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Follicular Histomorphometry and Evaluation of Ovarian Apoptosis in Queens of Different Age Groups

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ABSTRACT

***Background*:** In humans and bitchs the age is another factor that may affect the size of ovarian structures, verifying alterations in the quality of the pool and size of follicular structures, which can compromise the use of these structures for *in vitro* maturation. There are no reports correlating the morphometric characteristics of the follicles and ovarian apoptosis at different ages in cats. The aim of this study was to evaluate the histomorphometric parameters of follicular growth and the relationship with the occurrence of apoptosis in ovarian tissue of young, adult and senile queens.

***Materials, Methods & Results*:** Eighteen domestic queens, multiparous, of different breeds and age groups were used in this study and divided into three groups according to their ages: five months to one year - young; (7.8 ± 1.0 months); one to six years - adults (2.8 ± 0.5 years); and more than six years - senile (8.0 ± 0.9 years). Vaginal cytology was performed in order to characterize the estrous phase associated with plasma concentrations of progesterone. The morphology and percentage of the vaginal epithelium cells were evaluated and queens were classified into estrous and non-estrus and plasma concentrations of progesterone were determined. Ovarian samples were collected after ovariohysterectomy to routine histological processin and all follicles were counted and categorized into two groups, non-atresic and atresic. The mean follicular and oocyte diameters were calculated between the measurement of the largest diameter and perpendicular diameter. The relationship between follicle and oocyte were determined using the measurements of diameter, area and perimeter. The apoptotic cells were detected and cells were considered positive when TUNEL reaction was detected. The morphometric index of 1039 follicles were evaluated. Primordial follicles in young animals showed larger diameter, follicular area and perimeter than the structures of adult queens, as well as the unilaminar primary follicles of the same group were larger compared with senile animals ($P < 0.05$). Comparing adult and younger queens, the first showed a significant decrease of oocyte diameter in primary and unilaminar primary follicles, as well as oocyte area and perimeter ($P < 0.05$). The values for follicular diameter, oocyte area and perimeter for multilaminar primary, secondary and pre-ovulatory structures did not present statistical differences between the groups ($P > 0.05$). For the pre-ovulatory follicles there was no positive correlation between the oocyte growths regarding the follicles ($P > 0.05$). Only in senile animals positive markers for apoptosis were identified in nuclei of primordial follicles. No significant differences concerning the number of follicles and Tunel positive cells were observed between groups ($P > 0.05$).

***Discussion*:** Considering the importance of this study for greater knowledge in the basic aspects for reproductive biotechnologies, we verified that secondary follicles showed the largest diameters and younger animals the largest values for diameter, area and perimeter, suggesting that this age group could be ideal for the use and manipulation of oocytes. The process of follicular atresia is characterized by the occurrence of apoptosis, or programmed cell death when the organism begins to efficiently eliminate dysfunctional cells. The study of follicular apoptosis in small animals, especially in cats, is very important for the development of reproduction biotechnologies. Phenomenon of apoptosis showed no relationship with age in queens, occurring in a physiological, continuous and proportionate manner considering the number of non-dominant follicles involved in each estrous cycle.

Keywords: feline, follicle, reproduction.

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INTRODUCTION

The domestic cat is an important and necessary experimental model for reproduction biotechnologies. Reliable methods for the *in vitro* maturation and in vitro fertilization have been developed through the use of oocytes obtained from domesticated cats [18]. The study of ovarian folliculogenesis provides better understanding of the reproductive physiology and may be useful for improving the preservation of endangered wild cats [3].

Recent study using prepubertal and sexually mature queens evaluated the ovarian condition and follicle growth *in vitro* maturation and concluded that size and quality of follicles and oocytes can affect maturational ability [16]. According to studies in humans [17] and bitches [5], age is another factor that may affect the size of ovarian structures, in other words, the morphometric characteristics of the follicles are related to the age of individuals, verifying alterations in the quality of the pool and size of follicular structures, which can compromise the use of these structures for *in vitro* maturation.

The process of follicular atresia in the ovarian tissue is characterized by the occurrence of apoptosis of granulosa or theca cells [4,14]. To our knowledge, there are no reports correlating the morphometric characteristics of the follicles and ovarian apoptosis at different ages in cats.

The aim of this study was to evaluate the histomorphometric parameters of follicular growth and the relationship with the occurrence of apoptosis in ovarian tissue of young, adult and senile queens.

MATERIALS AND METHODS

Eighteen domestic queens, multiparous, of different breeds and age groups, with mean body weight of 3 kg were used in this study. None of the animals had any report of reproductive diseases and were considered healthy after physical and clinical examinations. The animals were divided into three groups according to their ages: five months to one year - young; (7.8 ± 1.0 months); one to six years - adults (2.8 ± 0.5 years); and more than six years - senile (8.0 ± 0.9 years). The age was estimated by analyzing the dental arch [6].

Vaginal cytology was performed in order to characterize the estrous phase associated with plasma concentrations of progesterone.

These samplings were performed prior to the ovariohysterectomy of the queens. Pre-anesthetic medication consisted of intramuscular administration of acepromazine (0.05 mg/kg; Acepran®)[1], midazolam (0.2 mg/kg; Dormire®)[2], and morphine (0.3 mg/kg; Dimorf®)[2]; induction protocol involved intravenous Ketamine (5 mg/kg; Vetaset®)[3] and xylazine (0.5 mg/kg; Dopaser®)[4] and epidural anesthesia consisted of (0.3 mL/kg = lidocaine 2% of epinephrine; Xylestesin®)[2], bupivacaine 0.5% with epinephrine (Neocaína®)[2] and fentanyl (2 mcg/kg; Fentanest®)[2].

Vaginal smears were obtained using a cotton sterile swab previously moistened with saline solution. The smears were stained with a modified Wright-Giemsa stain (Diff-Quick®)[5] and analyzed by light microscopy (Olympus BX61)[6]. The morphology and percentage of the vaginal epithelium cells were evaluated [15] and queens were classified into estrous and non-estrus.

Blood samples (3 mL) were collected by jugular vein puncture. The blood was centrifuged immediately and plasma was stored at -18°C until hormone analysis. Plasma concentrations of progesterone were determined, in duplicate, by solid-phase I125 radioimmunoassay[7].

In this study only animals classified as in a non-estrus period were enrolled, based on the results of progesterone levels and vaginal cytology evaluations [15].

After ovariohysterectomy the ovaries were fixed in paraformaldehyde solution at 5% (pH of 7.2 - 7.4) for 24 h and samples submitted to routine histological processing. Five-micron serial sections were mounted onto plain glass slides and stained with hematoxylin and eosin for light microscope (Olympus BX61)[6] evaluation. All follicles were counted and categorized into two groups, non-atresic and atresic, following the morphological criteria established [2].

The non-atresic follicles were those with intact granulosa membrane and the presence of a few pyknotic nuclei (≤ 5% pyknotic nuclei) and atresic follicles showed attenuated granulosa membrane, rupture of granulosa cells and increased number of pyknotic cells (≥ 15% pyknotic nuclei) according to literature [2].

Only non-atresic follicles were considered and classified [7]: primordial, primary oocytes surrounded by a single layer of follicular cells; unilaminar primary, oocytes surrounded by a single layer of cuboidal fol-

licular cells already with the formation of the zona pellucida; primary multilaminar or pre-antral, follicles characterized by the enlargement of the granulosa cells, after the formation of the zona pellucida and theca interna; secondary or antral follicles, enlargement of the structures' sizes, presence of follicular fluid and antrum, theca interna and externa, organization of granulosa cells begins; mature, pre-ovulatory or Graafian follicles, presence of the antrum, organized granulosa cells, theca interna and externa, corona radiata and oophorus cumulus.

For the morphometric study, histological sections were observed and photomicrographed by light microscopy (Olympus BX61)[6] using the Image J 1.45 software. For each follicular stage, ten follicles were examined (or what was available in two histological sections). The mean follicular and oocyte diameters were calculated between the measurement of the largest diameter (maximum diameter) and perpendicular diameter (minimum diameter) [Figure 1].

The relationship between follicle and oocyte were determined using the measurements of diameter, area and perimeter. For each follicular stage three linear equations were established, representing the patterns of follicle and oocyte growth, where x = follicular measurement and y = oocyte measurement.

The samples were 10% buffered formalin-fixed and processed for further paraffin embedding. The apoptotic cells were detected by a commercial kit for in situ detection of apoptosis (FragEL TM DNA Fragmentation Detection Kit Colorimetric - TdTEnzyme)[8], Sections of 5 µm mounted on signalized glass microscope slides were deparaffinized and washed in TBS (Tris-buffered saline). The sections were treated with Proteinase K for 8 min, and washed again in TBS. After washing, endogenous peroxidase was inactivated by using 9% hydrogen peroxide and then washed in TBS and incubated in equilibration buffer for 20 min at room temperature. Subsequently, the sections were incubated with the TdT enzyme (terminal deoxynucleotidyltranferase) at 37°C for one-half h in a moist chamber. The reaction was interrupted and the sections incubated, with conjugated antibody, in the moist chamber for 1 h. After washing with TBS, the reaction was detected using diaminobenzidine (DAB) for 30 min. The sections were counterstained with methylgreen during 10 min. Images were scanned by Image-ProPlus 7.0 software, and obtained through camera (Q Color 5 Olympus)[6], attached to an Olympus BX43 microscope[6]. Cells were considered positive when TUNEL reaction was detected.

Data was submitted to analysis of variance for group comparison, with the means being compared by Tukey test. Data was tested for normality and homogeneity of variances and in non-normal distribution the Kruskal-Wallis test was performed for group comparison, followed by Dunn multiple comparison test. The correlation coefficient (r) of determination (r2) and the regression equation were calculated for the selected variables [20]. The values were considered significantly different when $P < 0.05$. Statistical analysis was performed using the program "GraphPad version 3.10".

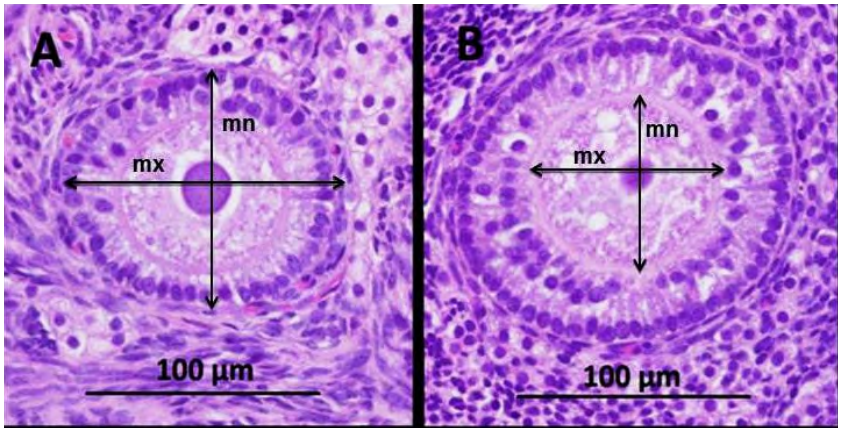

Figure 1. Maximum (mx) and minimum (mn) diameter of multilaminar primary follicle in domestic cat: (A) follicular diameter and (B) oocyte diameter.

RESULTS

The morphometric index (maximum and minimum diameter, oocyte and follicular area and perimeter) of 1039 follicles were evaluated, including primordial follicle (n = 318), unilaminar primary (n = 271), multilaminar primary (n = 271), secondary (n = 124) and pre-ovulatory (n = 55).

Primordial follicles in young animals showed larger diameter, follicular area and perimeter than the structures of adult queens, as well as the unilaminar primary follicles of the same group were larger compared with senile animals ($P < 0.05$). Comparing adult and younger queens, the first showed a significant decrease of oocyte diameter in primary and unilaminar primary follicles, as well as oocyte area and perimeter ($P < 0.05$). The values for follicular diameter, oocyte area and perimeter for multilaminar primary, secondary and pre-ovulatory structures did not present statistical differences between the groups ($P > 0.05$) [Tables 1, 2 & 3].

For the pre-ovulatory follicles there was no positive correlation between the oocyte growths regarding the follicles ($P > 0.05$). For primordial follicles, unilaminar and multilaminar primary and secondary, a correlation was observed between the measurements of oocytes as to the follicles (Table 4).

A total of 1975 follicles were histologically prepared and evaluated. The apoptosis signs of primordial follicles (1664), unilaminar primary (120), multilaminar primary (134), secondary (41) and pre-ovulatory follicles (16) were studied using Tunel analysis. Positive apoptosis was identified in oocytes and pre-granulosa cells of primordial follicles and in other follicle stages positive apoptosis was observed in both granulosa and theca cells. Only in senile animals positive markers for apoptosis were identified in nuclei of primordial follicles (Figure 2).

No significant differences concerning the number of follicles and Tunel positive cells were observed between groups ($P > 0.05$). Table 5 shows the number of follicles and Tunel positive cells at each follicular stage of 18 queens according to their age.

Table 1. Mean values (x), standard error of the mean (SEM) and median (Md) of the diameter (μm) of follicle and oocyte for female cats according to age.

	Stage	G1 (young)		G2 (adults)		G3 (senile)	
		x ± SEM	Med	x ± SEM	Med	x ± SEM	Med
Follicle	P	45.13 ± 0.71a	45.25	41.51 ± 0.65b	41.4	43.24 ± 0.66ab	43.55
	UP	70.35 ± 1.35	70.30A	64.43 ± 1.53	63.35B	68.11 ± 3.1	62.25B
	MP	127.60 ± 4.3	114.95	132.53 ± 5.3	121.3	128.51 ± 6.6	119.43
	SE	452.66 ± 19.46	423.3	400.51 ± 18.28	371.6	388.11 ± 22.05	395.7
	OP	893.18 ± 43.49	885.45	887.99 ± 37.63	930.4	1014.0 ± 100.99	1036.1
Oocyte	P	40.55 ± 0.56a	40.15	37.57 ± 0.59b	36.6	39.41 ± 0.57ab	40.25
	UP	47.97 ± 0.89	47.1A	43.00 ± 1.02	42.45B	45.24 ± 1.51	43.45AB
	MP	62.35 ± 1.56	59.8	65.77 ± 2.55	61.22	62.05 ± 2.36	58.72
	SE	112.80 ± 7.1	103	98.620 ± 10.72	91.55	88.70 ± 6.11	89.3
	OP	96.63 ± 3.56	94.35	101.35 ± 3.77	97.6	107.89 ± 12.95	116.7

Primordial (P), unilaminar (UP), multilaminar primary (MP), secondary (SE) and pre-ovulatory (OP); a, b: means followed by different small letters in line differ significantly by Tukey test ($P < 0.05$); A, B: means followed by different uppercase letters in line differ significantly by Dunn's test ($P < 0.05$).

Table 2. Mean values (x), standard error of the mean (SEM) and median (Md) of the area (μm2) of follicle and oocyte for female cats according to age.

	Stage	G1 (young)		G2 (adults)		G3 (senile)	
		x ± SEM	Med	x ± SEM	Med	x ± SEM	Med
Follicle	P	1941 ± 51.85a	1908.8	1652.4 ± 46.89b	1590.3	1776.9 ± 48.44ab	1723.3
	UP	4043.9 ± 159.05	3810.3A	3375.6 ± 165.22	3137.9B	4185.8 ± 541.1	3086.7B
	MP	14239 ± 1061.6	9807	15522 ± 1508.3	11130	15046 ± 1606.4	10759
	SE	169435 ± 14726	134712	137060 ± 13732	107104	126038 ± 13570	125592
	OP	672390 ± 63002	634085	647261 ± 54270	675397	921159 ± 170350	956801
Oocyte	P	1320.4 ± 35.92a	1305.1	1134.3 ± 36.25b	1036.7	1241.3 ± 32.81ab	1270.2
	UP	1851.9 ± 67.46	1702.2A	1497.0 ± 71.02	1341.9B	1721.0 ± 138.22	1461.8B
	MP	3225.1 ± 162.81	2782.2	3823.3 ± 351.1	2892.9	4061.6 ± 848.16	2669.6
	SE	10157 ± 1119.1	8336.8	6688.2 ± 548.44	5903	6311.8 ± 876.32	4949.4
	OP	7537.9 ± 529.42	7046.5	8518.6 ± 656.64	7721	10087 ± 2399.5	10333

Primordial (P), unilaminar (UP), multilaminar primary (MP), secondary (SE) and pre-ovulatory (OP); a, b: means followed by different small letters in line differ significantly by Tukey test ($P < 0.05$); A, B: means followed by different uppercase letters in line differ significantly by Dunn's test ($P < 0.05$).

Table 3. Mean values (x), standard error of the mean (SEM) and median (Md) of the perimeter (μm) of follicle and oocyte for female cats according to age.

	Stage	G1 (young)		G2 (adult)		G3 (senile)	
		x ± SEM	Med	x ± SEM	Med	x ± SEM	Med
Follicle	P	157.24 ± 2.13a	158	145.56 ± 2.02b	144.55	150.63 ± 2.02ab	150.9
	UP	272.82 ± 34.55	222.4A	204.29 ± 4.86	200.9B	217.01 ± 9.71	201.05B
	MP	407.25 ± 13.65	364	424.68 ± 17.21	390.3	395.65 ± 20.52	348.5
	SE	1487.5 ± 63.62	1405.7	1534.4 ± 2227.5	1210.1	1253.9 ± 72.45	1274.1
	OP	2958.2 ± 144	2995.9	2940.0 ± 128	3092.7	3430.8 ± 355.48	3749.4
Oocyte	P	129.90 ± 1.81a	129.5	120.58 ± 1.88b	116.45	126.37 ± 1.67ab	128.5
	UP	152.99 ± 2.73	150.3A	135.76 ± 3.3	132.75B	143.18 ± 4.82	137.9B
	MP	198.53 ± 5.07	194.1	208.80 ± 8.15	194.9	197.29 ± 7.34	187.35
	SE	342.28 ± 16.62	329.2	279.81 ± 12.65	277.5	282.70 ± 18.48	284.6
	OP	311.63 ± 11	302.65	327.35 ± 12.41	323.4	344.74 ± 43.82	363.9

Primordial (P), unilaminar (UP), multilaminar primary (MP), secondary (SE) and pre-ovulatory (OP); a, b: means followed by different small letters in line differ fsignificantly by Tukey test ($P < 0.05$); A, B: means followed of different uppercase letters in line differ significantlyby Dunn's test ($P < 0.05$).

Table 4. Linear equations (x = follicular measurement and y = oocyte measurement) and r2 values for follicular and oocyte growth of female cats according to age.

Stage	Measure	Linear equations	r^2
Primordial follicles	Diameter	y = 0.7405x + 7.0833	0.7655
	Area	y = 0.6598x + 49.767	0.8608
	Perimeter	y = 0.8043x + 3.9923	0.8582
Unilaminar primary follicles	Diameter	y = 0.506x + 11.301	0.7695
	Area	y = 0.2702x + 657.73	0.7677
	Perimeter	y = 0.0422x + 134.89	0.0887
Multilaminar primary follicles	Diameter	y = 0.3033x + 24.166	0.549
	Area	y = 0.1504x + 1401	0.2163
	Perimeter	y = 0.3024x + 77.633	0.5464
Secondary follicles	Diameter	y = 0.0782x + 70.059	0.0297
	Area	y = 0.0369x + 2603.7	0.3275
	Perimeter	y = 0.0248x + 270.62	0.0616

Table 5. Mean values (x), standard error of the mean (SEM) and median (Md) of follicles and apoptosis positive cells of primordial (P), unilaminar (UP) and multilaminar primary (MP), secondary (SE) and pre-ovulatory (OP) follicles of female cats in different age groups.

Structure	Type	G1 (young)		G2 (adult)		G3 (senile)	
		x ± SEM	Med	x ± SEM	Med	x ± SEM	Med
Follicles	P	100.17 ± 12.44	95	89.5 ± 16.48	95.5	87.66 ± 11.52	87
	UP	8.66 ± 1.38	8.0	5.33 ± 1.97	3.0	6.00 ± 2.04	4.5
	MP	1.32 ± 0.54	7.0	3.86 ± 1.57	8.5	5.92 ± 2.41	7.0
	SE	2.66 ± 0.49	2.5	2.16 ± 0.30	2.0	2.00 ± 0.57	2.0
	OP	0.83 ± 0.30	1.0	1.33 ± 0.33	1.0	0.50 ± 0.34	0.0
Positive cells	P	2.00 ± 1.26	0.0	0.00 ± 0.00	0.0	5.16 ± 3.68	1.0
	UP	2.16 ± 1.64	0.0	0.00 ± 0.00	0.0	0.33 ± 0.33	0.0
	MP	4.00 ± 2.11	2.0	0.66 ± 0.66	0.0	0.66 ± 0.42	0.0
	SE	2.83 ± 1.81	0.5	2.16 ± 0.47	2.0	1.16 ± 1.16	0.0
	OP	4.16 ± 2.56	1.5	13.16 ± 5.88	8.5	2.50 ± 1.58	0.0

Significance level of 5%.

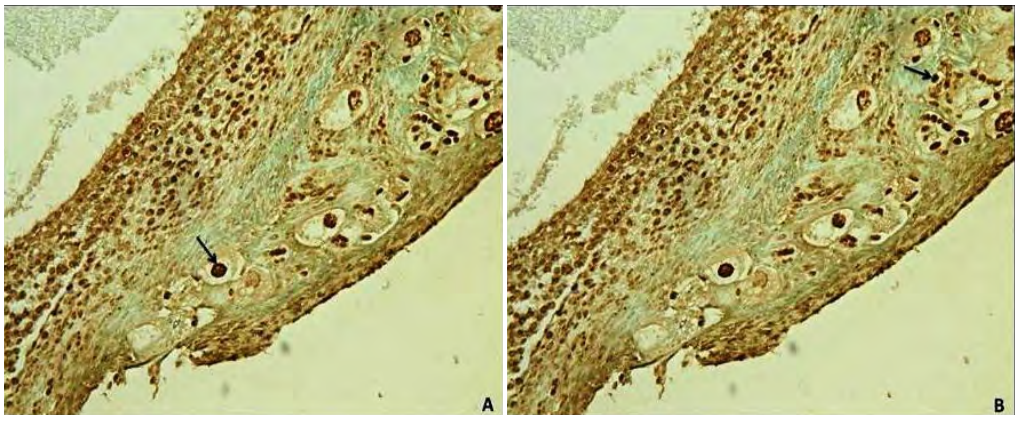

Figure 2. Tunel positive cells in primordial follicles of senile domestic cat ovary (longitudinal section 200x; A - oocyte; B - pre-granulosa cells).

DISCUSSION

Considering the importance of this study for greater knowledge in the basic aspects for reproductive biotechnologies, we verified that secondary follicles showed the largest diameters and younger animals the largest values for diameter, area and perimeter, suggesting that this age group could be ideal for the use and manipulation of oocytes. These characteristics were also observed in female dogs by literature [5], who evaluated the effects of age and oocyte size upon subsequent oocyte maturation (IVM). They verified that oocytes smaller than 100 µm in diameter had less nuclear material and meiotic competence and younger bitches showed optimized oocyte maturation.

Age had an important effect on oocyte morphometry in queens, given that remaining follicles and their respective oocytes decrease in size with the process of ageing. This fact is justified through the morphometric parameters of unilaminar primary follicles, which are smaller in older cats compared with the younger group (diameter - G1: 70.30 µm; G3: 62.25 µm) (area - G1: 3810.3 μm^2; G3: 3086.7 µm2) (Perimeter- G1: 222.4 µm; G3: 201.05 µm). This pattern was also observed in women [17], who found a reduction in mean follicular diameter of primordial and primary follicles in women older than 36 years (39.4 µm) in comparison to younger groups (20 to 27 years: 41.9 µm; 27 to 36 years: 41.5µm).

As described by literature [11], the oocyte and follicle growth in cats showed a biphasic pattern. During the first stage of follicular development, a significant correlation between follicle and oocyte growth was verified, resulting in a linear equation y = 0.3048x + 25.018, r2 = 0.7239 (x = follicular diameter y = oocyte diameter). After antral formation and development of secondary and pre-ovulatory follicles, the oocyte growth pattern significantly decreased compared to follicular growth, resulting in a new linear correlation: y = 0.0072x + 98.001, r2 = 0.0015. These results are superior to those described in cats [11].

In adult animals, the mean follicular diameter was lower in primordial follicles, unilaminar and multilaminar primary follicles and higher in secondary or antral follicles (41.51 µm, 64.43 µm, 132.53 µm and 400.51 µm, respectively), when compared to the results of literature [11] (44.3 µm, 86.2 µm, 155.6 µm and 223.8 µm, respectively). In contrast, the results in this study were superior when comparing to other works, where queens showed a decrease in the mean diameter of primordial follicles, unilaminar primary and secondary follicles of 28.3 µm,41 µm and 74.6 µm, respectively [3], and 38.8 µm, 63.9 µm and 98.7 µm, respectively [1]. Different values were also observed in the mean follicular diameter of other species such as adult sheep, in which primordial follicles showed a mean of 39.4 µm [9] and bitches with values for primordial, unilaminar and multilaminar primary follicles of 44.3 µm, 50.7 µm and 148.9 µm, respectively [1].

When we compared our findings with the results of other species we observed that the mean follicular diameter was higher in primordial follicles, unilaminar and multilaminar primary follicles (45.13 µm, 70.35 µm and 127.60 µm) than diameters obtained in other species as in pre-pubertal gilts (33.8 µm, 40.4 µm and 84.5 µm) [13] and young women (< 13 years) (39 µm and 44.1 µm) [17]; the mean follicular

diameter of older queens was higher in primordial and unilaminar primary follicles (43.24 μm and 68.11 μm, respectively) compared to post-pubertal female buffaloes (7 - 10 years) (35 μm and 41.8 μm, respectively) [10]; the mean oocyte diameter in young animals was higher in primordial, unilaminar and multilaminar primary follicles (40.55 μm, 47.97 μm and 62.35 μm, respectively) when comparing with pre-pubertal gilts (26 μm, 27.3 μm and 39.1 μm) [13] and women (< 13 years) with values for primordial and unilaminar primary follicles (34.3 μm and 41 μm, respectively) [17].

The process of follicular atresia is characterized by the occurrence of apoptosis, or programmed cell death when the organism begins to efficiently eliminate dysfunctional cells [12]. The study of follicular apoptosis in small animals, especially in cats, is very important for the development of reproduction biotechnologies.

The TUNEL technique is widely used to detect DNA fragmentation as an indicative factor of apoptosis. Cells that had suffered apoptosis were detected in human embryos using the chromatin condensation state and DNA fragmentation, which was subsequently confirmed by TUNEL [8]. To our knowledge, this is the first study evaluating the frequency of apoptosis with the TUNEL assay in ovarian tissue in queens according to their age. The TUNEL method was effective labeling oocytes of primordial follicles and pre-granulosa cells in older cats and multilaminar primary and secondary follicles or antral follicles in granulosa cells of young animals.

In our study, there was no significant difference of apoptosis in the different age groups, indicating that none of the older, adult or younger queens showed an increase or decrease of apoptosis. These results in felines are contrary to those verified by literature [19], who demonstrated that apoptosis rate of human oocytes was significantly higher in a group of older women (41 - 50 years) compared to younger women (21 to 40 years).

CONCLUSION

Young queens presented larger values for unilaminar primary follicles than older animals. We suggest that the pool of remaining small follicles and their respective oocytes decrease with age and in senile phase these follicles are of lower quality, when compared with the beginning of reproductive life. On the other hand, the phenomenon of apoptosis showed no relationship with age in animals, occurring in a physiological, continuous and proportionate manner considering the number of non-dominant follicles involved in each estrous cycle.

The results of this study regarding histomorphometry, morphology and apoptosis of ovarian tissue may contribute as a model for the study of physiological parameters or set up of assisted reproductive technologies of endangered wild cats.

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Ethical Approval. The trial was conducted following approval by the Institutional Animal Care and Use Committeel of the UNESP Araçatuba, SP, Brazil (protocol number 00717-2012).

Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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