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Population estimate and morphometry of ovarian preantral follicles from three recently recognized squirrel monkey species: a comparative study

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Summary

We describe morphological and morphometrical characteristics of preantral ovarian follicles from three recently recognized *Saimiri* species: *S. macrodon*, *S. cassiquiarensis* and *S. vanzolinii*; the last one a threatened species. Ovaries from four adult monkeys were evaluated: one pair from a pregnant *S. macrodon*, two ovarian pairs from *S. cassiquiarensis* females (one of them pregnant), and one left ovary from a senile *S. vanzolinii*, applying classical histology. Follicular preantral population was quantified and morphology and morphometry of primordial, primary and secondary follicles were evaluated. Follicular preantral population varied among species, being 347,153 in the ovaries of the *S. macrodon*, 270,342 and 278,376 in the ovaries of both adult non-pregnant and pregnant *S. cassiquiarensis* females, and 28,149 in the ovary from a senile *S. vanzolinii.* Most follicles were at primordial or transition stages, except for the senile *S. vanzolinii* female, which presented the lowest percentages of primordial and transition follicles when compared with primary and secondary ones. Most preantral follicles (>70%) were morphologically normal in the ovaries from all studied *S. macrodon* and *S. cassiquiarensis* females, but the ovary of the senile *S. vanzolinii* female presented a significant decrease in the percentage of normal follicles (primordial: 61%, transition: 52%, primary: 54%, and secondary: 48%). In general, follicular diameter increased significantly from primordial to transition, and subsequently from primary to secondary follicles.

Keywords: Morphology, Morphometry, Neotropical, Ovarian follicles, Primate, *Saimiri sp.*

Introduction

Squirrel monkeys (*Saimiri* spp.) are neotropical primates with a lifespan of approximately 30 years (Helvacioglu *et al.*, 1994). Female reproductive life starts at the age 2.5 to 3.5 years (Baldwin, 1969; Taube, 1980; Boinski, 1992), and at approximately 8 years old a massive depletion in the number of reserve gametes is initiated, resulting in the complete follicular loss when the females are about 20 years old (Walker *et al.*, 2009). Monkeys from this genus present a seasonal reproduction and an extremely short ovarian cycle, lasting for 7 to 9 days (Lang 1967, Wolf *et al.*, 1977; Schiml *et al.*, 1999; Trevino, 2007), that is different from other neotropical primates that present longer ovarian cycles, such as 18–23 days for *Sapajus apella* (Nagle & Denari, 1983), 20–24 days for *Ateles geoffroyi* (Campbell *et al.* 2001), 21 days for *Brachyteles hypoxanthus* (Strier & Ziegler 1994, 1997), 24–30 days for *Callithrix jacchus* (Kendrick & Dixson 1983; Dixson, 2001), 15 days for *Aotus trivirgatus* (Dixson, 1983), and 22 days for *Saguinus oedipus* (Brand, 1981). Also,

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progesterone peak levels are usually higher in *Saimiri* species (399 ng ml^{-1}) (Wolf *et al.*, 1977) than in other neotropical primates such as *Sapajus apella* (60–100 ng ml^{-1}) (Nagle & Denari, 1983), suggesting species-specific differences. As observed, information on ovarian cycle and late folliculogenesis is abundant for this genus. However, knowledge related to the early folliculogenesis focusing on preantral follicles of *Saimiri* sp. or other neotropical species is still needed.

Preantral follicles are the reserve of female gametes, and will decrease with age until females become senile and enter menopause. These follicles represent 90% of the total ovarian follicle population (Gougeon & Chainy, 1987). Besides nutritional and health status, the total population of preantral follicles in the ovary is species specific and age dependent. For instance, the number of follicles per ovary from adult women ranges of 76,000 (19 years old) to 27,000 (46 years old) (Gougeon & Chainy, 1987), each ovary from an adult female of *Sapajus apella* contains about 51,000 preantral follicles (Domingues *et al.*, 2004), while in *Macaca nemestrina* preantral follicular population was found in the range of 30,900 (8 months old) to 9940 (12–13 years old) (Miller *et al.*, 1999), and in *Macaca mulatta* preantral follicular population was found to be 910,000 follicles at birth (Baker & Wai, 1976). Almeida *et al.* (2012) estimated the population of primordial, primary, and secondary follicles in a senile *Saimiri sciureus* as 915, 230 and 115 follicles, respectively. Besides the number of follicles, morphometric characteristics of preantral follicles can vary with species (Gougeon, 1996), which can be used to study female reproduction in different animal species. Furthermore, such information can be used to determine characteristics of follicular atresia, infertility, and menopause (Almeida *et al.*, 2012). Knowledge on ovarian morphology and follicular population size may support the development of protocols for the conservation *in situ* and *ex situ* of endangered primate species (Mayor *et al.*, 2013). Moreover, knowledge contributes to the understanding of the processes related to formation, growth and maturation of oocytes enclosed in preantral follicles (Scalercio *et al.*, 2014).

Information on population estimates, with morphological and morphometric characterization of preantral follicles are limited for neotropical primate species, such as *Sapajus apella* (Domingues *et al.*, 2004), *Saimiri sciureus* (Walker *et al.*, 2009; Almeida *et al.*, 2012), and *Alouatta caraya* (Lopes *et al.*, 2006), and absent for the three *Saimiri* species recently recognized: *Saimiri macrodon*, *Saimiri cassiquiarensis* and *Saimiri vanzolinii.* The last one is a threatened species (IUCN, 2008). Therefore, in the present study, we aimed to estimate the population of preantral ovarian follicles, as well as to characterize the morphology and morphometry of these three *Saimiri* species: *Saimiri macrodon*, *Saimiri cassiquiarensis* and *Saimiri vanzolinii.*

Materials and methods

Animals

All animals were captured and collected during field expeditions at the Mamirauá Reserve, Amazonas, Brazil (Lopes *et al.*, 2017). Animals were euthanized as part of a large research program to investigate different biological aspects of these primate species. All experimental procedures of this study were approved by the Research Ethics Committee and the Mamirauá Institute for Sustainable Development Ethics Committee, under protocol number 002/2012. The license for collection was granted by the Brazilian Institute of Environment and Renewable Natural Resources, through the System of Authorization and Information on Biodiversity (SISBIO 29906-1). All the biological material used in this investigation are deposited at the Mammals Section of the scientific collection of Mamirauá Institute for Sustainable Development.

The ovarian samples were separately maintained in paraffin sections for histological analysis (Lopes *et al.*, 2017). Ovarian sections from four adult squirrel monkeys were evaluated: one pair from an adult pregnant *S. macrodon*, two ovarian pairs from adult *S. cassiquiarensis* females (one of them pregnant), and one left ovary from a senile *S. vanzolinii.* The approximate age of the females was estimated based on the phenotype of dental chronology (Smith, 1989), and they were classified as adults (ages ranging from 1.5 to 5 years old) or senile (older than 5 years). No pathologies or abnormal findings regarding the ovarian donors were reported.

Histological analysis

The ovaries were fixed in 10% formalin and subsequently prepared for classical histology. Serial sections (5 µm thick) were cut and every 10th section was mounted and stained with hematoxylin and eosin and examined under a converted light microscope (Leica, Wetzlar, Germany).

Preantral follicles were classified according to Domingues *et al.* (2004) in which the oocyte is surrounded by a layer of flattened granulosa cells (primordial follicle), by one layer of flattened and cuboidal granulosa cells (primordial), by one layer of cuboidal granulosa cells (primary), or by two or more layers of cuboidal granulosa cells (secondary). Preantral follicles were characterized as morphologically normal or atretic as described by Domingues *et al.*

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(2004) and Brito *et al.* (2013), in which the normal follicles presented intact basal membrane, absence of pycnotic bodies in the oocyte nucleus, no signs of oocyte and/or granulosa cells degeneration, as well as no shrunken oocyte, nor detachment between oocyte and granulosa cells.

The total number of each type of follicle was estimated using a correction factor as described by Gougeon & Chainy (1987):

$$Ni = No * St * Ts/So * dn$$

where Ni is the total corrected number of follicles of one class; No is the number of follicles observed in the analyzed sections; St is the total number of sections in the ovary; Ts is the thickness of the section (5 μm); So is the number of observed sections; and dn is the mean diameter of the nucleus of the oocyte for each follicle class.

Follicular, oocyte and oocyte nucleus diameters were measured using a digital camera (Moticam® 5, 5.0) coupled to a computer and a morphometric analysis program (Motic Images Plus 2.0ML, Australia). For this, the follicular dimensions were measured with a micrometric eye lens in an optical microscope (×400 magnification). The largest and the smallest diameters of the oocyte nucleus, oocyte and follicle were measured. The mean diameter of each structure was calculated. The thickness of the granulosa layer was estimated in all preantral follicles categories, subtracting the follicular diameter from the oocyte diameter (Domingues *et al.*, 2004). Follicles were only considered when the nucleus of the oocyte was visible. At least 400 follicles per animal species were measured.

Statistical analysis

Statistical analysis was performed applying the programs BioEstat 5.3 and StatView 5.0 (SAS Institute Inc., Cary, NC, EUA). Comparisons between the number of follicles per left and right ovary within each species were performed with the Wilcoson–Mann–Whitney test. Morphometric data were compared among different follicular class within a same species using ANOVA and Tukey as a post-hoc test. Results are expressed as mean ± standard error of the means (SEM) and differences were considered significant when the *P*-value was < 0.05.

Results

The ovaries were characterized by two regions: cortex and medulla. Most blood vessels were found in the medulla, but the cortical area also presented vascular irrigation, as expected (Fig. 1*A*). The ovarian cortex was characterized by the presence of ovarian follicles at different developmental stages (Fig. 1*B*, *D*), including those multi-oocyte follicles (MOFs), which is follicle containing two or three oocytes (Fig. 1*C*). This type of follicle was present in all evaluated ovaries (0.4% in *S. macrodon*, 1.2% in *S. cassiquiarensis* and 0.2% in *S. vanzolinii* ovaries), and at all developmental stages of preantral follicles. All ovaries presented a *corpus luteum* (Fig. 1*E*).

The major population of preantral follicles (mean number of 347,153) was encountered in the ovaries of the *S. macrodon*, which presented a greater number of follicles in the left ovary (426,607) than in the right one (267,699); thus, a difference >150,000 preantral follicles (Table 1). Both adult non-pregnant and pregnant *S. cassiquiarensis* females presented similar preantral follicular population, i.e. a mean number of 240,906–299,778 and 237,187–319,565 preantral follicles per ovary respectively. From the senile *S. vanzolinii*, only the left ovary was recovered and evaluated, presenting the lowest follicular population (28,149); see Table 1.

Table 2 depicts the total number of evaluated follicles and the proportions of follicles per class, as well as the number of morphologically normal preantral follicles within each follicular class, with their respective percentages. The ovaries of *S. macrodon* presented similar percentages of total primordial (32%) and secondary (30%) follicles, being higher than the percentages of transition (20%) and primary (18%) follicles. *S. cassiquiarensis* females presented ovaries containing mostly primordial (38–39%) and transition (25–27%) follicles, when compared with the percentages of primary (18–19%) and (16–18%) secondary follicles. The ovary from the senile *S. vanzolinii* female presented the lowest percentages of primordial (15%) and transition (24%) follicles when compared with primary (31%) and secondary (30%) ones. Most preantral follicles (>70%) were morphologically normal in the ovaries from all studied *S. macrodon* and *S. cassiquiarensis* females. As expected, the ovary of the senile *S. vanzolinii* female presented a significant decrease in the percentage of morphologically normal preantral follicles, especially those classified as transition (52%), primary (54%), and secondary (48%) follicles, when compared with the primordial (61%) ones.

Tables 3, 4 and 5 present the measured mean diameter (μm) of follicles, oocytes, oocyte nucleus, as well as thickness of granulosa cells layer per follicular class when evaluating ovaries from non-pregnant and pregnant *S. cassiquiarensis*, as non-pregnant *S. macrodon* and senile *S. vanzolinii*, respectively. In ovaries from non-pregnant and pregnant *S. cassiquiarensis* the only remarkable difference observed was the larger

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Table 1 Mean (±SE) number of ovarian preantral follicles present in the ovaries from *S. macrodon* (pregnant), *S. cassiquiarensis* (pregnant and non-pregnant), and *S. vanzolinii* (senile)

	Ovary		
Species	Right (±SE)	Left (±SE)	Mean (±SE)
S. macrodon (pregnant)	267,699 ± 10,410[a]	426,607 ± 17,789[b]	347,153 ± 79,454
S. cassiquiarensis (non-pregnant)	299,778 ± 13,172[a]	240,906 ± 14,854[a]	270,342 ± 29,436
S. cassiquiarensis (pregnant)	237,187 ± 18,182[a]	319,565 ± 16,820[b]	278,376 ± 41,189
S. vanzolinii (senile)	–	28,149 ± 303	–

[a,b]Different letters indicate significant differences between right and left ovaries within the same *Saimiri* species and category; $P < 0.05$

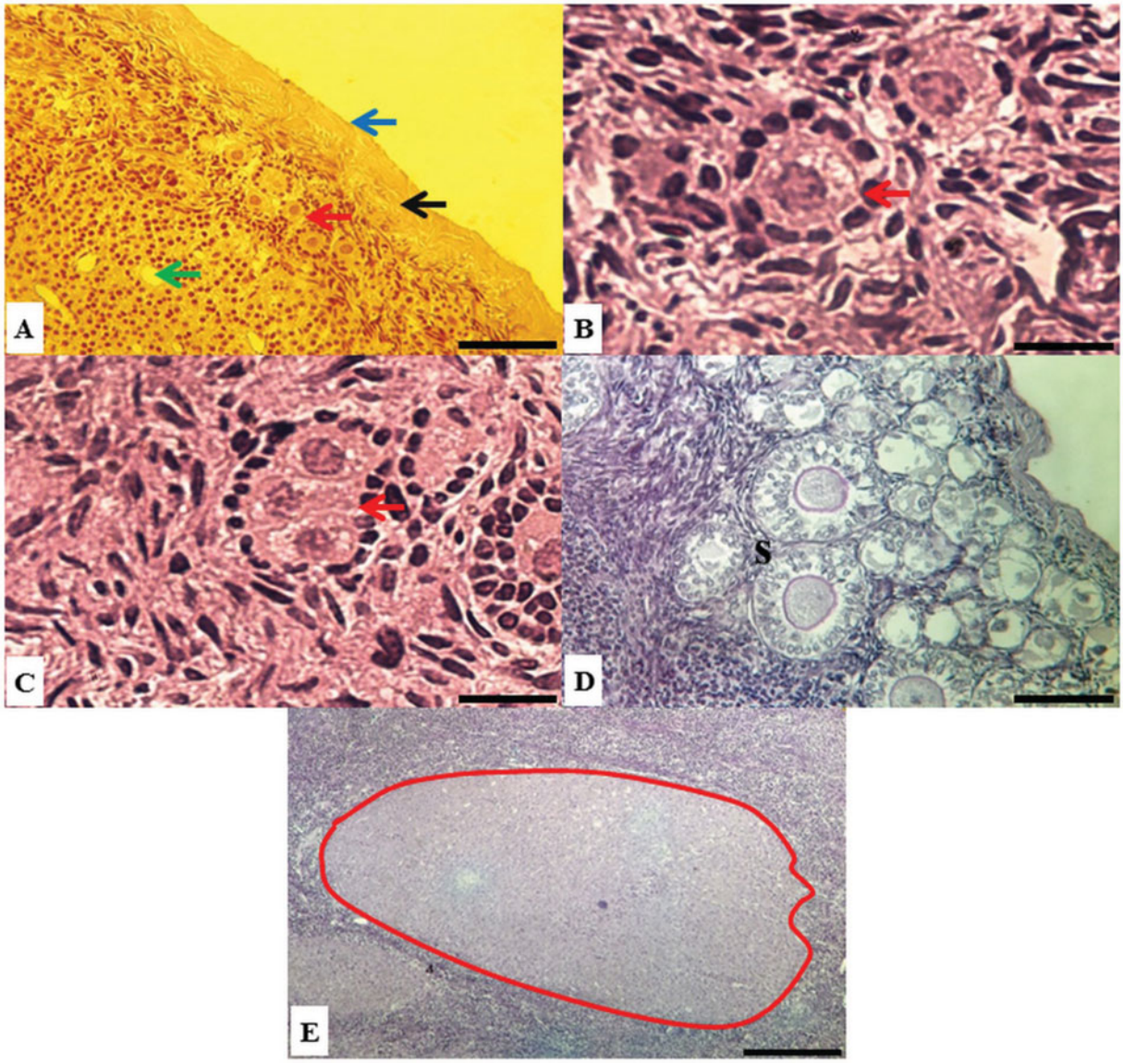


Figure 1 Photomicrography of *Saimiri* ovaries. (*A*) Histological section of ovarian tissue showing epithelium (blue arrow), tunica albuginea (black arrow), cortex (red arrow), blood vesser (green arrow); ×40 magnification. (*B*) A transit in follicles is pointed out with a red arrow; ×100 magnification. (*C*) Multi-oocyte follicle (MOF) enclosing three oocytes (red arrow); ×40 magnification. (*D*) Preantral follicles at different developmental stages, including secondary follicles (marked with an 's'); 100 magnification. (*E*) A delimited corpus luteum; ×40 magnification. Scale bar: 100 μm.

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Table 2 Number and mean (±SE) percentage of total and morphologically normal ovarian preantral follicles per class and animal species/category, i.e. in the ovaries from *S. macrodon* (pregnant), *S. cassiquiarensis* (pregnant and non-pregnant), and *S. vanzolinii* (senile)

	Preantral follicular classes							
	Primordial		Transition		Primary		Secondary	
Species	Total n (% ± SE)	Normal (% ± SE)	Total n (% ± SE)	Normal (% ± SE)	Total n (% ± SE)	Normal (% ± SE)	Total n (% ± SE)	Normal (% ± SE)
S. macrodon (pregnant)	111,471 (32 ± 4^{a})	81,373 (73 ± 13^{A})	69,118 (20 ± 3)^{b}	52,530 (76 ± 13^{A})	61,158 (18 ± 3^{b})	49,538 (81 ± 13^{A})	105,406 (30 ± 4^{a})	82,217 (78 ± 12^{A})
S. cassiquiarensis (non-pregnant)	105,239 (39 ± 5^{a})	88,401 (84 ± 14^{A})	71,822 (27 ± 4)^{b}	53,867 (75 ± 12^{A})	49,989 (18 ± 3^{c})	35,492 (71 ± 13^{A})	43,292 (16 ± 2^{c})	33,335 (77 ± 12^{A})
S. cassiquiarensis (pregnant)	105,782 (38 ± 5^{a})	93,088 (88 ± 14^{A})	69,582 (25 ± 3)^{b}	50,099 (72 ± 12^{A})	53,986 (19 ± 3^{c})	39,950 (74 ± 13^{A})	49,027 (18 ± 3^{c})	37,751 (77 ± 12^{A})
S. vanzolinii (senile)	4255 (15 ± 3^{a})	2596 (61 ± 11 ^{A})	6874 (24 ± 3)^{b}	3574 (52 ± 11^{B})	8617 (31 ± 4^{c})	4653 (54 ± 12^{B})	8403 (30 ± 4^{c})	4033 (48 ± 11^{B})

$^{a–d}$Different lower-case letters indicate significant differences among the percentages of total follicles per follicular class within the same *Saimiri* species and category; $P < 0.05$.
$^{A–D}$Different upper-case letters indicate significant differences among the percentages of morphologically normal follicles per follicular class within the same *Saimiri* species and category; $P < 0.05$.

follicular and oocyte diameter of secondary follicles from pregnant compared with non-pregnant females (Table 3). However, because only one specimen per group was evaluated, no statistical conclusion can be drawn. Morphometric evaluation resulted in similar results for both females, where follicular diameter increased significantly from primordial to transition, and subsequently from primary and to secondary follicles. Oocyte diameter started to increase significantly only in primary and secondary follicles, and oocyte nucleus increased significantly in diameter only when follicles were at the secondary stage. Thickness of the granulosa cells layer increased significantly in each follicular class after activation (Table 3). In ovaries from *S. macrodon* (Table 4) and *S. vanzolinii* (Table 5), follicular and oocyte diameter increased significantly from primordial to transition, and subsequently to primary and to secondary follicles. The same was observed when the thickness of the granulosa cells' layer was compared within *S. vanzolinii* follicles (Table 5). In *S. macrodon* follicles, diameter of oocyte nucleus, as well as the layer formed by granulosa cells increased in thickness from the transition stage to further primary and secondary ones (Table 4). In *S. vanzolinii* follicles, diameter of oocyte nucleus increased significantly only from the primary to the secondary stage (Table 5).

Discussion

Information regarding the reproductive aspects of threatened species is crucial to increase effectiveness of conservation programmes (Andrabi & Maxwell, 2007; Mayor *et al.*, 2013). Obviously, the paucity of biologic material for evaluation is more accentuated for endangered species (Pukazhenthi & Wildt, 2004). Therefore, analyses must be performed with a limited number of animals. This study is the first to describe morphological and morphometric aspects of initial folliculogenesis in the threatened species *S. vanzolinii*, as well as of *S. macrodon* e *S. cassiquiarensis*, all of them living in the same geographic location.

Follicular population varied between left and right ovaries in each species studied here, as well as among species. The remarkably different follicular population from left and right ovaries found in the *S. macrodon* female and in the pregnant *S. cassiquiarensis* female was also previously described in ovaries of *Sapajus apella* (Domingues *et al.*, 2004). These later authors studied the ovaries of four adult *Sapajus apella* females, and recorded more follicles present in the right ovary than in the left one (Domingues *et al.*, 2004). They suggested that in *S. apella* the right ovary has more follicles because it is less functional than the left one,

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Table 3 Mean (± SEM) follicular, oocyte, oocyte nucleus diameter and granulosa cells layer thickness (μm) in primordial, transition, primary and secondary follicles from *S. cassiquiarensis* (non-pregnant and pregnant)

Follicular class	Follicle mean diameter (μm) (range)	Oocyte mean diameter (μm) (range)	Oocyte nucleus mean diameter (μm) (range)	Granulosa cell layer thickness mean diameter (μm) (range)
Non-pregnant				
Primordial	19.7 ± 0.1^{a} (12.7–25.0)	14.4 ± 0.1^{a} (9.8 - 18)	7.8 ± 1.1^{a} (5.2–10.3)	5.1 ± 0.3^{a} (1.8–7.2)
Transition	23.1 ± 0.2^{b} (17.0–30.1)	16,07 ± 0.2^{ab} (10.3–21.7)	8.1 ± 0.1^{a} (2,47–10.8)	7.0 ± 0.2^{b} (3.3–10.6)
Primary	27.5 ± 0.3^{b} (20.3–37.0)	17.9 ± 0.3^{b} (12.1–30.9)	8.4 ± 0.1^{a} (5.9–10.9)	9.3 ± 0.3^{c} (2.5–17)
Secondary	156.7 ± 11.0^{c} (23.7–581.6)	59.5 ± 4.6^{c} (14.2–320.3)	24.2 ± 1.7^{b} (6.9–87.8)	83.1 ± 11.0^{d} (7.1–487.8)
Pregnant				
Primordial	19.0 ± 0.1^{a} (12.7–22.5)	13.7 ± 0.1^{a} (9.4–16.5)	7.7 ± 0.1^{a} (5.6–8.9)	4.3 ± 0.3^{a} (1.8–7.2)
Transition	23.6 ± 0.1^{b} (19.9–28.6)	15.6 ± 0.1^{ab} (11.9–19.0)	7.6 ± 0.1^{a} (6.2–10.1)	6.7 ± 0.4^{b} (1.5–8.0)
Primary	25.6 ± 0.3^{b} (19.5–36.0)	17.7 ± 0.3^{b} (12.9–30.3)	8.0 ± 0.1^{a} (6.2–9.1)	8.9 ± 0.4^{c} (2.5–15.0)
Secondary	239.8 ± 13.4^{c} (26.7–583.8)	90.1 ± 6.0^{c} (14.4–318.6)	35.0 ± 2.3^{b} (7.8–134.1)	96.6 ± 18.0^{d} (8.2–462.2)

$^{a–d}$Different letters indicate significant differences among follicular classes within the same evaluated parameter, i.e. follicular, oocyte, oocyte nucleus, and granulosa cell diameter, and specimen, i.e. non-pregnant or pregnant female; $P < 0.05$.

Table 4 Mean (± SEM) follicular, oocyte, oocyte nucleus diameter and granulosa cells layer thickness (μm) in primordial, transition, primary and secondary follicles from *S. macrodon* (pregnant)

Follicular class	Follicle mean diameter (μm) (range)	Oocyte mean diameter (μm) (range)	Oocyte nucleus mean diameter (μm) (range)	Granulosa cell layer thickness mean diameter (μm) (range)
Primordial	19.8 ± 0.1^{a} (15.8–21.9)	14.3 ± 0.1^{a}(10.7–16.9)	7.9 ± 0.1^{a}(5.3–9.1)	7.4 ± 0.1^{a}(4.9–10.7)
Transition	23.3 ± 0.2^{b}(19.6–27.6)	15.9 ± 0.2^{a}(12.4–19.2)	7.9 ± 0.1^{a}(5.2–10.5)	7.4 ± 0.1^{a}(5.0–11.3)
Primary	26.6 ± 0.3^{c}(21.1–33.2)	17.2 ± 0.3^{b}(12.9–25.4)	8.2 ± 0.1^{b}(4.9–10.2)	9.4 ± 0.2^{b}(5.7–13.1)
Secondary	230.7 ± 9.4^{d}(76.3–525.5)	106.8 ± 4.5^{c}(46.6–249.7)	34.2 ± 1.6^{c}(11.5–87.8)	123.9 ± 5.7^{c}(28.5–275.7)

$^{a–d}$Different letters indicate significant differences among follicular classes within the same evaluated parameter, i.e. follicular, oocyte, oocyte nucleus, and granulosa cell diameter; $P < 0.05$.

Table 5 Mean (± SEM) follicular, oocyte, oocyte nucleus diameter and granulosa cells layer thickness (μm) in primordial, transition, primary and secondary follicles from *S. vanzolinii*

Follicular class	Follicle mean diameter (μm) (range)	Oocyte mean diameter (μm) (range)	Oocyte nucleus mean diameter (μm) (range)	Granulosa cell layer thickness mean diameter (μm) (range)
Primordial	19.8 ± 0.3^{a} (12.7–21.9)	14.9 ± 0.2^{a} (10.9–16.9)	8.1 ± 0.1^{a} (5.3–9.1)	4.9 ± 0.1^{a} (1.8–7.0)
Transition	22.7 ± 0.3^{b} (17.0–26.8)	15.6 ± 0.2^{a} (12.4–19.5)	7.9 ± 0.1^{a} (6.2–10.1)	7.0 ± 0.2^{a} (4.0–10.3)
Primary	27.1 ± 0.4^{c} (21.8–35.0)	17.3 ± 0.4^{b} (12.1–25.1)	8.3 ± 0.1^{b} (6.9–10.9)	9.8 ± 0.3^{b} (5.5–15.1)
Secondary	86.3 ± 2.9^{d} (53.6–160.8)	35.8 ± 1.5^{c} (12.6–53.6)	17.1 ± 0.5^{c} (11.1–30.4)	50.5 ± 3.3^{c} (11.6–116.1)

$^{a–d}$Different letters indicate significant differences among follicular classes within the same evaluated parameter, i.e. follicular, oocyte, oocyte nucleus, and granulosa cell diameter; $P < 0.05$.

since Nagle *et al.* (1994) observed the lowest ovulation rates in the right ovaries *S. apella.* In women, however, no differences in ovulation rates are observed between right and left ovaries (Lass *et al.*, 1997), although some authors describe a more frequent ovulation rate in the right ovary (Potashnik *et al.*, 1987; Fukuda *et al.*, 2000). Although we found the opposite in the present research, our data are restricted to one animal only. However, all together, it can be confirmed that ovarian follicular population is also variable between right and left ovary in different individuals (Miller *et al.*, 1999).

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Importantly, the low number of follicles encountered in the ovary from the senile *S. vanzolinii* female was most probably a physiologic response of ovarian aging (Tardif *et al.*, 1985; Walker *et al.*, 2009; Almeida *et al.*, 2012). Besides aging, reproductive state, nutrition, and genetic factors are also known to affect follicular population (Erickson, 1966; Erickson *et al.*, 1976; Cahill *et al.*, 1979; Scaramuzzi *et al.*, 1993; Forman *et al.*, 2013).

Primordial, transition, primary and secondary follicles presented morphometric differences as shown before for other species (Domingues *et al.*, 2004), being indicated to determine follicular morphological quality. Our results confirm that morphometric information supports follicular classification in different developmental stages (Domingues *et al.*, 2004; Lopes *et al.*, 2006). Differences observed among species were previously found when studying other mammals (Koering, 1983; Gougeon, 1996). The changes in the diameters of oocyte nucleus, oocyte, follicle and granulosa cells layer thickness indicate that preantral follicular development follows two distinct phases (Gougeon & Chainy, 1987; Domingues *et al.*, 2004). In the first phase (activation), the pre-granulosa cells surrounding the oocyte are differentiated to granulosa cells, which is characterized by the change in their flattened to cuboidal structure. In this period, the oocyte size increases (Braw-Tal, 2002). In a second phase, increase in both follicular and oocyte diameters take place together with the proliferation of the granulosa cells (Gougeon & Chainy, 1987; Domingues *et al.*, 2004). Correlations are difficult to interpret, once herein we have not the possibility to obtain several samples per species.

Multi-oocyte follicles were observed and previously described in other primates (Harrison, 1949; Graham & Bradley, 1971; Domingues *et al.*, 2004; Lopes *et al.*, 2006). It was suggested that MOFs are a result of an incomplete ovarian organogenesis, i.e. defected follicular formation (Domingues *et al.*, 2004). It was believed that all those follicles become atretic (Hartman, 1926; Harrison, 1949). However, MOFs can be ovulated (Bysted *et al.*, 2001; Reynaud *et al.*, 2005; Silva-Santos & Seneda, 2011) as an exceptional phenomenon (Gougeon, 1981). These follicles present a high concentration of estradiol and low progesterone concentration, which may influence steroidogenesis (Stankiewicz *et al.*, 2009).

In conclusion, for the first time the morphometry and morphological characteristics of preantral follicles were described for three recently recognized squirrel monkey species (*S. macrodon*, *S. cassiquiarensis* and *S. vanzolinii*), and revealed that specific follicular development patterns are present, as well as are age dependent. These differences among species in their early folliculogenesis will be important during the development of reproductive technologies applied to ovarian preantral follicles, especially to protect threatened species such as *S. vanzolinii.*

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Conflict of interest

The authors declare that they have no conflict of interest.

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