle and anestrus phases**

	Anestrus		Menstrual phase		Follicular phase		Luteal phase	
	$\alpha$	$\beta$	$\alpha$	$\beta$	$\alpha$	$\beta$	$\alpha$	$\beta$
ROL (cm)	0.76 $\pm$ 0.33 <sup>a</sup>	0.55 $\pm$ 0.14 <sup>bA</sup>	0.77 $\pm$ 0.16	0.64 $\pm$ 0.31 <sup>AB</sup>	0.91 $\pm$ 0.22	0.77 $\pm$ 0.20 <sup>B</sup>	0.87 $\pm$ 0.22	0.68 $\pm$ 0.21 <sup>B</sup>
ROH (cm)	0.67 $\pm$ 0.11 <sup>a</sup>	0.47 $\pm$ 0.07 <sup>bA</sup>	0.71 $\pm$ 0.05	0.50 $\pm$ 0.16 <sup>AB</sup>	0.83 $\pm$ 0.11	0.67 $\pm$ 0.14 <sup>B</sup>	0.76 $\pm$ 0.09	0.63 $\pm$ 0.14 <sup>B</sup>
ROW (cm)	0.57 $\pm$ 0.16 <sup>a</sup>	0.39 $\pm$ 0.11 <sup>bA</sup>	0.52 $\pm$ 0.13	0.47 $\pm$ 0.26 <sup>AB</sup>	0.69 $\pm$ 0.19	0.57 $\pm$ 0.16 <sup>B</sup>	0.63 $\pm$ 0.19	0.55 $\pm$ 0.17 <sup>B</sup>
ROV ( $\text{cm}^3$ )	0.18 $\pm$ 0.02 <sup>a*</sup>	0.06 $\pm$ 0.01 <sup>bA</sup>	0.17 $\pm$ 0.03	0.09 $\pm$ 0.02 <sup>AB</sup>	0.25 $\pm$ 0.12*	0.17 $\pm$ 0.07 <sup>B</sup>	0.22 $\pm$ 0.09	0.14 $\pm$ 0.02 <sup>B</sup>
LOL (cm)	0.72 $\pm$ 0.20 <sup>a</sup>	0.54 $\pm$ 0.18 <sup>bA</sup>	0.81 $\pm$ 0.17	0.67 $\pm$ 0.20 <sup>AB</sup>	0.88 $\pm$ 0.26	0.84 $\pm$ 0.37 <sup>B</sup>	0.85 $\pm$ 0.26	0.76 $\pm$ 0.21 <sup>B</sup>
LOH (cm)	0.60 $\pm$ 0.11 <sup>a</sup>	0.46 $\pm$ 0.09 <sup>bA</sup>	0.69 $\pm$ 0.12	0.53 $\pm$ 0.17 <sup>AB</sup>	0.72 $\pm$ 0.18	0.69 $\pm$ 0.13 <sup>B</sup>	0.75 $\pm$ 0.15	0.61 $\pm$ 0.20 <sup>B</sup>
LOW (cm)	0.52 $\pm$ 0.17 <sup>a</sup>	0.37 $\pm$ 0.11 <sup>bA</sup>	0.41 $\pm$ 0.03	0.48 $\pm$ 0.14 <sup>AB</sup>	0.59 $\pm$ 0.15	0.49 $\pm$ 0.15 <sup>AB</sup>	0.60 $\pm$ 0.19	0.55 $\pm$ 0.22 <sup>B</sup>
LOV ( $\text{cm}^3$ )	0.13 $\pm$ 0.02 <sup>a***</sup>	0.05 $\pm$ 0.03 <sup>bA</sup>	0.15 $\pm$ 0.03	0.10 $\pm$ 0.03 <sup>AB</sup>	0.20 $\pm$ 0.11 <sup>**</sup>	0.15 $\pm$ 0.05 <sup>B</sup>	0.19 $\pm$ 0.08	0.13 $\pm$ 0.03 <sup>B</sup>

ROL = right ovary length, ROH = right ovary height, ROW = right ovary width, ROV = right ovary volume, LOL = left ovary length, LOH = left ovary height, LOW = left ovary width, LOV = left ovary volume; \*\*\* Indicate  $P < 0.05$  by the paired Student's t-test in the comparison between the right and left ovary, in the same variable, in the same column; values with different letters indicate significant differences ( $P < 0.05$ ) by the variance test (ANOVA): <sup>a,b</sup> lower case letters indicate different values between social classes during anestrus, <sup>A,B</sup> capital letters indicate differences in values of the same social class at each phase of the ovarian and anestrus cycle.

The ovaries presented minimum measurements of length, height, and width (cm) of 0.43, 0.22, and 0.17 observed in the anestrus of subordinate females and a maximum of 1.23, 0.98, and 0.87 observed in the follicular phase of dominant females. The minimum measurements (cm) measured in the ovaries of the dominant females for length, height, and width (cm) were 0.51, 0.39, and 0.28, respectively, also observed in the anestrus while the maximum measurements (cm) obtained in the ovaries of subordinate females were observed follicular phase, being 1.12, 0.91, and 0.82 in length, height, and width (cm), respectively. The smallest and largest ovarian volumes were observed respectively in the left ovary of a subordinate subordinate female in the anestrus (0.03cm<sup>3</sup>) and the right ovary of a dominant female in the follicular phase (0.32cm<sup>3</sup>). Regarding this parameter, in the case of dominant females, the lowest observed was 0.10cm<sup>3</sup> in the left ovary in the anestrus, and in the subordinate females, the largest was 0.26cm<sup>3</sup> in the follicular phase.

Measurable ovarian structures were present in 69.23% (n=72/104) of the examinations and ranged from 0.10 to 0.62cm in diameter, with structures larger than 0.40cm (dominant follicles and corpus luteum) being present in 49.01% (n=51/104) of the comments. When we separated by classes, in dominant females, ovarian structures were observed in 87.50% (35/40) of the ultrasound exams, and 55.00% (22/40) had diameters greater than 0.40cm. In the subordinate females, ovarian structures were observed in 57.81% (37/64) of the examinations, and those with a diameter greater than 0.40cm represented 48.57% (29/64) of the observations.

As for the side in which the structures were present, we observed 40.27% (29/72) of them in the right ovary, 43.06% (31/72) in the left, and 16.67% (12/72) in both ovaries. Structures more significant than 0.40cm showed a distribution of 49.02% (25/51) in the right ovary and 50.98% (26/51) in the left ovary. When we separated classes, we observed that dominant females had structures larger than 0.40 cm in diameter in the right ovary in 59.09% (13/22) of the examinations and the left ovary in 40.91% (9/22). Already in the class of subordinate females, when also considered structures of this same diameter, they were observed in the right ovary in 41.38% (12/29) of the examinations while in the left ovary represented 58.62% (17/29) of the observations.

Follicles and corpus luteum were differentiated by ultrasound in most collections (86.11%). Cystic corpus luteum (probably early-stage luteal bodies) were not differentiated from follicles in 90.0% of cases, as evidenced by vaginal cytology. During anestrus, no measurable ovarian structures were observed in both ovaries, either in dominant females or in the subordinate females. However, it should be noted that in the dominant females, during the anestrus, it was observed that the ovarian echotexture was more heterogeneous when compared to the ovarian echotexture of the subordinate females. This difference was due to the many anechoic points present in both ovaries that structurally resembled tiny immeasurable follicles (<0.08cm).

## DISCUSSION

Although this study involved two species of *Sapajus* (*Sapajus xanthosternos* and *Sapajus robustus*), the data were analyzed together as a single group, since measurements of the uterus, endometrium, ovaries and ovarian structures did not differ

( $p>0.05$ ). The animals are phylogenetically close and have a geographical distribution with intercession between their endemic territories, and there may be natural hybridization of individuals, as already observed by Martins et al. (2017). They are also similar in size and weight, and this is relevant since the individual's weight directly interferes with uterine and ovarian measurements and volume (Coutinho et al. 2011). The most important - that could bring variations to the study - would be different breeding conditions, management, and environment, a fact that did not occur, since the groups were kept in the same place, under the same conditions.

In these primate species, as well as in *Sapajus apella* (Ortiz et al. 2005, Domingues et al. 2004, Domingues et al. 2005, Alves et al. 2007), in *Macaca mulatta* (Bishop et al. 2009) and in *Aotus azarae infulatus* (Monteiro et al. 2009) the ultrasound examination was efficient in the evaluation and measurement of the uterus and ovaries during the ovarian cycle phases. The ultrasound examination was also efficient in the evaluation and measurement of the uterus and ovaries during anestrus periods that interspersed with the recorded ovarian cycles in some females during the research.

The uterine and ovarian measurements of the subordinate females of *S. xanthosternos* and *S. robustus* of the present study are similar to those of *S. apella* described by Ortiz et al. (2005). However, dominant females, showed higher measurements than those of Ortiz et al. (2005) and were closer to those of Domingues et al. (2007) also in *S. apella*. This fact may be related to the category of females used in both studies, i.e. Ortiz et al. (2005) probably also used nulliparous and/or subordinate females, while Domingues et al. (2007), monkeys that had already calved and/or dominant that were always cyclical. Both authors kept females separated in individual cages during their research, i.e., there was no establishment of dominance among individuals as occurs in a flock, which is an important point in articles involving uterine measurements in primates. These measures can also be very useful when we are unfamiliar with the previous reproductive life of captive or free-living females, providing insight into whether the female has already given birth or not, and providing insight into her social class.

It is noteworthy the lack of citations in the scientific literature on uterine and ovarian ultrasound measurements of the species in this study, being necessary to compare with other species of the genus *Sapajus* to discuss the results obtained. It should also be taken into account that different ultrasound equipment, methodologies used by the researchers and the equipment operator, may provide small differences between the measures, requiring careful comparison of the results of various researches (Mercadante et al. 2010).

The uterine diameters and volume of the subordinate females in the anestrus were lower ( $P<0.05$ ) than the other values observed in this research. This is explained by cyclic-interspersed anestrus periods more frequently recorded in the subordinate females compared to dominant females (unpublished data), which highlighted the differences between uterine measurements in these periods, as hormonal influence is directly related the variation of uterine volume (Mauad Filho et al. 2001). Dominant females, which generally presented more constant cycles and rare periods of anestrus intercalated between them, revealed greater and more constant hormonal stimulus, maintaining uterine measurements in

the anestrus and in the next phases of the cycle, not differing between each other ( $P > 0.05$ ).

Regarding uterine volume, we observed differences ( $P < 0.05$ ) between dominant and subordinate females in the anestrus and all phases of the cycle except for the follicular phase. This is due to the multiple pregnancies through which the dominant females have passed through their reproductive life, making their womb larger, while the subordinates were nulliparous. The number of births is the most critical factor for increased uterine measurements in sexually mature females (Coutinho et al. 2011). In the follicular phase, due to the edema caused by the estrogen hormone (Ortiz et al. 2005), which increased uterine measurements in both dominant and subordinate females, this difference in volume was "masked", which led to similar measurements between social classes ( $P < 0.05$ ).

There were no significant changes in uterine measurements between dominant females during different phases of the cycle, as observed in other neotropical primates (Ortiz et al. 2005, Domingues et al. 2007, Monteiro et al. 2009). Uterine measurements of subordinate females also did not vary during the phases of the cycle, a fact not commented on in other studies.

The smallest uterine measurements observed during the research occurred in the anestrus, due to the total absence of hormonal stimuli that characterizes this period. The largest uterine measurements occurred during the follicular phase, due to the action of the estrogen produced by the ovarian follicles on the uterus, as previously explained. However, when evaluating the smallest uterine measurements measured during the phases that make up the ovarian cycle of females *Sapajus* spp. (i.e., menstrual, follicular and luteal phases), these were observed during the menstrual phase (Table 1). During this phase, endometrial flaking occurs, and edema resolves, leading to shrinkage of the uterus as a whole (Dixon 2012).

The mean endometrial measurement during the anestrus period was higher in the dominant females when compared to the subordinate females ( $P < 0.05$ ) (Table 1). This is probably due to the longer periods without hormonal stimulation that the latter experienced, as the gonadal hormones are the same responsible for stimulating the proliferative and secretory phases and cause significant morphological changes in the endometrium (Dixon 2012). Subordinate females showed differences in endometrial measurements between the anestrus period and the follicular and luteal phases ( $P < 0.05$ ). As in anestrus measurements were extremely low, remembering that in some females the endometrium could not be measured during anestrus, the difference between this period and the measurements of cyclic females became more evident. In dominant females, even with smaller anestrus measurements, no measurable differences were observed between the cyclic and acyclic periods. In these females it was also possible to measure it even in the anestrus, i.e., the endometrium did not suffer significant metric and visual changes. Such a fact can be explained by the most constant hormonal stimulus observed in this social class during the research (unpublished data).

There were no significant differences in endometrial measurements between ovarian cycle phases in both alpha and subordinate females, as mentioned in other studies (Ortiz et al. 2005). The largest endometrial measurement

was observed in a female at the beginning of the luteal phase, which still had edema caused by the estrogen hormone from the follicular phase and already had a small influence of progesterone, which stimulates stromal water retention and endometrial gland secretion (Dixon 2012). The lowest endometrium measurement was observed in the anestrus due to the absence of hormonal stimulation.

Regarding the ovaries, the average ovarian measurements of dominant females were higher than those of subordinates during anestrus, probably due to a larger population of preantral follicles observed in this social class, as already observed in *Leontopithecus* spp. (Brasil 2008). Besides, the more extended periods of anestrus observed in the subordinate females, when compared to the dominant ones occurred during the experiment (unpublished data), i.e., the most prolonged periods in which their ovaries did not suffer hormonal stimulation. Ovarian volume is known to decrease significantly over long periods without hormonal action (Wallace & Kelsey 2004, Nichols et al. 2005). Mean ovarian measurements did not vary between short anestrus periods and cycle phases in alpha females. However, in the subordinate females, differences ( $P < 0.05$ ) were observed in these measurements between the acyclic periods (anestrus) and all other phases of the ovarian cycle. Such difference between the dominant and subordinate females is explained: even in the periods when they were in anestrus, confirmed by vaginal cytology, homogeneous echotexture of their ovaries and absence of ultrasound measurable structures, a greater amount was observed of tiny anechoic points when compared to the ovaries of the subordinate females, which contributed positively to the ovarian measurements. Perhaps these small structures are several early/early antral/antral follicles. According to a report by Brasil et al. (2007) in *Leontopithecus* spp., dominant females have a lower rate of follicular atresia than subordinate females.

Right ovarian volume was greater than left ovarian volume in dominant females during anestrus and follicular phase ( $P < 0.05$ ), probably because there is a larger follicular population in this ovary as observed in *S. apella* (Domingues et al. 2004) and/or preantral follicles as reported in *Leontopithecus* spp. (Brasil et al. 2006). Another fact to be considered is that this difference may be related to a larger increase of interstitial glandular tissue in the right ovary when compared to the contralateral as observed in *A. azarae infulatus* (Monteiro et al. 2009). As for the difference between the volume of the right and left ovaries observed in the dominant females in the follicular phase, it may be associated with a larger number of dominant follicles and consequently of ovulation occurring in the right ovary during the research period, as observed by Domingues et al. (2007) in *S. apella*. However, it is known that the development of dominant follicles/ovulation in *S. apella* does not alternate between right and left ovary in consecutive cycles, ovulation in the same ovary being observed for up to five consecutive cycles (Ortiz et al. 2005). It can, therefore, be assumed that, during the study period, there was a greater formation of dominant follicles and ovulation in the right ovary of the dominant females, which caused the volume difference between them during the follicular phase ( $P < 0.05$ ). Subordinate females showed greater alternation regarding the side of follicular dominance and ovulation,

which provided ovaries with similar volumes also during the follicular phase ( $P > 0.05$ ).

It was not possible to differentiate follicles from cystic corpus luteum, which were probably observed at the beginning of luteogenesis, using 2D ultrasound, without daily monitoring of the development of the structures. Although vaginal cytology specimens clearly determined the luteal phase, the sonographic image of the ovarian structure was not elucidative for the definition of the structure as the corpus luteum, mainly due to the presence of fluid inside it. Differentiation between these structures might be possible with the use of 4D Power Doppler, as performed by Bishop et al. (2009).

This differentiation would also be possible if one follows the cycles daily as performed by some authors (Domingues et al. 2005, Ortiz et al. 2005), although even so, initial corpus luteum is usually difficult to identify structures (Morgan et al. 1987). However, the daily manipulation of the monkeys in the present study could increase their stress level, causing hormonal changes and consequently in the ovarian cycles, besides interfering even more in the behavior of the colony, modifying these results.

## CONCLUSIONS

The methodology used in this study allowed following the ovarian cycle of dominant and subordinate infants of *Sapajus* spp. efficiently, quickly and noninvasively.

The social class influenced the uterus and ovarian measures during the ovarian cycle and not anestrus.

However, many aspects of the sonographic anatomy, reproductive cycle, and behavior of several New World primate species still need further study to clarify issues that improve the results of conservation programs for these species.

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