ACTA
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Histological study of capuchin monkey (*Cebus apella*) ovarian follicles.

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ABSTRACT

The present study aimed to obtain quanti-qualitative data about the follicular ovarian population in *Cebus apella* females. Seven ovaries were obtained from 4 *C. apella* adult females. The ovaries were subjected to light microscopy. The number of preantral and antral follicles for each ovary was estimated using the Fractionator method. The preantral follicles were classified into primordial, transitional, primary and secondary follicles. Antral follicles were those that presented an antral cavity. All counted follicles were classified as normal or degenerated. The diameter of the follicles, oocytes and their nuclei were determined to accompany the follicular development. All results were represented as mean ± SE. The number of preantral follicles was 56,938 ± 21,888 and 49,133 ± 26,896 for the right and left ovaries, respectively. The percentage of normal follicles was 80 ± 4.95%. The follicular diameter ranged from 22 ± 0.5 µm to 61.2 ± 4.0 µm. Regarding the antral follicles, the number of normal and degenerate follicles per ovary were 60.0 ± 19.0 and 3 ± 1.8 follicles, respectively. The antral follicular diameter was 514.4 + 56.6 µm. In conclusion, the information obtained in this study can be used as a parameter for subsequent *in vivo* or *in vitro* studies about folliculogenesis in non-human neotropical primates of the *C. apella* species.

KEY WORDS

Ovary, histological study, *Cebus apella*, ovarian follicles.

Estudo histológico de folículos ovarianos de Cebus apella.

RESUMO

O objetivo do presente trabalho foi obter dados quantitativos e qualitativos da população folicular ovariana de fêmeas de Cebus apella*. Foram obtidos 7 ovários de 4 fêmeas adultas de* C. apella *através de ovariectomia. Os ovários foram submetidos à preparação para histologia ótica de rotina. O número de folículos pré-antrais e antrais por ovário foi estimado utilizando o Método Fracionador. Os folículos pré-antrais foram classificados em primordial, transição, primário e secundário. Foram considerados folículos antrais todos aqueles que apresentavam uma cavidade antral. Todos os folículos contados foram classificados em normais ou degenerados. Com o intuito de acompanhar o desenvolvimento folicular, os diâmetros médios folicular, oocitário e do núcleo do oócito foram determinados. Todos os resultados foram apresentados em Média ±Erro Padrão. A população média de folículos pré-antrais foi de 56.938 ± 21.888 e 49.133 ±26.896 para os ovários direito e esquerdo, respectivamente. A percentagem de folículos pré-antrais estimados normais foi de 80,00 ± 4,95 %. O diâmetro médio folicular variou de 22,0 ± 0,5 µm a 61,2 ± 4,0 µm. No tocante aos folículos antrais, a população média de folículos normais e degenerados por ovário foi de 60,0 ± 19,0 e 3 ± 1,8 folículos, respectivamente. O diâmetro médio folicular foi de 514,4 ± 56,6 µm. Para concluir, as informações obtidas neste trabalho poderão servir como parâmetro para posteriores estudos* in vivo *ou* in vitro *da foliculogênese de primatas não-humanos neotropicais da espécie* C. apella.

PALAVRAS-CHAVE

Ovário, estudo histológico, Cebus apella*, folículos ovarianos.*

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INTRODUCTION

In the last 30 years, the primatology has been receiving great attention, certainly because of the anatomical and physiological similarity with the human species. As studies develop, the fragility of most primates species has been found caused mainly by destruction of the natural primate habitat (Aurichio, 1995). In this context, captive breeding programs for endangered primates need to be developed (Hearn, 1994). However, even for those species that reproduce naturally, it is necessary to understand the processes involved in reproduction, before developing an artificial reproduction program with success (Asa, 1996).

At present, many reproductive biotechnology have been developed in domestic animals, which includes the manipulation of oocytes enclosed in preantral follicles, this process seeks to isolate, culture and/or conserve preantral follicles, to optimize the use of oocitary potential with high genetic value or from endangered species (Figueiredo *et al.,* 1993). However, for successful development of this biotechnique, it is important to know histological aspects of the ovarian follicles from the species under study. The aim of this work was to obtain morphometric and histological data of the ovarian follicular population in capuchin monkey females (*Cebus apella*). In the future, these data will support other studies with manipulation of oocytes enclosed in preantral follicles from the *Cebus apella* species.

MATERIAL AND METHODS

1. Ovarian collect and histological treatment

Ovaries (n=7) from 4 adult *C. apella* females were used. All ovaries were obtained through ovariectomy. These females were part of the animal surplus from the Municipal Zoological Park (Fortaleza-Ceará, Brazil). The animals had been recovered by IBAMA (Brazilian Wildlife Police). The sterilization was part of the contraception management control of the *C. apella* population.

Each whole ovary was submitted to histological analysis. The ovaries were fixed in Carnoy and sectioned serially at a thickness of 7 mm. Each 10th section of ovarian tissue fragment was mounted and stained with periodic acid Schiff and hematoxylin (PAS-Hematoxylin).

2. Ovarian follicle classification

The preantral follicles were classified according to Lintern-Moore (1974), in: (i) primordial, that present a layer of flattened granular cells surrounding the oocyte; (ii) transitional follicle, in which the oocyte is surrounded by a layer of flattened and cuboidal granulosa cells; (iii) primary, that are formed by a layer of cuboidal granular cells, and (iv) secondary, that possess two or more layers of cuboidal cells surrounding the oocyte. Antral follicles were considered those that presented an antral cavity replete of follicular fluid (Dellman & Brown, 1982).

3. Follicular population estimative

Each 50th section was evaluated to estimate the population of preantral follicles. Only follicles that presented a visible nucleus from the oocyte in the analyzed section were counted. The antral follicles were counted by analyzing each 10th histological section. The ovarian follicle populations were estimated using the Fractionator Method (Gundersen *et al.,* 1988).

4. Qualitative analysis of follicular population

All follicles counted were classified as normal or degenerated for the qualitative evaluation. The normal follicles were those that presented the complete basal membrane, absence of pycnotic bodies in the oocyte nucleus, without signs of oocyte and/or granular degeneration (Bailey, 1976).

Follicular dimensions

The follicular dimensions were measured with a micrometric eye lens in an optical microscope (400X). The largest and the smallest diameters of the oocyte nucleus, oocyte and follicle were measured. The mean diameter of each structure was calculated. For this end, 10 preantral normal follicles of each category per ovary were measured. The antral follicular dimensions were also determined. The approximate diameter of the granulosa surface was estimated in all preantral follicles categories, subtracting the follicular diameter from the oocyte diameter. To accompany the follicular growth, the cell and layer number of the granulosa surface from 5 preantral follicles per ovary (n=7) were counted, totaling a number of 35 follicles per category.

5. Statistical data analysis

The results were demonstrated on mean ± SE. The Whitney-Mann test was used to compare the number and the diameters of the different preantral follicle categories, between right and left ovaries. The Friedman test was used to compare the total percentage and the percentage of normal and degenerate follicles among the different categories. The Kruskal-Wallis test was used to compare the number of granulosa cells and the follicular, oocitary, oocyte nucleus and granulosa surface diameters among the preantral follicles. Simple linear regression was performed to follow the follicular growth, using the diameter oocyte nucleus as an independent variable in relation to the follicular, oocitary and granulosa surface diameters. The results were considered significant when $P < 0.05$.

RESULTS

The presence of *corpus luteum* was observed in all *C. apella* females studied. Table 1 shows the total mean of preantral follicle population of the right and left ovaries from 4 adult C. apella females. A great variation in the parameters of both the right and left ovaries was observed. The mean number of preantral follicles present *in situ* was higher in the right ovary.

Table 2 shows the mean percentage of primordial, transition, primary and secondary follicles in 7 ovaries from 4 adult *C. apella* females. The largest percentage of follicles present *in situ* was the transition follicle, followed by the primordial, primary and secondary.

From the total number of follicles, 80 ± 4.95% was normal and 20 ± 4.95% was considerate degenerate. Table 3 shows the percentage of normal and degenerate follicles by follicular category. It was observed that the transition follicle has the grater percentage of normal follicles, followed by the secondary, primary and primordial. The percentage of normal follicles was significantly greater than the percentage of degenerate follicles in all categories.

Table 1 - Preantral follicular mean (± SEM) population in the right and left ovaries from 4 adult *C. apella* females.

Animal	Ovary	
	Right	Left
I	34,500	-
II	24,550	18,550
III	47,650	26,100
IV	121,050	102,750
Mean (± SEM)	56,938 ± 21,888[a]	49,133 ± 26,896[b]

Among lines: a, b (P< 0.05).

Table 2 - Percentage of primordial, transition, primary and secondary ovarian follicles in 4 adult *C. apella* females.

Follicular Category			
Primordial (%)	Transition (%)	Primary (%)	Secondary (%)
30 ± 4.3[a]	60 ± 4.8[b]	6 ± 0.96[c]	4 ± 0.67[d]

Table 3 - Percentage of normal and degenerated preantral follicles from 4 adult *C. apella*.

	Follicular category			
	Primordial (%)	Transition (%)	Primary (%)	Secondary (%)
Normal	72 ± 10[a*]	83 ± 10[b*]	80 ± 13[c*]	82 ± 10[d*]
Degenerated	28 ± 10[a]	17 ± 10[b]	20 ± 13[c]	18 ± 10[d]

Among columns: a, b, c, d and (*) between lines (P< 0.05)

Table 4 shows the follicular, oocyte, its nucleus and granulosa surface mean diameters from preantral follicles. The mean diameters of follicles and granulosa surface were statistically different from each other. There was no statistical difference between the transition and the primary follicle in relation to the oocyte diameter. The nuclear diameter of the secondary follicle was greater than all nuclear diameters from other preantral follicles. There was no statistical difference among the diameters of any preantral follicular parameters between the right and left ovaries.

Figure 1 shows the relationship between three morphometric values, i.e. follicular, oocitary and granulosa surface diameters in the largest cross-section, and oocyte nucleus diameter. The follicular development in *C. apella* is separated in two different phases. The primordial and transition follicles are in included the left graphs are . Until the 15 μm nuclear diameter, the three follicular dimensions showed little change. There were significant ($P < 0.001$) linear relationships and the slopes of the regression lines possess low values, 1.17, 0.94, 0.22, respectively. The primary and secondary follicles are in the right graphs. In phase II there is fast growth in the three follicular measurements. There were significant linear relationships ($P < 0.001$). The slopes of the regression values are higher than in the previous phase.

The numbers of granulosa cells in relation to the preantral follicular category are shown in Table 5. The significant increase in the number of granulosa cells occurs among the different follicular categories, according to the follicular development.

The transformation from flattened to cuboidal granulosa cells in relation to the number of granulosa cells in the largest cross-section is shown in Figure 2. In the transition follicle, the numbers of flattened and cuboidal granular cells are 6 ± 0.35 and 7 ± 0.44, respectively. The cubic cells appear when the follicle presents approximately 11 cells in the granulosa cell layer. The flattened cells are present until the follicular 15-cell stage. The complete transformation from flattened to cuboidal cells was achieved at about the 16-cell stage.

Figure 3 (A, B, C and D) illustrates the different categories of the morphology of the preantral follicles analyzed in histological cross-section. Many polyovular follicles were found (Figure 3 E). The pellucid zone was first localized in the primary follicles. However, the pellucid zone just became easily shown in the secondary follicle with more than two layers of granulosa cells. The immature theca cells were already present in some secondary follicles with two layers of granulosa cells.

Figure 3 (F) shows an antral follicle in histological cross-section. The mean population of normal and degenerate follicles per ovary was 60.0 ± 19.0 and 3

± 1.8, with the percentage of 95.24% and 4.76% respectively. The follicular, oocitary and oocyte nucleus mean diameter were 514.4 + 53 mm, 56.6 + 1.5 mm and 17.7 + 1.2 mm. The minimum and maximum diameters of the antral follicles were 262 mm and 983 mm, respectively.

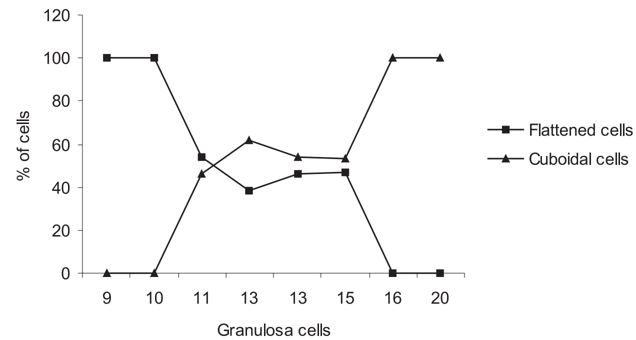

Figure 2 - Relationship between granulosa cell number in the largest cross-section and its pattern of flattened and cuboidal cells distribution. Each point is the mean value of 35 follicles from 7 ovarian samples.

DISCUSSION

The results of this study have shown an estimation of ovarian follicular population and data about the folliculogenesis in the *C. apella* female. The follicles have been classified as proposed by Lintern-Moore (1974). This classification considers a transitional follicular stage (category C/B), which we have called transitional follicle. This preantral follicle has characteristics of follicular activation, with transformation of the flattened to cuboidal granulosa cells, characterizing that the follicle left the quiescent state (Erickson, 1986).

There was a great variation in the number of follicles per ovary. In human and non-human primates, it has been reported that the number of follicles varies with age. Gougeon & Chainy (1987) showed that in women of 19 and 46 years, the number of follicles per ovary can vary from 79,600 to 27,000, respectively. As age increases, the number of follicles per ovary is reduced. Miller et al. (1999) reported that in the Pigtailed monkey, with ages varying from 0.85 to 12.50 years, the number of follicles per ovary varied from 30,900 to 9,940. In the present study, just *C.apella* adult females were used. However, the animals´ age was unknown, so that it was not possible to determine the effect of age on the follicle population per ovary in *C. apella*. Besides age, a variety of factors has been described which may affect the ovarian follicular population, including race (Cahill *et al.,* 1979; Driancourt *et al.,* 1985), reproductive stage (Erickson, 1976), nutrition (Scaramuzzi *et al.,* 1993) and genetic factors (Erickson *et al.,* 1966; Cahill *et al.,* 1979). Gougeon et. al. (1994) affirmed that cyclicity affects the follicular ovarian population in women. According to these authors, cyclic women of a certain age present a follicular population 10 times larger than acyclic women in the same age. In the *C. apella* females used, the presence a *corpus luteum* was observed on the surface of the ovary at the ovariectomy moment.

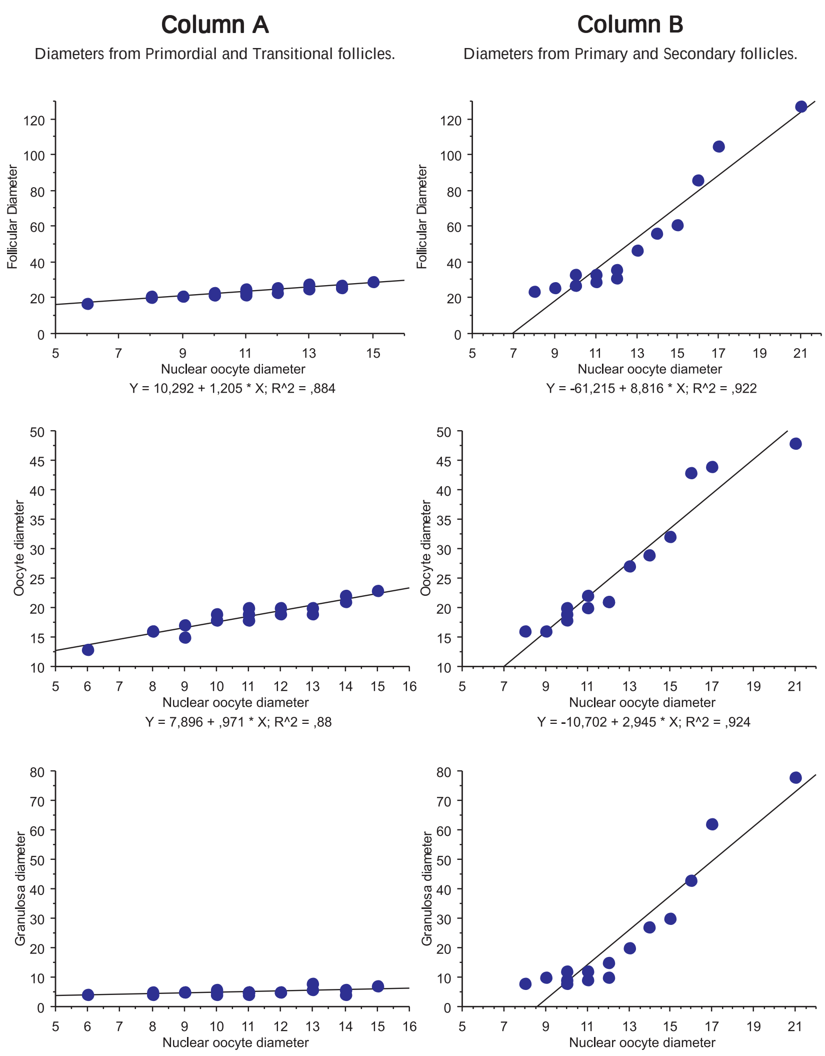

Figure 1 - Relationships between oocyte nuclear diameter with follicular, oocyte and granulosa layer diameters of 280 preantral follicles: b= slope of the regression line (y = a + bx).

There was a statistical difference between the right and left ovaries for the follicular population in adult *Cebus apella* female. The right ovary had a larger follicular population compared to the left. Miller et al. (1999) found no difference between the left and right ovary for the number of follicles in the Pigtailed Monkey. In *C. apella*, a great number of follicles in the right ovary can suggest a lower functionality of this compared to the left ovary. Nagle et al. (1994) showed that the ovulation rate in the left ovary is 62.5% and 37.5% for the right ovary in the *C. apella*. In this study, the follicular ovarian population was found, without verifying the ovulation rate. In women controversy exists about the functionality of the ovaries. According to Potashnik et al. (1987), ovulation happens more frequently in the right ovary in cyclic women. However, more recently, Lass et al. (1997) observed that the ovulation rate is the same for both ovaries.

Concerning the percentage of follicles by follicular category, in the present study a larger percentage of transitional follicles was found in relation to the other preantral follicles. This result is in agreement with the data presented by Block (1951), Gougeon & Chainy (1987) and Gougeon et al. (1994). These authors state that in adult woman, there is a larger proportion of preantral follicles that were activated and are growing.

The greatest percentage of degenerate preantral follicles was the primordial. According to Erickson (1966), as age advances, the number of degenerated primordial follicles increases in cows. The percentage of degenerated primordial follicles varies from 18 to 100% in animals aged from 1-14 days to 15-20 years. Considering that all the *C. apella* females were adult, the obtained results are in agreement with those reported by Erickson (1966).

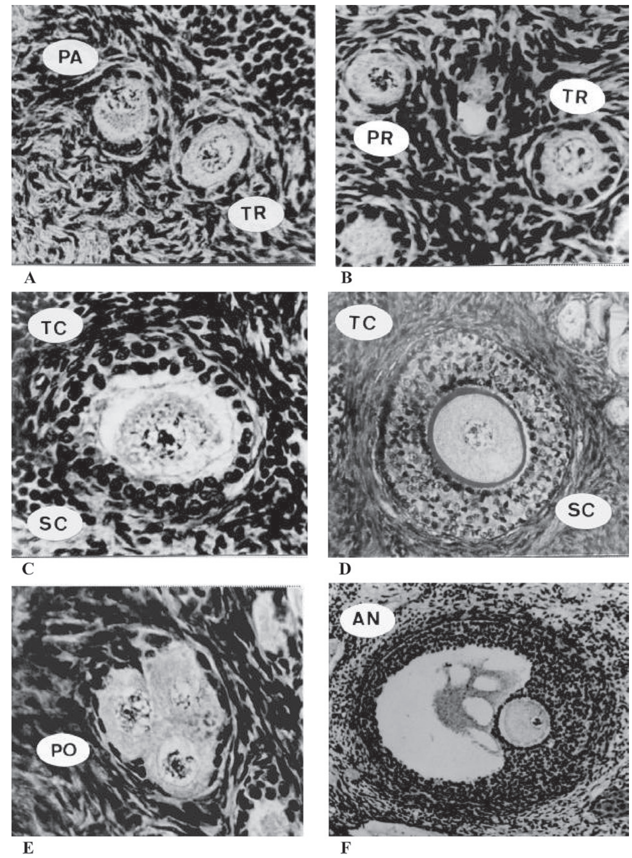

Figure 3 - Illustration of ovarian follicles. A, B, C, D, E (x 400) and F (x 100). PR - Primordial, TR – Transitional, PA – Primary, SC – Secondary, PO – Polyovular, AN – Antral and TC – Thecal cells.

Table 4 - Mean diameter (mm) of follicles, oocytes, their nuclei and the granulosa layer of preantral follicles from ovaries of 4 adult *C. apella* females.

Follicle	Fol. Diam.	Ooc. Diam.	Nuc. Diam.	Gran.Diam.
Primordial	22.1 ± 0.5^a	17.9 ± 0.4^a	10.6 ± 0.2^a	4.1 ± 0.2^a
Min-Max	(16-47)	(12-39)	(6-15)	(2-18)
Transition	24.2 ± 5.0^b	18.9 ± 0.3^{bc}	11.0 ± 0.2^a	5.3 ± 0.2^b
Min-Max	(17-33)	(12-23)	(6-16)	(2-11)
Primary	27.3 ± 0.5^c	19.3 ± 0.3^c	11.0 ± 0.2^a	8.1 ± 0.3^c
Min-Max	(20-38)	(12-26)	(7-16)	(4-16)
Secondary	61.2 ± 4.0^d	30.4 ± 1.2^d	13.7 ± 0.4^b	30.6 ± 3.0^d
Min-Max	(26-193)	(18-58)	(8-24)	(7-141)

Among lines: a, b, c, d ($P< 0.05$)

Table 5 - Granulosa cell number of preantral follicles (n=35) according to their category from *C. apella*.

Primordial	Transition	Primary	Secondary			
			Cell layers			
			2	3	4	5
9 ± 0.3^a	$13 \pm 0{,}5^b$	23 ± 1.0^c	64 ± 1.6^d	133 ± 8.1^e	240 ± 14^f	400 ± 27^g

Comparisons among columns: a, b, c, e, f, g ($P<0{,}05$).

The statistical difference found in the follicular diameter show that is possible to use it to aid in the classification of the different follicular categories. These results found in *C. apella* are similar to those found by Hulshof *et al*. (1994) for the bovine species. There was no statistical difference between the oocyte diameters of the transitional and primary follicles. This result suggests that the main responsible component for follicular growth in these stages is the granulosa surface.

The changes in the three follicular diameters observed in relation to the oocyte nucleus diameter, show that there are two phases of follicular growth. The first phase corresponds to transformation from flattened to cuboidal granulosa cells. At the second growth phase, there is an increase in the oocyte size and in the number of granulosa cells, with the increase in follicular diameter. These data are similar to those found by Gougeon & Chainy (1987). However, in the first phase, the small follicular growth is caused mainly by the increase in the oocyte size, while in the second phase of follicular growth, there is a larger participation of the granulosa cells.

The incidence of in situ polyovular follicles has been verified (with up to 4 oocytes). Forabosco *et al*. (1991) reported the presence of polyovular follicles in human ovaries during the neonatal period. According to these authors, the organogenesis of the ovary is not complete in humans at birth, and oogonias and follicles can be found in the same ovary. Such condition only happens close to birth in great primates, but it extends until the adult life in prosimians (Anand Kumar, 1974). In the present study, we did not find oogonias, but the presence of polyovular follicles in the ovaries of adult animals may suggest an incomplete organogenesis in the adult ovary.

The pellucid zone can be first visualized in the primary follicle in *C. apella*. This result is in agreement with Hirshfield (1991). According to this author, the pellucid zone begins to be formed soon after the primordial follicle begins growth. Regarding the theca, this does not appear until follicular growth is well underway. In the secondary follicles, a signal is generated and causes a stream of mesenchymal cells to migrate to the basal follicular membrane, where they become parallel to one another, forming a radial arrangement of fibroblast-like cells around the entire follicle. These cells will eventually develop into the theca intern and extern (Erickson, 1986). In the mouse, the first recognizable theca cells are reported to appear when the fully grown oocytes and the follicle have 2-3 layers of granulosa cells (Peters, 1969). In hamsters, the theca cells are not evident until the follicle reaches the 7-8 layers of granulosa cells stage (Roy and Greenwald, 1985). However, in female rats, a different theca is already evident in small follicles. These immature theca cells proliferate along with the granulosa cells during the follicular growth (Hirshfield, 1991).

In the present study, the antral follicles present in the ovaries were counted and measured, to provide more information about the folliculogensis in *C. apella*. Comparing the results in *C. apella* with other primates, in *Aotus trivirgatus* (owl monkey) the diameter of the antral follicles can vary from 0.3 mm to 3 mm (Herting *et al.,* 1976). Oerke (1995) reported that in *Callitrichix jacchus*, the preovulatory follicles diameter can vary from 2.1 to 3.2 mm. In women, the preovulatory follicle can reach 20 mm (Baker & Wai, 1976). In *C. apella*, the mean diameter of the preovulatory follicle can reach 10-12 mm (Nagle *et al.,* 1980). The 983 µm is not the maximum diameter that an antral follicle can reach in *C. apella* females and this diameter does not correspond to the final reached at follicular maturation. Regarding the quality of the antral follicles, a small number was degenerate, which may be because the antral follicles are more susceptible to follicular atresia in the final differentiation stage of the granulosa and theca cells than the antral follicles at the initial stage of development (Lussier *et al.,* 1987).

Taking into consideration the results obtained in the present study, it can be concluded that the ovarian follicular population in adult *C. apella* females is variable. The number of preantral follicles is larger in the right ovary. The primordial follicle is the more susceptible to atresia. During the preantral stage, there are two phases of follicular development. In the first phase the oocitary growth has larger participation in the follicular growth, while in the second phase, the proliferation of the granulosa cells is predominant for the follicular growth. The follicular diameter can be used as a parameter to aid the classification of preantral follicles. Regarding the antral follicles, more studies are necessary to understand the folliculogenesis dynamics of this development stage in the *C. apella* species. The information obtained in this study can be used as a parameter for subsequent in vivo or in vitro studies about the folliculogenesis in neotropical primates of the *C. apella* species. The *C. apella* female may be used as an experimental model in subsequent studies on manipulation from oocytes of ovarian preantral follicles, to develop multiplication programs for endangered non-human primates in the future.

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