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Morphometric characteristics of preantral and antral follicles and expression of factors involved in folliculogenesis in ovaries of adult baboons (*Papio anubis*)

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Results A total of 2221 follicles were counted and measured. Proportions of primordial, primary, secondary, small antral, and large antral follicles were 49, 26, 23, 1, and 1 %, respectively. The increase in follicle diameter was due not only to the increase in oocyte diameter but also to granulosa cell proliferation. Almost all antral follicles were positive for Cx43 (89.8 %), aromatase (84.8 %), and ZP3 (100 %). Most secondary follicles were positive for Cx43 (65 %) and ZP3 (64.5 %), and some primary follicles were positive only for Cx43. No primordial follicles stained positive in any of these immunohistochemical analyses. Only antral follicles showed aromatase activity.
Conclusions On the basis of these results, we can conclude that folliculogenesis in baboons appears to be similar to that in humans, and this animal therefore constitutes a valuable model.

Keywords Baboon · Folliculogenesis · Ovarian follicles · Oocyte · Granulosa cells · Theca cells

CA Amorim and CF Moya are joint first authors.

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Introduction

Non-human primates are widely used as models for studies on human reproduction, as they share many similarities in their reproductive biology. Mechanisms involved in gametogenesis, hormone patterns during estrous cycles, fertilization, embryo implantation, and maintenance of early pregnancy are common to these species, but not other mammals. Like humans, some non-human primates also menstruate (menstrual cycles of 28 days in macaques and 33 days in baboons) and undergo menopause [1, 2], hence have proved vital to studies on infertility, contraception, pregnancy [2], and endometriosis [3, 4] that can be extrapolated to humans.

More recently, baboons have also been used as a model to study follicle recruitment and development in vitro [5, 6], ovarian tissue cryopreservation [7, 8] and transplantation [9], and endometriosis [3, 4], since they are similar to humans in terms of ovarian function, histology, and anatomy [9]. However, there are only a few existing studies on folliculogenesis in these animals, notably on follicle

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formation in fetal ovaries [10–12]. The lack of studies on folliculogenesis and oogenesis in baboons is nevertheless understandable, given the limited availability of these animals [13]. Can studies that involve follicle recruitment and development in these animals also be extrapolated to humans?

The aim of this study was to assess follicle morphometry, evaluating factors such as follicle proportion, follicle and oocyte diameter, granulosa cell number, and theca cell layer thickness, as well as expression of essential proteins involved at different stages of folliculogenesis in baboons, namely c-kit, kit ligand (KL), connexin 43 (Cx43), aromatase, and zona pellucida glycoprotein 3 (ZP3).

Materials and methods

Collection of ovarian tissue

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed and approved by the Committee on Animal Research of the Université Catholique de Louvain. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institute of Primate Research (Nairobi, Kenya) where the experiments were conducted. Approval for this study was obtained from the Animal Care and Use Committee of the Institute of Primate Research (IRC/03/14).

Four adult female baboons between 5 and 15 years of age (mean weight 13 ± 1.9 kg) were studied at the Institute of Primate Research. All tested negative for common pathogens (bacterial and viral infections as well as parasites) and were screened for tuberculosis, simian T-lymphotropic virus-1, and simian immunodeficiency virus. After this period, the animals were housed one per cage, fed commercial monkey pellets (Gold Star Products, Kenya) and seasonal fruit and vegetables, with ad libitum access to water.

For the surgical procedure, the animals were anesthetized with a mixture of ketamine (Anesketin, 15 mg/kg; Eurovet NV/SA, Heusden-Zolder, Belgium) and xylazine (Bomazine 2 %, 2 mg/kg; Bomac Laboratories Ltd, Auckland, New Zealand) administered intramuscularly for induction, and 1–2 % halothane (Halothane; Nicholas Piramal India Ltd, Andhra Pradesh, India) with N_2O/O_2 (70/30 %) for maintenance.

Video-assisted laparoscopy was performed by qualified gynecologists (J. Donnez and M.M. Dolmans) by introducing a 10-mm scope (Karl Storz Company, Tuttlingen, Germany) into the umbilicus after CO_2 pneumoperitoneum induction. Two trocars (Karl Storz Company) measuring 5 mm in diameter were placed in the suprapubic area to facilitate pelvic exploration once the baboons were in the Trendelenburg position. Half an ovary was removed from each animal, immediately transported to the laboratory in minimal essential medium + Glutamax™ (MEM, Gibco, Carlsbad, USA) and then fixed in formalin (VWR Chemical, Leuven, Belgium).

After surgery, the animals received antibiotics for 1 week (Clamoxyl LA; Pfizer, Paris, France), and pain was controlled with ibuprofen (Ketofen; Merial, Lyon, France) for 3 days.

Histological analysis

After fixation, to evaluate follicular morphology, the ovarian fragments were dehydrated and embedded in paraffin. Each sample was cut into 5-μm serial sections and every sixth section was stained with hematoxylin–eosin (Merck, Darmstadt, Germany) for histological evaluation, while the others (Superfrost® Plus slides, Menzel-Glaser, Braunschweig, Germany) were kept for immunostaining.

Follicle morphometry

For morphometric characterization, sections were scanned using the Mirax Scan system (Zeiss, Jena, Germany). Follicles were counted and set aside for further analysis (follicle and oocyte diameters, granulosa cell counting and calculation of theca cell layer thickness) only when the oocyte nucleus was present. They were classified according to stage into primordial, primary, secondary, small antral, or large antral (Fig. 1). For follicle distribution in ovarian tissue, at least four sections (one from each extremity of the sample and two or more from the middle) from each animal were used to count and classify at least 500 follicles. Primordial follicles showed one layer of flattened granulosa cells, primary follicles one layer of flattened and cuboidal or only cuboidal granulosa cells, secondary follicles two or more layers of cuboidal granulosa cells [14], small antral follicles a segmented cavity with two or more compartments, and large antral follicles one large continuous antral cavity [15].

Follicle and oocyte measurements

Diameters were computed using integrated measuring tools in Mirax Viewer and calculated according to Griffin et al. [15]; two measurements were taken and their average was recorded as the structure diameter (Fig. 2). Follicle diameters were measured from the outer layer of granulosa cells, while the oocyte was measured together with its zona pellucida, when present (Fig. 2) [15]. At least 100 follicles per animal were used for the measurements.

Granulosa cell counting

Granulosa cells were counted using ImageJ, a freely available image-processing and analysis program developed at the National Institutes of Health (http://rsb.info.nih.gov/ij/). Once selected with the freehand tool, each granulosa cell layer was

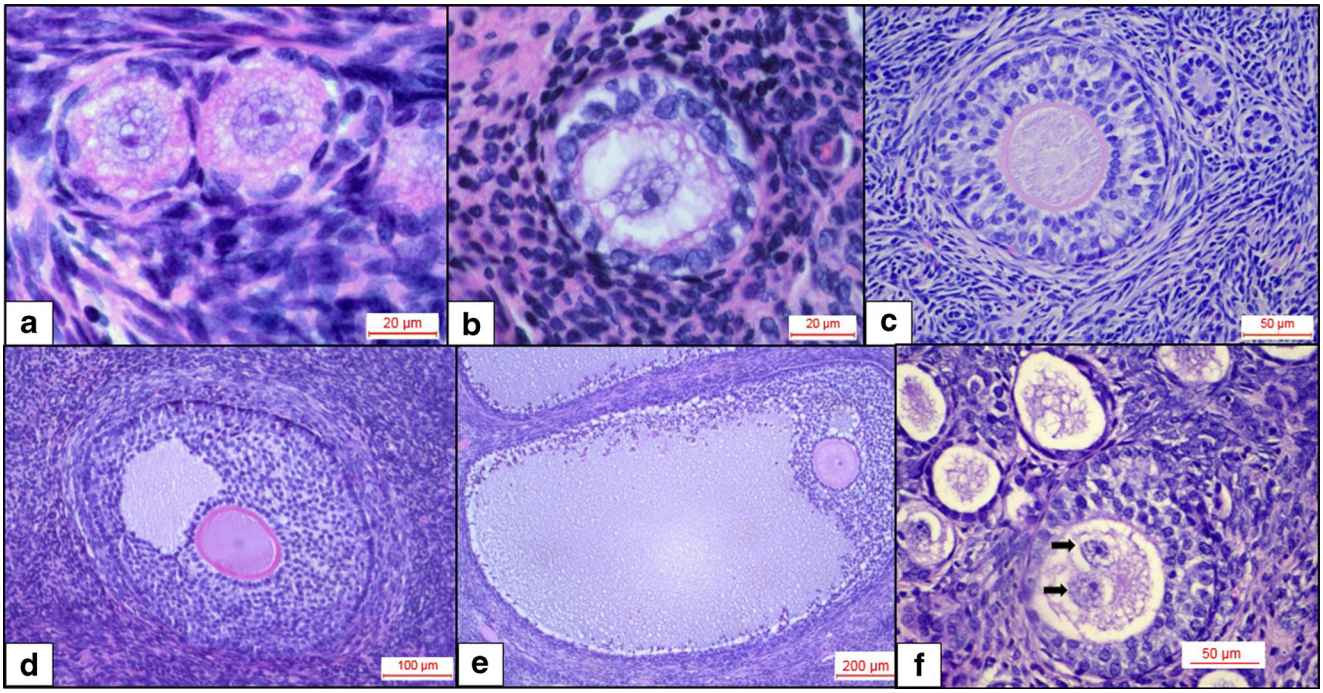

Fig. 1 Histology of follicles at different stages of development in the baboon ovary (hematoxylin–eosin staining). Primordial (**a**), primary (**b**), secondary (**c**), small antral (**d**), and large antral (**e**) follicles. A few binucleated oocytes (*black arrows*) were also observed in secondary follicles (**f**)

transformed into a grayscale image (Fig. 2) and cells were counted using the particle analyzer function. Due to the amount of work that cell counting entails, we used a minimum number of follicles (at least 20 per animal) to obtain a representative example.

Theca cell thickness

To calculate theca layer thickness, a total of 360 follicles were used (90 follicles/animal). To this end, we applied the Mirax Viewer program, measuring from the follicle basement membrane to the end of the visible difference between theca and stromal cells using the most representative part/area of this layer around the follicle [16] (Fig. 2). In order to confirm the accuracy of this measurement, some slides containing secondary and antral follicles were stained with Masson's trichrome to differentiate between the theca layer and stromal tissue through collagen staining, present in larger amounts in the latter [16].

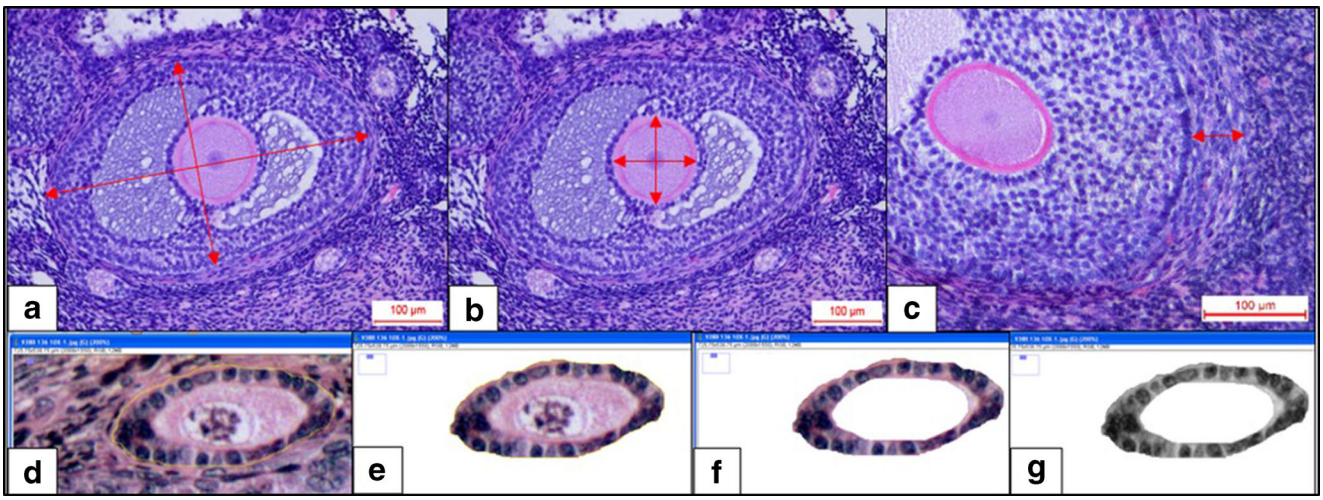

Fig. 2 Morphometric measurements (hematoxylin–eosin staining). Small antral follicle at its largest cross section (**a**), its oocyte diameter (**b**), and thickness of its theca layer (**c**). For granulosa cell counting using ImageJ, the follicle was selected (**d**), then the surrounding tissue (**e**) and the oocyte were removed (**f**) in order to avoid counting stromal cells and the oocyte. Before applying the "analyze particles" command, the picture was converted into grayscale (**g**)

Immunohistochemistry

The following markers were selected to assess folliculogenesis: KL and its receptor c-kit, aromatase, ZP3, and Cx43.

Paraffin sections were deparaffinized with Histosafe (Yvsolab SA, Beerse, Belgium) and rehydrated in alcohol series. After blocking endogenous peroxidase activity with 0.3 % H_2O_2 diluted in methanol (for c-kit, ZP3 and Cx43) or 3 % H_2O_2 diluted in methanol (for KL and aromatase), a demasking step was performed for 75 min at 98 °C with citrate buffer and Triton X100 (for KL, ZP3 and Cx43) and 20 min at 96 °C with Tris-EDTA buffer and 0.1 % Tween 20 (for c-kit and aromatase). All antibodies were raised against human proteins. Antibody dilutions and incubation conditions are summarized in Table 1. An extra step to block endogenous biotin was required for aromatase. The slides were then counterstained with hemalum and mounted with DPX neutral mounting medium (Prosan, Merelbeke, Belgium). Negative controls consisted of the dilution solution without any primary antibody. For positive controls, we used human ovarian follicles (for KL), testicular germ cell tumor tissue (for c-kit), heart (for Cx43), ovarian follicles (for ZP3), and placenta (for aromatase).

Follicles were considered positive for KL, Cx43, and aromatase when at least one granulosa cell was immunostained. Oocytes were considered positive for ZP3 when the whole membrane was clearly immunolabeled, and for c-kit when its oolemma was immunostained.

Results

Follicle distribution in the ovary

While primordial and primary follicles are homogeneously distributed along the periphery of the ovary, a few secondary and antral follicles are present in the inner parts of the ovary, between the layers of small follicles and the medulla (Fig. 3). A total of 2221 follicles were counted and classified into the different stages of folliculogenesis, identifying the proportion of each stage found in the baboon ovary. Primordial follicles were the most numerous (49 %), primary and secondary follicles occupied similar proportions (26 and 23 %, respectively), and there was a small percentage of antral follicles (1 % small antral and 1 % large antral). Follicle distribution per animal is shown in Table 2.

Morphometric characterization

The hematoxylin–eosin slides used to define follicle distribution were the same as those used to measure follicle and oocyte diameters. A total of 1792 follicles were utilized for these measurements. Because of the small number of small and large antral follicles detected in the sections, all of them were taken into account. For the other follicle categories, we measured at least 100 follicles/category/animal (primordial, 931; primary, 447; secondary, 400). Figure 4 shows follicle and oocyte diameters at all follicle stages, and Table 2 the measurements per animal.

It is interesting to observe that while follicle diameter was found to increase almost 20-fold from the primordial to the large antral stage, oocyte diameter increased only 3-fold. The number of granulosa cells, counted in 357 follicles, increased mainly in the last stages of follicle development (Fig. 4).

The theca cell layer starts to appear at the secondary stage in baboon follicles. As soon as two layers of granulosa cells form around the oocyte, a concentric layer of flattened theca cells appears around the follicular basement membrane (Fig. 5). In small antral follicles, theca interna cells are hypertrophied and some vascularization is already present. In large antral follicles, it is possible to observe vessels and distinguish two different populations of cells, the theca interna, with larger cells and vessels, and the theca externa, which is loosely organized between the theca interna and the stromal cells (Fig. 5). Theca cell layer thickness, which was measured when it was most visible (in late-term secondary and antral follicles), was found to double from the secondary to the small antral stage, and then from the small to the large antral stage (Fig. 4).

Immunohistochemical analyses

Table 3 summarizes the proportion of follicles positive for each immunostaining.

Table 1 Summary of immunohistochemical protocols applied to assess the presence of KL, c-kit, ZP3, aromatase, and Cx43 in the different follicle compartments

Antigen	Antibody type	Dilution	Incubation
KL (sc-13126; Santa Cruz Biotechnology, Santa Cruz, USA)	Monoclonal	1:50 in 2 % NGS + 0.2 % BSA	4 °C O/N
C-kit (A4502; Dako, Glostrup, Denmark)	Polyclonal (rabbit)	1:200 in 1 % NGS + 0.1 % BSA	4 °C O/N
Cx43 (sc-59949; Santa Cruz Biotechnology, Santa Cruz, USA)	Monoclonal	1:300 in 1 % NGS + 0.1 % BSA	4 °C O/N
ZP3 (ARP35855_T100, Aviva Systems Biology, San Diego, USA)	Polyclonal (rabbit)	1:50 in 1 % NGS + 0.1 % BSA	4 °C O/N
Aromatase (3599-100; Biovision/Gentaur, Brussels, Belgium)	Polyclonal (rabbit)	1:50 in 2.5 % BSA + 1 % milk	4 °C O/N

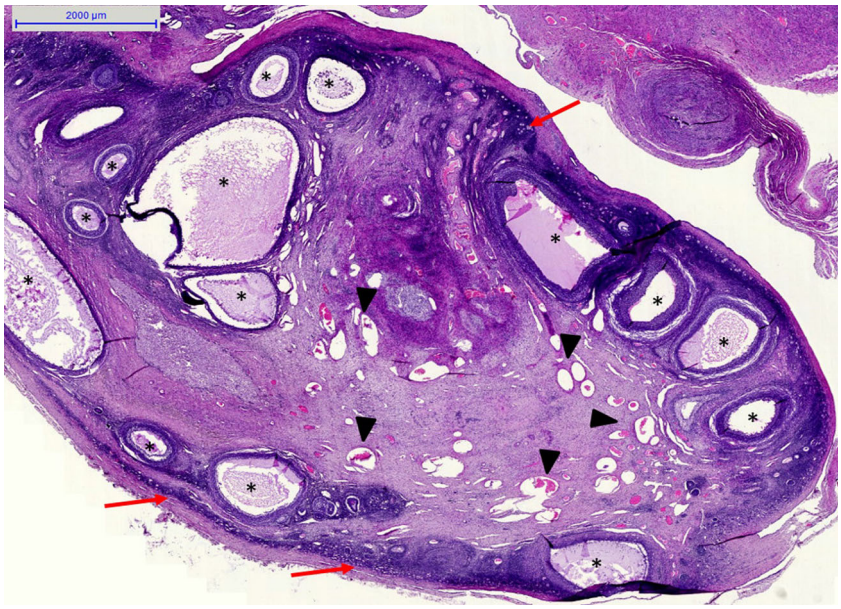

Fig. 3 Largest cross section of the baboon ovary, showing follicle distribution (hematoxylin–eosin staining). A thin layer of primordial and primary follicles (*red arrow*) can be seen in the cortex, while antral follicles (*asterisk*) are found in the deeper layers of the ovary. The medulla is characterized by a significant number of large vessels (*black arrow head*)

KL expression was observed in granulosa cells at all follicle stages in all four groups (Online Resource 1) and also in occasional stromal cells. C-kit immunolabeling ranged in intensity from faint to strong, staining the oolemma and ooplasm of oocytes from all follicle stages (Online Resource 2). It was also expressed in the cytoplasm of granulosa cells from primordial, primary, and small secondary follicles, but not in granulosa cells from large secondary or antral follicles. While KL stained between 42 and 67 % of the population of small follicles (primordial and primary, respectively) and its expression increased with follicle development, c-kit stained the vast majority of preantral follicles and all antral follicles.

Cx43 was present from primary follicles onwards. Although it was found in only a few primary follicles, it showed strong staining between the granulosa cells of secondary and antral follicles (Online Resource 3) and was also observed at the border between the granulosa cells and the oocyte. In antral follicles too, some theca cells were Cx43 positive.

ZP3 immunolabeling intensity varied from faint to strong, but all primordial and primary follicles were negative,

Table 2 Follicle and oocyte diameters (mean ± SD) and proportion of each follicle class (in percent) per baboon

Follicle class	Measurements	Animals			
		Baboon 1	Baboon 2	Baboon 3	Baboon 4
Primordial follicle	Follicle diameter	44.1 ± 6.1	49.2 ± 6.4	49.3 ± 6.4	45.8 ± 6.3
	Oocyte diameter	33.3 ± 4.6	35.9 ± 5.4	36.8 ± 6.0	37.0 ± 7.3
	Proportion (%)	50	48	45	53
Primary follicle	Follicle diameter	51.5 ± 8.8	56.1 ± 10.4	61.0 ± 10.2	62.0 ± 14.6
	Oocyte diameter	37.1 ± 6.1	41.6 ± 8.7	39.7 ± 6.8	42.0 ± 11.4
	Proportion (%)	20	28	30	26
Secondary follicle	Follicle diameter	108.8 ± 38.6	106.5 ± 43.8	115.9 ± 44.6	113.0 ± 47.5
	Oocyte diameter	55.2 ± 16.3	59.0 ± 21.4	59.6 ± 20.1	57.4 ± 19.1
	Proportion (%)	26	22	24	20
Small antral follicle	Follicle diameter	235.5 80.1	379.7 ± 221.4	345.3 ± 129.0	383.3 ± 190.3
	Oocyte diameter	104.3 ± 22.6	105.3 ± 22.4	111.9 ± 19.8	105.4 ± 11.5
	Proportion (%)	3	1	0	0
Large antral follicle	Follicle diameter	719.6 ± 198.6	937.8 ± 204.1	1064.5 ± 338.4	1011.1 ± 585.2
	Oocyte diameter	106.4 ± 27.3	107.5 ± 22.7	127.0 ± 14.9	104.2 ± 20.4
	Proportion (%)	1	1	1	1

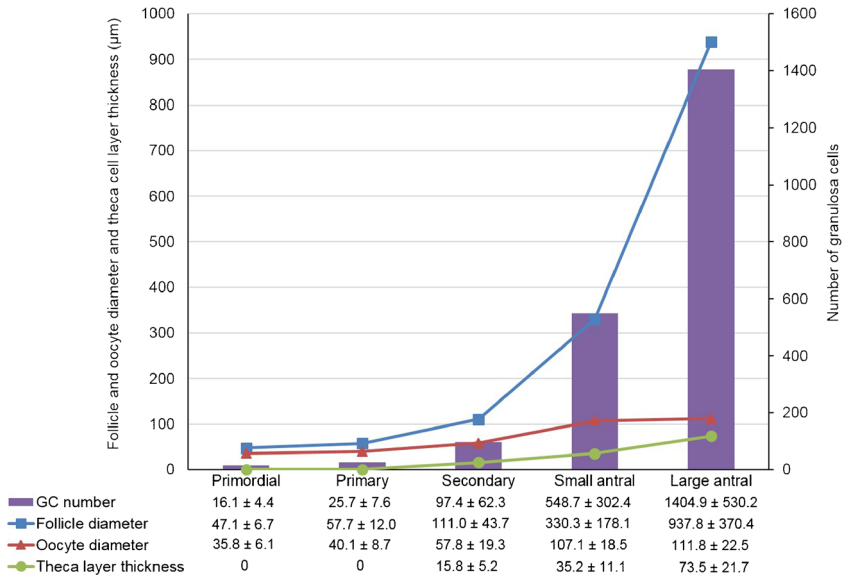

Fig. 4 Follicle and oocyte diameters, theca cell thickness, and number of granulosa cells in the different follicle categories

showing that the zona pellucida does not develop until the later stages of preantral follicles (Online Resource 4).

Aromatase expression was only encountered in granulosa cells (cytoplasm) from antral follicles (Online Resource 5), and large antral follicles exhibited stronger aromatase staining than small antral follicles. No immunolabeling was observed in preantral follicles.

Discussion

It is important to stress that the ovarian tissue biopsies used in this study were obtained from young, healthy, and regularly cycling baboons. The presence of follicles at different stages of development, as well as corpora lutea, testifies to the reproductive health of the animals.

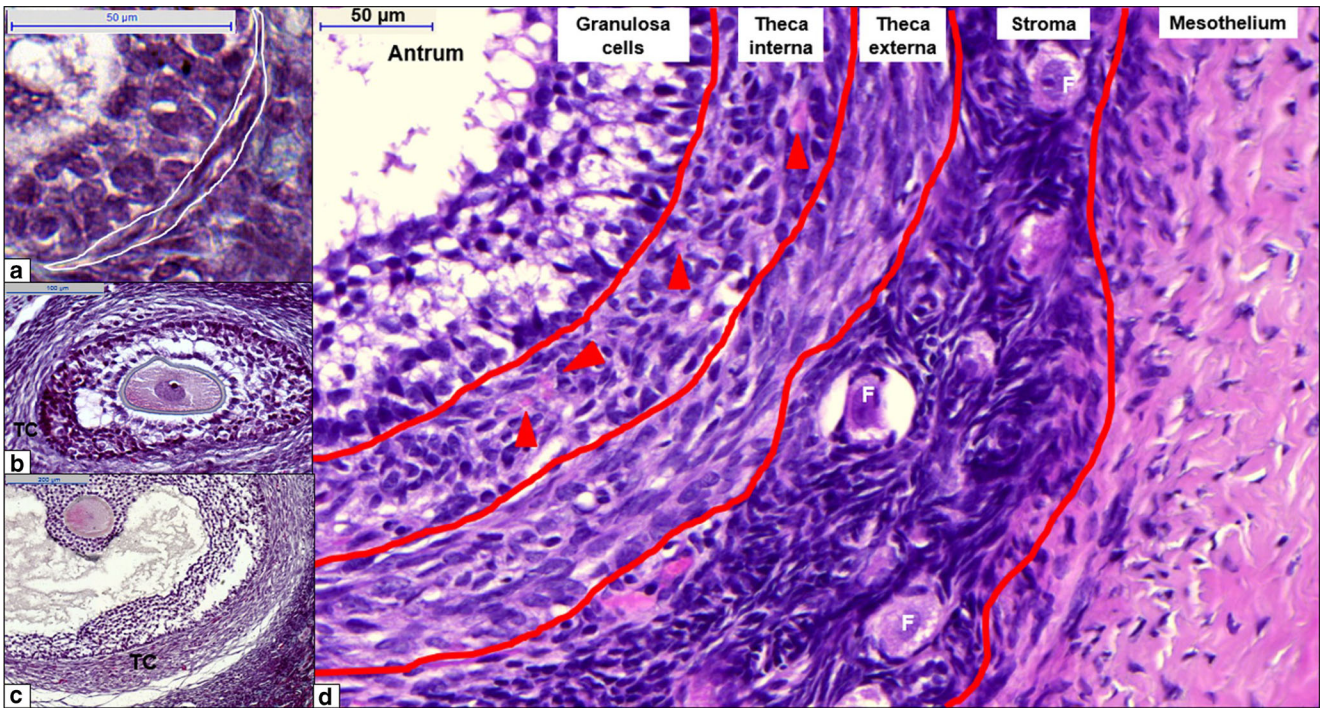

Fig. 5 Theca cell layer in baboon follicles (hematoxylin–eosin staining). A concentric layer of flattened theca cells (*circled in white*) surrounds the follicular basement membrane of a small secondary follicle (**a**). In small (**b**) and large (**c**) antral follicles, the theca layer (*TC*) is thicker and vascularized. In large antral follicles, it is possible to distinguish two different populations of theca cells (**d**): the theca interna, a richly vascularized (*red arrow heads*) layer with endocrine-like cells that border the basement membrane of the follicle, and the poorly defined theca externa, between the theca interna and the stromal tissue (*F*: follicle), with some cells that resemble fibroblasts and others with a wavy appearance, similar to contracted smooth muscle cells

Table 3 Proportion of positive-stained follicles with each immunostaining protocol

Immunostaining	Follicle classes				
	Primordial	Primary	Secondary	Small antral	Large antral
KL	42.2 % (103/244)	66.9 % (121/181)	94.9 % (37/39)	100 % (3/3)	100 % (3/3)
C-kit	86.5 % (83/96)	76.0 % (92/121)	72.4 % (21/29)	100 % (5/5)	100 % (3/3)
Cx43	0 % (0/89)	26.6 % (41/154)	61.3 % (65/106)	100 % (7/7)	87.5 % (28/35)
ZP3	0 % (0/22)	0 % (0/29)	64.5 % (20/31)	100 % (7/7)	100 % (8/8)
Aromatase	0 % (0/32)	0 % (0/28)	0 % (0/15)	100 % (11/11)	80 % (28/35)

To our knowledge, this is the first study to analyze the morphometric and immunohistochemical aspects of baboon ovarian follicles. There are some existing studies on baboon ovaries investigating other immunohistochemical markers, such as anti-Müllerian hormone (AMH) and growth differentiation factor 9 (GDF-9), to evaluate follicle function after vitrification, warming, and long-term grafting [7]. Morphological studies have also been conducted on monkey follicles [17, 18], as have studies of various factors (GDF-9 [19, 20], AMH [20–22], KL and c-kit [20]) in primate ovaries. However, none of them specifically addresses folliculogenesis in baboons. Since among non-human primates, baboons are one of the species most akin to humans in terms of reproductive processes, our results provide important data on folliculogenesis and suggest that these animals indeed share many similarities in their reproductive biology with humans.

Localization of the different follicle categories in baboon ovaries was similar to that described in human ovaries. The striking difference was in the number and distribution of primordial follicles, which were more numerous and more uniformly distributed than the smaller heterogeneous population of primordial follicles found in ovaries from adult women [23]. Moreover, unlike in human ovaries, where primordial follicles are located no more than 0.8 mm from the mesothelium [24], these follicles can be encountered as far as 1.5 mm from the mesothelium in baboons (Fig. 6).

In general, the morphology of baboon follicles is similar to that of other primates [14–16, 18, 20]. Regarding follicle and oocyte diameters, our findings show that baboon ovarian follicles are comparable to human follicles [14–16, 25], but there is a difference regarding follicle classification between studies. What we classify as early and late antral follicles, Griffin et al. [15] termed incipient and early antral follicles, while Gougeon [14] defines them as class 2 and 3, respectively. Despite the difference in classification, both these follicle categories have a similar morphology and size. It is also important to point out that the growth rate of baboon follicles is progressive, not linear, and this also resembles human follicle development [15]. Moreover, theca layer thickness in large antral baboon follicles is similar to human follicles [16].

Primordial follicles contain an oocyte surrounded by a small number of flat granulosa cells. Changes in granulosa cell shape from flat to cuboidal signal activation of the follicles. At the primary stage, baboon granulosa cells double in number. However, up to this point, the size of follicles is barely larger than that of oocytes, bordered by granulosa cells in one thin layer. In humans, the number of granulosa cells in the largest cross section ranges from 8 to 13 in primordial follicles and from 27 to 76 in primary follicles [25, 26]. Our findings in baboons are similar to results obtained by Gougeon and Chainy [26] and Westergaard et al. [25], showing comparable populations of primordial and primary follicles, respectively. Once they reach the secondary stage, granulosa cells proliferate appreciably, forming more than one layer around the oocyte. However, the period of fast growth starts at the end of the pre-antral phase. Antral follicles increase considerably in size due to exponential multiplication of the granulosa cells and marked development of the oocyte. A noticeable increase in oocyte diameter is also observed in humans at this stage [15], as well as in sheep [27] and cows [28], two other mammalian species commonly used as models for studies on human reproduction.

Similarly to humans [29], the theca cell layer in baboons starts to appear in small secondary follicles. They are essential for further follicle development, as they offer structural support and produce ovarian androgens required for estradiol production by granulosa cells [29]. Although of comparable aspect, human theca interna cells look rounder and larger than baboon cells. Moreover, measurement of the theca cell layer in antral follicles revealed a further difference [16], this layer being thinner in large antral follicles from baboons, even if their diameter was almost three times greater than in humans. It would be interesting to perform further morphological and ultrastructural analyses to understand if such a difference may be linked to the function or simply the number of these cells.

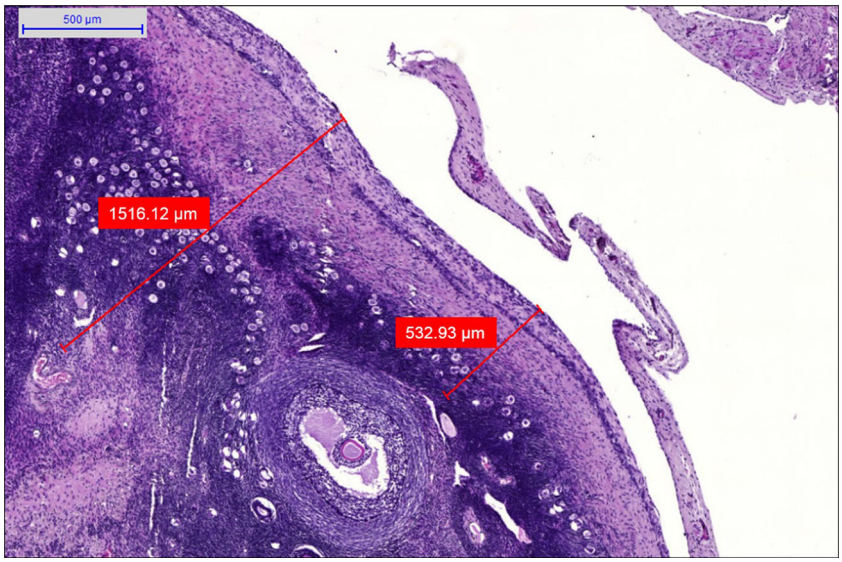

Fig. 6 Ovarian cortical biopsy showing the distance between the mesothelium and primordial follicles, which can reach 1.5 mm (hematoxylin–eosin staining)

In our study, KL expression was observed in follicles at all stages of development, as previously described by our team for human follicles [30]. However, while KL expression was limited to granulosa cells in baboon follicles, it was also found in oocytes in humans [30, 31]. Indeed, both KL and c-kit expression in baboon follicles were similar to findings in humans [30, 32]. Furthermore, as in our previous study with human follicles [30], KL expression increased according to follicle development. Because of such similarities, it is very likely that in baboon follicles, the KL/c-kit system is also implicated in primordial follicle activation, follicular development, formation of the antrum, theca cell differentiation and steroidogenesis, as well as oocyte cytoplasmic maturation [33].

Of the different connexins present in ovarian follicles [34], we decided to study Cx43 as it is known to make a significant contribution to intercellular coupling in follicles [35–37]. Baboon primary follicles did not show any Cx43-positive granulosa cells. Although there are no data on Cx43 expression in human preantral follicles, in cows, Cx43 was not observed in primordial follicles [38], but only from the primary stage onwards. Gap junction proteins in general have been poorly studied in human follicles. Xu et al. [39] and Wang et al. [36] reported Cx43 expression by granulosa cells in small and large antral follicles, respectively. Expression patterns of Cx43 in the different follicle categories in baboons suggest that this connexin plays an important role in oocyte health, either by transferring nutrients in the early stages of follicular development, or by maintenance of meiotic arrest in large antral follicles [38].

The zona pellucida of human oocytes is composed of four glycoproteins (ZP1, ZP2, ZP3, and ZP4) and has an essential role in sperm binding, prevention of polyspermy, and embryo protection before implantation [40]. Among these glycoproteins, ZP3 plays a key role in oocyte–sperm recognition, as it is the ligand for primary sperm binding and induces the acrosome reaction [41]. Unlike in humans, where ZP3 is detected in all follicle categories [42], only oocytes from secondary and antral follicles were found to be positive for ZP3 immunostaining in the present study. Moreover, while ZP3 expression in baboon follicles was limited to the oocyte, in human follicles, granulosa cells also appear to be able to express ZP3. Such a disparity could be due to a real difference between species, but may not have any actual impact on the role of ZP3 in these two species, as ZP3 is required only after ovulation. However, it could also be due to the use of a polyclonal antibody against human ZP. Similarly to our study, Grootenhuis et al. [43] used monoclonal antibodies against recombinant human ZP3 in marmosets and reported that small dots of immunoreactive ZP were found in only 60 % of primordial follicles, while granulosa cells did not stained at all. More recently, Bogner et al. [44, 45] showed by immunohistochemistry and RT-PCR that ZP3 is expressed in primordial follicles (oocytes and granulosa cells).

Aromatase is an essential enzyme in follicular selection that occurs in the final stages of follicle development. In the mid-to-late follicular phase of the menstrual cycle, because of FSH stimulation, antral follicles acquire aromatase activity that is responsible for biosynthesis of estradiol from androgens. Such an increase in estradiol concentrations leads to suppression of FSH secretion, which in turn has a negative impact on the survival of less mature follicles [44–46]. In humans, Bøtkjær et al. [47] observed that while small antral follicles (5–6 mm) showed weak or absent aromatase expression in theca and granulosa cells, their larger counterparts (8–9 mm) and preovulatory follicles exhibited strong aromatase staining in granulosa cells. The follicle sizes studied by Bøtkjær et al. [47] correspond to initial cyclic recruitment of early antral follicles and selection of dominant follicles [48], confirming the increase in aromatase activity in final follicle selection. As

in humans, antral follicles from rhesus monkeys showed ever greater aromatase expression, being the dominant follicles (>2 mm), namely those with the highest levels of aromatase [49]. Interestingly, aromatase was not expressed in all large antral follicles. Such an intriguing finding could be due to when in the menstrual cycle the biopsies were collected. Aromatase expression in healthy large antral follicles varies according to the period of cycle phase [50]. Since we did not record the phase of the cycle at the moment of biopsy retrieval and it is very unlikely that the females were all in the same phase, aromatase expression in large antral follicles in our study may not be representative of the follicular class.

In conclusion, this study provides new insights into the morphology, growth, and function of baboon follicles, showing them to be similar to human follicles. Indeed, aspects such as follicle distribution in the ovary, proportions of different follicle classes and their growth based on their morphometry, and expression of several proteins necessary for follicle survival and development are all comparable. These data corroborate the idea that this non-human primate species is a very good model not only for study of human ovarian function but also for research into early follicle recruitment and development.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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