

ORIGINAL ARTICLE OPEN ACCESS

Cytologic Characteristics of the Feline Ovary at Different Sexual Stages

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Received: 15 July 2025 | **Revised:** 17 February 2026 | **Accepted:** 25 May 2026

Keywords: corpus luteum | cytology | histology | ovarian follicles | ovary

ABSTRACT

Background: Cytology is a valuable diagnostic tool in veterinary medicine, allowing rapid and minimally invasive diagnosis. However, its application in the reproductive tracts of small animals is limited, primarily used to assess mammary masses and stage the estrous cycle.

Objective: This study evaluated the cytologic characteristics of healthy queen ovaries throughout the estrous cycle.

Animals: Twenty-four domestic shorthair queens were included and submitted to a conventional ovariohysterectomy.

Methods: A blood sample was obtained to assess the serum concentration of estradiol and progesterone. One ovary was immersed in paraformaldehyde for histology purposes, whereas the other was used for cytology. Blood parameters and histology were used to classify the queens into 4 different groups: anestrus, follicular, luteal phase, and luteal cell phase groups.

Results: When comparing the groups, statistically significant ($p < 0.05$) differences were observed only in the percentage of luteal and mesenchymal cells and mitotic figures. Luteal cells were only present in those ovaries belonging to the luteal phase group. Mesenchymal cells were present in the follicular and the luteal phase groups. Mitotic figures were observed in the anestrus and follicular phases.

Conclusions: It can be concluded that the ovarian cytology in the queen varies according to the reproductive cycle.

1 | Introduction

The ovary is an “always-changing” structure. Both anatomy and endocrine functions are modified under the influence of follicle-stimulating (FSH) and luteinizing (LH) hormones. Ovaries carry two main tasks: the production, differentiation, and release of female gametes, and the production of hormones involved in the reproductive functions of the female, such as sexual cyclicity, pregnancy, and parturition [1].

Anatomically, the ovary is divided into two main structures, namely (1) the ovarian medulla, which houses the vasculature, the innervation, and lymphatic vessels; and (2) the ovarian cortex, which houses the population of oocytes, corpora lutea (CL), and corpora albicantia [1]. Oocytes are surrounded by follicular

cells, thus composing the ovarian follicles. The size and shape of the ovarian follicles will vary depending on the follicular development and maturity stage. According to this, the types of follicles observed in the ovary are primordial, primary, secondary, and tertiary or antral. Primordial follicles comprise the oocyte and a single layer of surrounding flattened cells. Primary follicles present a single layer of cuboidal cells surrounding the oocyte. Secondary follicles present two or more layers but without an antrum. Finally, tertiary follicles present several layers of surrounding cells and a fluid-filled antrum [1].

Once ovulation occurs, the follicle collapses, and theca interna and granulosa cells undergo luteinization [2, 3]. The CL is composed of luteal cells, leukocytes, fibroblasts, endothelial cells, and pericytes [4]. Its morphology has been previously described

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in detail in several species such as the Canadian porcupine, sheep, pigs, cattle, and women [5–10]. The queen is not an exception, and ovarian morphology has been recently described and updated using imaging diagnosis techniques [11] and histology [12, 13].

Cytology is a recognized technique in human medicine that is specially used to obtain preoperative cytodiagnosis of ovarian masses [14]. In veterinary species, cytology is commonly used to evaluate various tissues. In large animals, like mares and cows, the cytology of the endometrium is frequently used to assess reproductive health. However, in small animals like dogs and cats, the use of cytology in the reproductive tract is much more limited. In these species, the inaccessibility of the reproductive tract makes it challenging for the clinician to obtain reliable diagnostic information. In dogs and cats, vaginal cytology is a highly recognized tool to stage the estrous cycle. Likewise, uterine cytology and biopsy samples can be obtained, but these procedures are more invasive in small animals than in other species and require higher expertise [15]. The ovaries are also very difficult to reach, and little is known about ovarian cytology. In dogs, some authors have described the specific cytologic features of the normal ovary [16], with cytology being considered a relevant tool for detecting ovarian neoplasia [17, 18]. However, according to the authors' knowledge, cytologic findings of the queen ovary obtained by fine-needle aspiration remain unknown.

Thus, the present study aimed to establish a baseline of the cytologic features of the feline ovary through the lens that the reproductive stage might influence its cytologic features, and to compare the findings with those from histologic examination of the ovaries and serum concentrations of steroid sexual hormones.

2 | Materials and Methods

2.1 | Animals

The present study included 24 domestic short-hair queens from a “trap, neuter, and return” program carried out by the Surgery Unit at the Veterinary Hospital of the Universitat Autònoma de Barcelona (UAB). All the included females were stray queens, thus impeding the obtaining of information about previous reproductive history, reproductive status, and age. Samples were obtained in two periods: the first from November to December, and the second from February to March.

Queens were included in the study if (1) the physical examination was considered within normal limits and (2) they tested negative against feline leukemia virus and feline immunodeficiency virus (Snap FIV/FeLV; IDEXX Laboratories, Westbrook, ME, USA).

Before the surgery, queens were sedated using an intramuscular combination of 5 mg/kg Ketamine (Ketamidol, Richter Pharma, Austria) with 20 µg/kg buprenorphine (Buprecare, Divisa FarmaVic, S.A., Spain) and 0.2 mg/kg midazolam (Midazolam, Normon S.A., Spain). After sedation, a blood sample from the jugular vein was collected and placed into a glass tube. The

blood was allowed to clot and then centrifuged at 2200 × g for 10 min at room temperature. The serum was immediately frozen and kept at –20°C until analyses were performed.

Anesthesia was then induced with 2–4 mg/kg propofol (Propovet TM, Zoetis Ecuphar, Spain). The anesthetic status was maintained using 1.5%–2% isoflurane (IsoFlo, Zoetis Ecuphar, Spain) in oxygen using a Mapleson F anesthetic breathing circuit, and a conventional ovariohysterectomy through ventral midline laparotomy was performed.

The study was approved by the UAB's Ethical Committee of Animal Care and Research (CEEAH, code 2939).

2.2 | Blood Samples

Blood samples were used to determine the serum concentration of estradiol and progesterone. Estradiol concentration was determined using the Estradiol-sensitive ELISA commercial kit (Demeditec Diagnostics GmbH, Kiel, Germany) [19], according to the manufacturer's instructions. The detection limit ranged from 1.40 to 200 pg/mL. Intra-assay coefficients of variation were 7.87%, 5.68%, and 5.52% for 8.21 pg/mL, 18.50 pg/mL, and 27.62 pg/mL, respectively. Inter-assay coefficients of variation were 8.78%, 7.25%, and 6.78% for 7.87 pg/mL, 17.87 pg/mL, and 26.71 pg/mL of estradiol, respectively. Those queens showing estradiol concentration above 20 pg/mL were considered to be undergoing the follicular phase [20]. This ELISA has been limitedly implemented for the measurement of feline estradiol in the literature but formal validation for cats has not been published.

The serum concentration of progesterone was determined using the Immulite 1000 equipment (Immulite; Siemens Healthcare Diagnostics, Cornellà del Llobregat, Spain). The detection limit of the equipment ranged from 0.2 to 40 ng/mL. Intra and inter-assay coefficients of variation of the instrument were 5.5 and 6.5, respectively. Queens with serum concentrations of progesterone below 1.5 ng/mL were considered non-ovulated, whereas those showing serum concentrations above 1.5 ng/mL were considered ovulated [21–24].

2.3 | Cytology

Immediately after the surgical removal of the reproductive tract, fine-needle aspiration (FNA) of one of the ovaries was performed using a 25-gauge needle and a 5-mL syringe. The needle was introduced inside the ovary, opposite to the ovarian hilum, and was randomly redirected multiple times to obtain ovary specimens. After the cytology sampling, the material obtained was squashed on a glass slide and allowed to air-dry, thus obtaining two smears from each queen ($n=48$ smears). Since the FNA technique may modify the physiologic architecture of the ovaries, the histologic assessment was performed with the contralateral ovary.

Dried samples were stained with a modified Giemsa stain (Auto-Hemacolor, Merck KGaA, Darmstadt, Germany) using an automatic dye (Hematek, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA). Smears were examined under

a light microscope at 100× to 1000× magnification (Nikon Eclipse Ci, Japan) by a board-certified pathologist and a resident of clinical pathology separately. Both pathologists performed a blind assessment of the samples. A consensual result was obtained after the potential discrepancies were revised. Smears were evaluated at low magnification (low power field (LPF)=100×) to assess general features related to the quality of the smear. This assessment included background, cellularity and cell preservation, the type of cells encountered, and blood contamination. After that, cytologic specimens were evaluated at higher magnification (high power field (HPF)=600×) to determine the cell arrangement, the characteristics of the specific cell types and the presence of mitotic figures. A higher magnification (1000×) was applied when a more defined detail of the nuclear and/or cytoplasmic characteristics was necessary. Finally, the number and distribution of leukocytes were also evaluated at high magnification (400×) (adapted from Piseddu et al. [16]). The main evaluated parameters are summarized in Table 1.

2.3.1 | Background

The background evaluation included the presence of debris, the presence of fat droplets, extracellular matrix or pink protein material, and the presence of artifacts (Table 1). The presence of debris was graded on a scale of 0–3, being graded as 0 for those smears with a clear background without debris, 1 for those preparations with some debris present, 2 for preparations with abundant debris but still adequate for diagnosis, and 3 for those with abundant debris and not adequate for diagnosis.

Fat droplets were graded on a scale of 0–4. A 0-score was assigned if no fat droplets were present, a 1-score was assigned for the preparations with scattered fat droplets, 2 for those with few droplets, 3 if a moderate number of fat droplets were present, and 4 if they were abundant.

The presence of extracellular matrix was also evaluated and graded on a scale of 0 to 3. A score of 0 was assigned when no material was found, 1 was given to preparations with scattered matrix, 2 to those with a moderate quantity of matrix, and 3 to those preparations showing abundant matrix.

Finally, the presence of artifacts, such as stain material, was evaluated using a score from 0 to 2. Smears graded with a 0 had no artifact present. Smears graded with 1 had some artifact but were still acceptable for diagnosis. Finally, smears with a grade of 2 had excessive artifacts and were considered unsuitable for diagnosis.

2.3.2 | Cellularity and Cell Preservation

Cellularity and cell preservation were evaluated according to the number of cells, cell distribution and overlapping, and the preservation of the cells (Table 1). Cellularity was scored on a scale of 0–3. A score of 0 was given when no cells were found. A score of 1 was given if scattered cells were present (1–50 cells/LPF). A score of 2 was given if a moderate number of cells were

found (50–100 cells/LPF), and finally 3 was given if the preparation had abundant cellularity (> 100 cells/LPF).

Cell distribution and overlapping were also evaluated and graded on a scale of 0–3. A score of 0 was given to the preparations that were uniformly distributed, with none or only minimal overlapping. A score of 1 was assigned to samples where cells had minimal to moderate overlap or were found in restricted areas. A score of 2 was assigned to samples exhibiting clumped areas and moderate to abundant cell overlap. Finally, a score of 3 was assigned to those preparations with highly overlapped cells or cells distributed only in clumps or for preparations with few areas of cells, making them unsuitable for diagnosis.

Cell preservation was also graded on a scale of 0–3. A score of 0 was assigned to preparations with perfect cell preservation, containing 0% of broken cells. Score 1 was considered good preservation and was assigned to those preparations with less than 25% of broken cells. Score 2 was assigned to those smears that showed moderate rupture and contained 25%–50% of broken cells. Finally, score 3 was given to those preparations with more than 50% of the cells broken and unsuitable for diagnosis.

2.3.3 | Cell Arrangement

Cell arrangement was evaluated for the number of individual cells and clusters present, and the specific type of arrangement (acinar, palisade, and papillary) (Table 1). Individual cells and clusters were graded on a scale of 0–4. A score of 0 was given when no individual cells or clumps were found, 1 for preparations with less than 10 individual cells or clusters formed by less than 10 cells/LPF, 2 for preparations with 10–40 individual cells or clusters formed by 10–40 cells/LPF, 3 for preparations with 41–80 individual cells or clusters formed by 41–80 cells/LPF, and 4 for those with > 80 cells/LPF.

The specific type of arrangement was evaluated and graded separately from 0 to 3 for each type. A score of 0 was determined when the particular type of cell arrangement was absent, 1 when there were scattered cells of the specific type (< 1/600×), 2 when few cells of the particular type of arrangement were present (1–3/600×), and 3 for the moderate presence of the specific type of arrangement (> 3/600×).

2.3.4 | Specific Cell Type

Cell type assessed the presence of recognizable, non-leukocyte, specific cells, namely luteal, granulosa, and mesenchymal cells. The presence of mitotic figures was also evaluated. Furthermore, large multinucleated structures were occasionally present in some of the samples, and their presence was also graded (Table 1).

The presence of the different specific cell types was evaluated separately and graded on a scale of 0–4. A score of 0 was assigned when the smear did not contain any specific identifiable cell type. A score of 1 was assigned when 1–10 cells/LPF of a

TABLE 1 | Cytologic features evaluated in the ovarian smears.

Cytological feature	Score				
	0	1	2	3	4
Background					
Debris in the background	Clear background	Debris present (AD)	Abundant debris	NAD	—
Fat droplets	Absent	Scattered	Few	Moderate	Abundant
Extracellular matrix	Absent	Scattered	Moderate	Abundant	—
Artifacts	Absent	Scattered (AD)	Excessive (NAD)	—	—
Cellularity/cell preservation					
Cellularity (cells/LPF)	Absent (0)	Scattered (1–50)	Moderate (51–100)	Abundant (> 100)	—
Cell distribution/overlapping	Uniform	Restricted to one area	Clumps through a slide	Clumps, very overlapped or	—
	Minimal/no overlapping	Minimal/moderate overlapping	Moderate/abundant overlapping	in only a few areas of cells	
Cell preservation (% of broken cells with chromatin strands)	Good (0%)	Adequate (< 25%)	Moderate rupture (25%–50%)	Poor (> 50%)	—
Cell arrangement (number/LPF)					
Individual epithelial cells	Absent (0)	Scant (< 10)	Few (10–40)	Moderate (41–80)	Abundant (> 80)
Clusters of epithelial cells	Absent (0)	Scant (< 10)	Few (10–40)	Moderate (41–80)	Abundant (> 80)
Acinar arrangement	Absent (0)	Scattered (1)	Few (2–3)	Moderate (> 3)	—
Palisade arrangement	Absent (0)	Scattered (1)	Few (2–3)	Moderate (> 3)	—
Papillary arrangement	Absent (0)	Scattered (1)	Few (2–3)	Moderate (> 3)	—
Specific cell type					
Luteal cells (cells/LPF)	Absent (0)	Scattered (< 10)	Few (10–20)	Moderate (21–50)	Abundant (> 50)
Granulosa cells (cells/LPF)	Absent (0)	Scattered (< 10)	Few (10–20)	Moderate (21–50)	Abundant (> 50)
Multinucleated (structures/LPF)	Absent (0)	Scattered (< 10)	Few (10–20)	Moderate (21–50)	Abundant (> 50)
Mesenchymal cells (cells/LPF)	Absent (0)	Scattered (< 10)	Few (10–20)	Moderate (21–50)	Abundant (> 50)
Mitosis (number of mitosis/HPF)	Absent (0)	Scattered (1)	Moderate (2–3)	Abundant (> 3)	—

Abbreviations: AD, adequate for diagnosis; HPF, high power field, 600× magnification; LPF, low power field, 400× magnification; NAD, not adequate for diagnosis.

specific type were encountered, a score of 2 was assigned when 10–20 cells/LPF of a specific type were found, and a score of 3 was assigned when 20–50 cells/LPF of a specific type were found. Finally, a score of 4 was assigned when > 50 cells/LPF of a specific type were encountered.

Finally, the presence of mitotic figures was graded on a scale of 0 to 3. A 0 score was given when no mitotic figures were found, a 1 score was given for the preparations that contained 1–3 mitotic figures/600×, and a score of 3 was given when > 3 mitotic figures/600× were found.

2.3.5 | Presence of Erythrocytes and Leukocytes

The presence of erythrocytes and leukocytes in the background was evaluated and graded following a similar approach as previously described by Piseddu et al. [16]. The scores are summarized in Table 2.

A numerical score from 0 to 3 was used to classify smears according to the degree of blood contamination. Smears with a score of 0 showed the absence of blood contamination. Smears scored with 1 contained mild blood contamination. Smears with a 2-score showed moderate blood contamination. Finally, the smears with a score of 3 showed marked blood contamination.

The number of leukocytes was determined by evaluating 10 fields at high magnification (400×) and was scored on a scale of 0–3. A score of 0 was assigned to the smears that had no leukocytes. A score of 1 was assigned to smears presenting 1–3/400× leukocytes. Similarly, score 2 was assigned to 4–6/400× leukocytes, and score 3 was assigned to 7–10/400× leukocytes. The leukocyte score was then compared with the erythrocyte score to determine the impact of blood contamination. If the leukocyte score was the same or lower than the erythrocyte score, the leukocyte presence was suggestive to be attributed to blood contamination. If the leukocytes were admixed with the ovarian cells, their presence was suggestive to be relevant in the tissue and not attributed to blood contamination.

2.4 | Histology

To corroborate the cytologic findings, a histologic assessment was performed on the contralateral ovary. Samples were embedded in paraffin blocks, and 4 μm-thick sections were obtained and stained with hematoxylin–eosin. Stained sections were scanned and viewed using the NDP View 2 software

(Hamamatsu Photonics, Japan). The biopsy examination consisted of the preliminary evaluation of the presence or absence of ovarian follicles and luteal tissue. Luteal tissue was classified into two categories, namely the luteal phase and the luteal cell phases. The luteal phase comprised those ovaries containing structurally defined CL, whereas the luteal cell phase comprised those ovaries that did not contain structurally defined CL, but luteal cells were present.

2.5 | Queens Classification

Queens were divided into four groups, namely the anestrus, the follicular, the luteal phase (either pregnant or pseudopregnant), and the luteal cell phases, according to the hormonal concentration and the ovarian histologic morphology. The anestrus group included those queens presenting low serum progesterone and estradiol concentrations, with no apparent CL or a small number of luteal cells. The follicular phase group included queens presenting serum estradiol concentration above 20 pg/mL, serum concentration of progesterone below 1.5 ng/mL, and no apparent CL or a few amounts of luteal cells. The luteal phase group included queens presenting serum concentration of progesterone above 1.5 ng/mL, regardless of the presence of an evident pregnancy (pregnant queens) or not (pseudopregnant queens), and evident CL. Finally, the luteal cell phase included those queens presenting high serum concentrations of progesterone with no apparent CL or few amounts of luteal cells [20–23].

2.6 | Statistical Analysis

Queens' CL lifespan was histologically divided into four groups based on histological characteristics and hormonal concentration. The four groups were anestrus, follicular, luteal phase, and luteal cell phases. The chi-squared test was used with Fisher's multiple-comparison post hoc test to compare the different groups. The statistically significant difference was set at $p < 0.05$. Statistical package SPSS (version 22, IBM Corporation, United States) was used for the analysis.

3 | Results

3.1 | Queens Classification

Fifteen out of the 28 queens were sampled between November and December. The other nine females were sampled between February and March (Table 3). According to hormonal concentration and histologic appearance of the ovaries, 5 animals were classified in the anestrus group, 7 queens were included in the

TABLE 2 | Presence of erythrocytes and leukocytes.

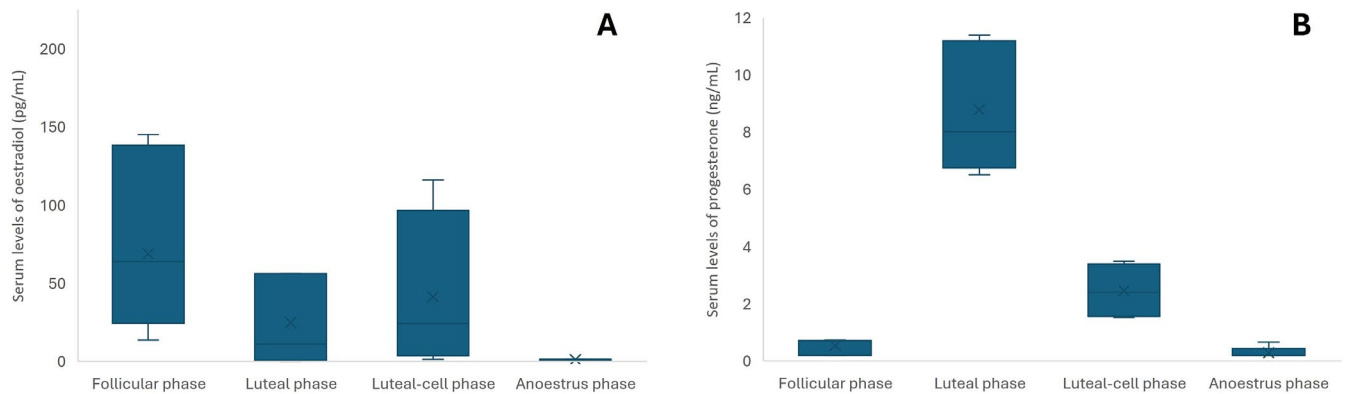
Cytological feature	Score			
	0	1	2	3
Erythrocyte content	Absent	Mild blood content	Moderate blood content	Marked blood content
Leukocyte content (cells/HPF)	Absent	1–3/400×	4–6/400×	7–10/400×

Abbreviation: HPF, high power field (400×).

TABLE 3 | Queens classification according to histology and hormonal concentrations.

Animal	Sampling period	Group	Histology F/CL	Estrogens (pg/mL)	Progesterone (ng/mL)
1	November	Follicular	+/-	70 568	0.2
2	November	Follicular	+/-	24 564	0.2
3	November	Follicular	+/-	138 393	0.708
4	November	Follicular	+/-	26 244	0.737
5	November	Follicular	+/-	13 646	0.71
6	November	Follicular	+/-	145 393	0.726
7	December	Follicular	+/-	63 832	0.511
8	March	Luteal phase	+/+	0.1	11
9	March	Luteal phase	+/+	NA	NA
10	March	Luteal phase	+/+	11 176	11.4
11	February	Luteal phase	+/+	56 008	6.52
12	February	Luteal phase	+/+	NA	NA
13	February	Luteal phase	+/+	1.4	8.02
14	March	Luteal phase	+/+	NA	NA
15	November	Luteal phase	+/+	56.18	6.99
16	December	Luteal cell	+/-	10 256	1.7
17	February	Luteal cell	+/-	116 126	3.12
18	November	Luteal cell	+/-	1.4	1.52
19	November	Luteal cell	+/-	38 149	3.49
20	March	Anoestrus	+/-	0.492	0.203
21	November	Anoestrus	+/-	1.4	0.2
22	November	Anoestrus	+/-	1.4	0.2
23	November	Anoestrus	+/-	1.4	0.2
24	November	Anoestrus	+/-	1.4	0.666

Abbreviations: CL, corpus luteum; F, follicles; NA, samples not available; +, presence; -, absence; $\pm$, luteal cells with no CL structure.

**FIGURE 1** | Serum hormonal concentration. (A) Serum concentration of estradiol. (B) serum concentration of progesterone. Follicular phase, $n = 7$. Luteal phase, $n = 8$. Luteal-cell phase, $n = 4$. Anestrus phase, $n = 5$.

follicular group, 8 queens were classified in the luteal phase, and 4 were classified as the luteal cell phase. Six out of the seven queens included in the luteal phase group were found to be pregnant.

3.2 | Blood Samples

The serum concentrations of progesterone and estradiol are shown in Figure 1A,B. Blood samples from three pregnant

queens in the luteal phase group were missing. For statistical purposes, progesterone values below the detection limit were considered 0.20 ng/mL ($n=5$), values above the detection limit were considered 40 ng/mL ($n=2$), and estradiol values below the detection limit were considered 1.4 pg/mL ($n=6$) (Table 3).

Progesterone concentration was significantly higher ($p<0.05$) in the luteal phase group (8.79 ± 2.27 ng/mL). Samples from follicular and anestrus phases showed serum concentrations of progesterone below 1 ng/mL. Finally, progesterone values in queens from the luteal cell phase ranged between 1.7 and 3.49 ng/mL, with a mean value of 2.46 ± 0.99 ng/mL.

Regarding estradiol, only queens from the anestrus group showed negligible values of this hormone. Follicular, luteal, and luteal cell phases all showed high mean values of estradiol.

3.3 | Cytology Findings

When comparing the different groups, non-statistically significant differences between groups were found for debris in the background ($p=0.417$), fat droplets ($p=0.815$), extracellular matrix ($p=0.289$), cellularity ($p=0.802$), cell distribution/overlapping ($p=0.371$), cell preservation ($p=0.752$), individual epithelial cells ($p=0.662$), clusters of epithelial cells ($p=0.060$), acinar arrangement ($p=0.153$), papillary arrangement ($p=0.830$), granulosa cells ($p=0.123$), multinucleated structures ($p=0.999$), erythrocyte content ($p=0.266$), and leukocyte content ($p=0.184$). No statistical study was performed for the parameters artifact because all samples were scored as 1, and palisade arrangement because it was absent in all samples. Statistically significant differences were observed only in

the percentages of luteal, mesenchymal cells, and mitotic figures (Table 4).

In general terms, all samples showed a light pink background with scant cellular debris and stain material that was considered artifacts. Only one of the queens, belonging to the anestrus phase group, had abundant debris, although still adequate for diagnosis. Likewise, the background of ovarian FNA contained low to moderate numbers of small, round, and clear vacuoles consistent with lipid droplets. The presence of amorphous, pink, and proteinaceous material consistent with extracellular matrix was also variable among slides, although no significant statistical differences were observed between groups (Figure 2).

Regarding cellularity, 12 out of 48 (25%) smears showed abundant cellularity, moderate cellularity was found in 26 (54.2%) smears, and 10 (20.8%) smears showed scant cellularity.

Regarding cell distribution and overlapping, of the 48 smears reviewed, 40 received a score of 1 (83.3%, no or minimal overlapping), 6 received a score of 2 (12.5%, moderate or restricted overlapping), and 2 received a score of 3 (4.16%, overlapped cells or cells distributed in clumps).

Cell preservation was variable, ranging from adequate, with less than 25% of ruptured cells in 12 (25%) preparations, to poor, with more than 50% of ruptured cells in 4 smears (8.3%). The remaining 32 samples (66.6%) showed moderate preservation of ruptured cells, with 25%–50% of cells broken.

The number of individual cells and cells distributed in clusters was generally similar, except in two queens from the luteal cell

TABLE 4 | Results of luteal and mesenchymal cells and mitotic figures at the different sexual phases evaluated in the study.

	Category 0	Category 1	Category 2	Category 3	Category 4
Luteal cells ($p=0.012$)					
Anoestrus ($n=5$) ^a	100 (5)	0	0	0	0
Follicular ($n=7$) ^a	100 (7)	0	0	0	0
Luteal phase ($n=7$) ^b	28.6 (2)	0	28.6 (2)	28.6 (2)	14.3 (1)
Luteal cell ($n=5$) ^a	100 (5)	0	0	0	0
Mesenchymal cells ($p=0.014$)					
Anoestrus ($n=5$) ^a	60 (3)	0	40 (2)	0	0
Follicular ($n=7$) ^b	14.3 (1)	85.7 (6)	0	0	0
Luteal phase ($n=7$) ^c	42.9 (3)	28.6 (2)	28.6 (2)	0	0
Luteal cell ($n=5$) ^d	0	20 (1)	60 (3)	20 (1)	0
Mitosis ($p=0.04$)					
Anoestrus ($n=5$) ^a	80 (4)	0	20 (1)	0	ND
Follicular ($n=7$) ^b	57.1 (4)	42.9 (3)	0	0	ND
Luteal phase ($n=7$) ^c	100 (7)	0	0	0	ND
Luteal cell ($n=5$) ^c	100 (5)	0	0	0	ND

Note: Values are expressed in percentages (n). Statistically significant differences in the distribution of the cell types among the different sexual phases are represented with different superscripts.

phase group and one from the anestrus group, which contained higher numbers of clusters than individual cells, and two queens from the luteal phase group, which contained higher numbers of individual cells compared with cells distributed in clusters.

Regarding the cell arrangement, acinar arrangement was absent in 14 smears (29.2%), scattered in 24 (50%), few in 4 (8.3%), and moderate numbers in 6 smears (12.5%). Palisade arrangement was absent in 32 smears (66.7%), scattered in 14 (29.2%), and few in 2 smears (4.1%). Finally, the papillary arrangement was absent in 16 smears (33.3%), scattered in 12 (25%), few in 18 (37.5%), and moderate in 2 (4.2%).

Finally, the most common cell types identified on smears were granulosa, luteal, and mesenchymal cells, and occasional leukocytes. Additionally, rare large multinucleated structures of unknown lineage were also found. Granulosa cells were distributed in different-sized aggregates, occasionally arranged in palisade or acinar-like structures. The cells were round to slightly cuboidal and small. Nuclei were round to slightly oval, centrally or paracentrally located, with finely reticular chromatin and

one or more small nucleoli. The nuclear to cytoplasmic ratio was moderate to high. The cytoplasm was generally scant, clear blue, and sometimes with low numbers of small, clear vacuoles. Anisocytosis and anisokaryosis were mild (Figure 3A,B).

Luteal cells were large, round to slightly polygonal, generally individually arranged or rarely in loose aggregates. Nuclei were round, central or paracentrally located, with finely reticular chromatin and, occasionally, with one nucleolus visible. The nuclear: cytoplasmic ratio was low. The cytoplasm was generally abundant, with distinct cell borders, finely granular, and blue to purple (Figure 4). Frequently, cells contained variable numbers of small to medium-sized clear vacuoles. Anisocytosis and anisokaryosis were mild to moderate, and scant binuclear cells were found. Luteal cells were only present in preparations from the luteal phase group, showing statistically significant differences among the groups ($p = 0.012$). Specifically, they were present in 71.4% of preparations from the luteal-phase group. (Table 4). In this sense, categories 2 and 3 were observed in 28.6% of the FNA each, whereas category 4 accounted for 14.3% of the samples.

Mesenchymal cells were fusiform, with thin wispy tails, and were generally exfoliated in loose aggregates around a variable amount of amorphous pink material compatible with the extracellular matrix. The nuclei were oval, centrally placed, and with finely reticular chromatin. The nuclear: cytoplasmic ratio was low to moderate. The cytoplasm was clear blue and presented well-defined cell borders. Anisocytosis and anisokaryosis were mild (Figure 5).

Mesenchymal cells were present in 100% of samples from the luteal cell phase, 85.7% in the follicular phase, 57.1% in the luteal phase, and 40% in the anestrus phase (Table 4). Category distribution showed that all samples from the anestrus phase were classified as category 2. In the follicular phase, all the samples were classified as category 1. Regarding the luteal phase, categories 1 and 2 accounted for 28.6% each. Finally, in the luteal cell phase, categories 1 and 3 accounted for 20% of the samples each, whereas category 2 accounted for 60% of the samples. Queens belonging to the follicular group had significantly ($p = 0.014$) lower numbers of mesenchymal cells.

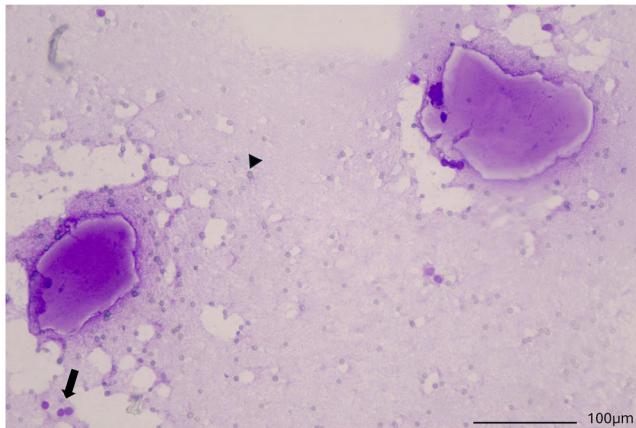


FIGURE 2 | Cytology from a normal feline ovary obtained by fine-needle aspiration. Modified Giemsa stain. Mild proteinaceous background with dense proteinaceous material (pink). Note the presence of erythrocytes (arrowhead) and granulosa cells (arrow).

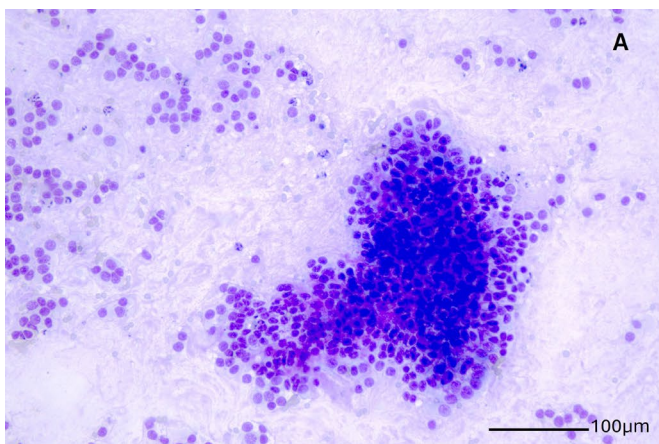
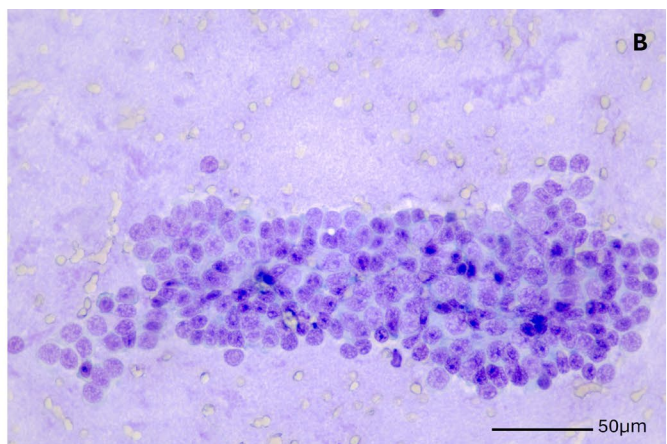


FIGURE 3 | Fine-needle aspiration of a normal ovary. Modified Giemsa stain. (A) Cluster of granulosa cells in a proteinaceous background. (B) Papillary distribution of granulosa cells showing scant, clear blue cytoplasm with mild anisocytosis and anisokaryosis.

Scant large multinuclear structures from unknown lineage were also observed in the preparations from seven animals (Figure 6). Specifically, two animals from the anestrus phase group, one from the follicular phase group, two from the luteal

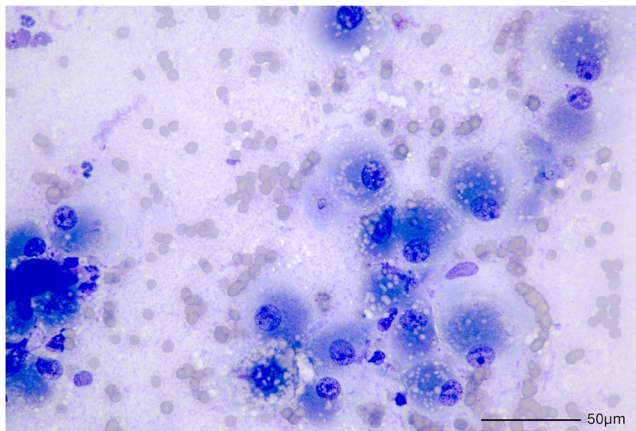


FIGURE 4 | Fine-needle aspiration of a normal ovary. Modified Giemsa stain. Luteal cells were characterized by clear blue, vacuolized cytoplasm. The nuclei were eccentrically located.

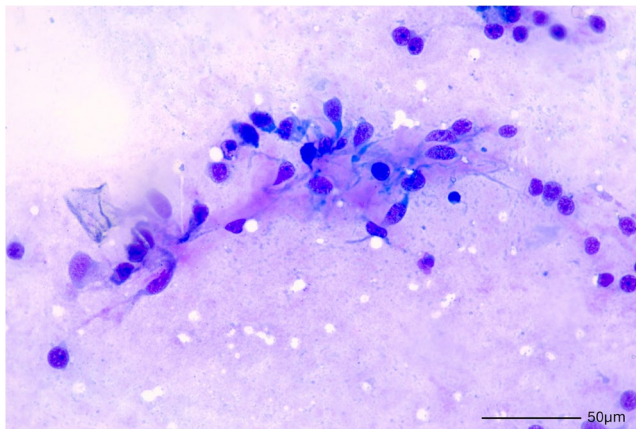


FIGURE 5 | Fine-needle aspiration of a normal ovary. Modified Giemsa stain. Occasional spindle cells with an oval nucleus and wispy cytoplasmic borders surrounding a small amount of pink matrix can be observed.

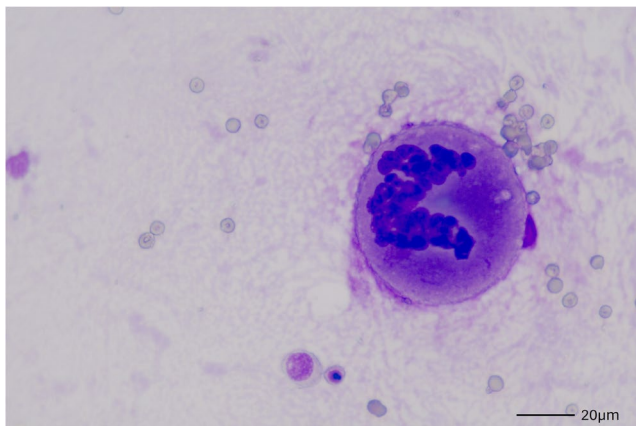


FIGURE 6 | Fine-needle aspiration of a normal ovary. Modified Giemsa stain. One single, large, and multinucleated structure of unknown origin.

phase group, and two from the luteal cell phase were identified. Cells were round to slightly polygonal and individually arranged. The nuclei were round, centrally located, and had finely reticular chromatin. The nuclear: cytoplasmic ratio was low. The cytoplasm was generally abundant, finely granular, and blue to purple. Anisocytosis and anisokaryosis were mild to moderate.

Mitotic figures also showed statistically significant differences among the groups ($p = 0.04$) (Table 4). Mitotic figures were present only in the follicular (42.9% of samples) and the anestrus (20% of samples) phases. Notably, in the anestrus group, 20% of the animals showing mitotic figures were classified in score 2. In the follicular group, 57.1% of the queens had no mitotic figures, and 42.9% were classified as score 1, thus having scattered mitotic figures in the smears.

Leukocytes were present in 42 specimens and were observed in all groups. Neutrophils were the predominant leukocyte identified. In most smears, leukocyte numbers were proportional to the degree of blood contamination, and their presence was therefore interpreted as consistent with peripheral blood contamination. However, in four smears from the luteal phase group, leukocytes were observed admixed with luteal cells and, occasionally, within the cytoplasm of luteal cells, suggesting emperipolesis and the presence of true tissue-associated leukocytes rather than contamination alone (Figure 7).

3.4 | Histology Findings

The presence of CL and ovarian follicles was evaluated on histologic sections from the contralateral ovary in every queen (Figure 8). All the queens in the luteal phase group (29.1% of the total population) had fully formed CL. Luteal cells were large, round to polyhedral, and contained a round nucleus. The cytoplasm was moderately to heavily vacuolated. The remaining queens (70.8% of the population) did not present a defined CL. Twelve queens (50%), 4 from the anestrus phase, 5 from the follicular phase, and 3 from the luteal phase, had a mixture of large and small luteal cells, accompanied by ovarian follicles. The remaining five (20.1%) queens, 1 from the anestrus phase, 2 from the follicular phase, and 1 from the luteal phase, had no relevant numbers of luteal cells.

Finally, all the ovaries evaluated in the present study contained variable numbers of ovarian follicles regardless of the stage of the sexual cycle.

4 | Discussion

In veterinary medicine, cytology has remarkably evolved in the last 15 years and is an extended diagnostic tool in general practice. Compared to histopathology, one of the main advantages of this diagnostic tool is obtaining rapid results with a less invasive and expensive technique [25]. When evaluating the reproductive system in small animals, cytology is more commonly used to assess mammary masses and to stage the estrous cycle. However, little information on the physiologic ovarian cytologic features in dogs and cats is available [18]. In those species, ovarian cysts

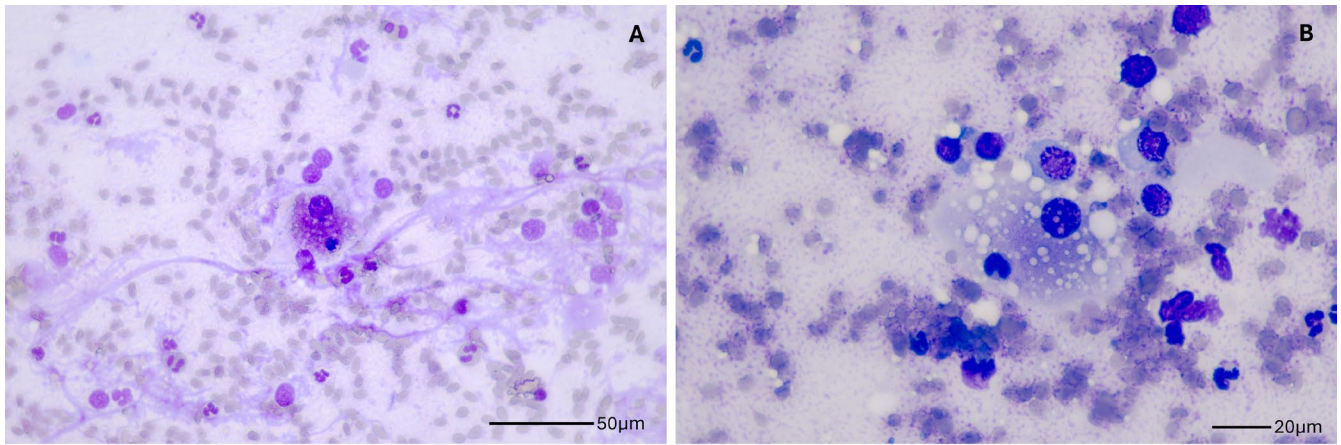


FIGURE 7 | Fine-needle aspiration of a normal ovary. Modified Giemsa stain. (A) Mixed population of granulosa and luteal cells. In the center of the image, a luteal cell with an intact leukocyte in the cytoplasm can be observed (emperipolesis). (B) Luteal and granulosa cells with naked nuclei in a background with red blood cells and leukocytes.

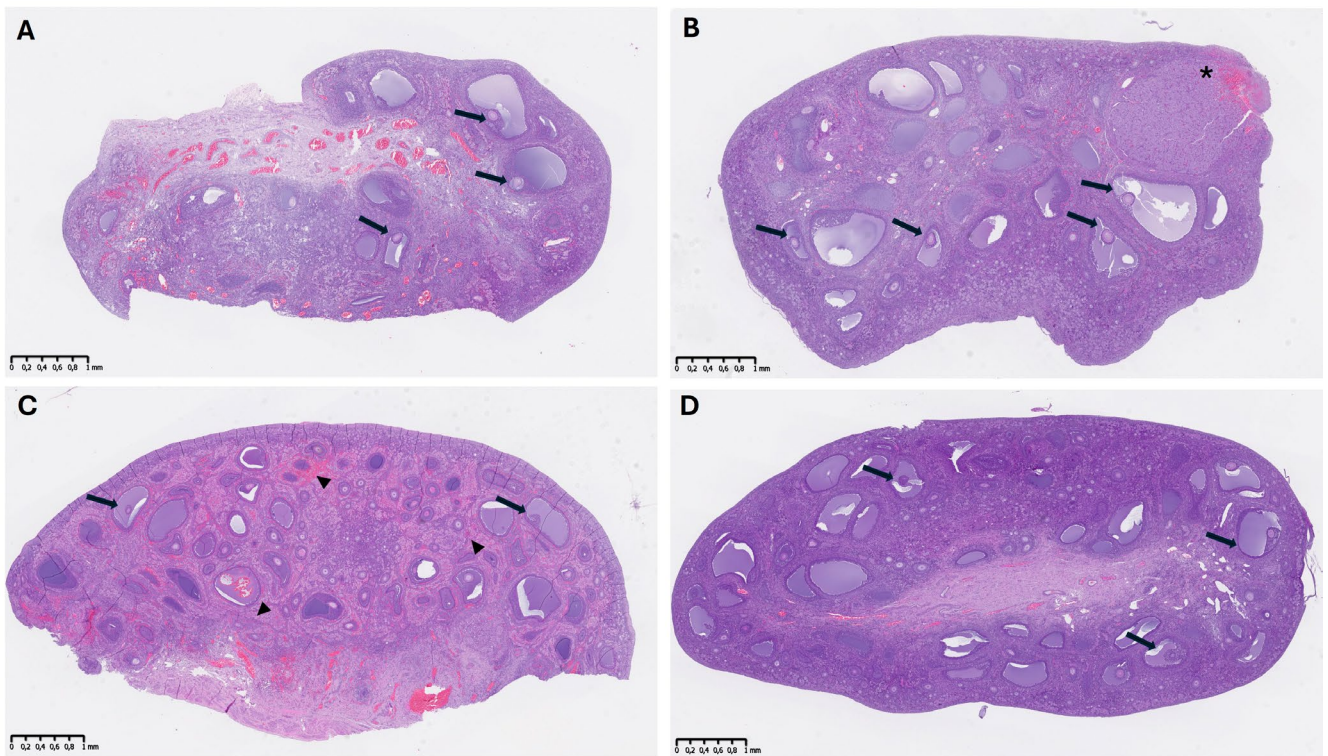


FIGURE 8 | Ovarian histology. (A) Follicular group, queen number 1. Antral follicles with visible oocytes are observed (arrows); (B) Luteal group, queen number 15. A corpus luteum (*) and antral follicles with visible oocytes (arrows) can be observed; (C) Luteal cells group, queen number 16. Antral follicles with visible oocytes (arrows) and luteal cells not organized as a CL (arrowheads) are observed; (D) Anestrus group, queen number 21. Antral follicles with visible oocytes are observed (arrows).

and tumors are the most commonly documented diseases diagnosed by cytology [17, 18], demonstrating that this tool has good agreement (94.7%) with histology to diagnose canine ovarian pathologies.

According to the authors' knowledge, this is the first time that cytological features of feline ovaries throughout different sexual phases, sustained by histologic and hormonal findings, are

described in the literature. In the present study, only the specific type of cells showed statistically significant differences among the groups. Thus, whereas granulosa cells were observed in all the sexual stages, luteal cells were present only in the cytology of those queens in the luteal phase. This is an expected result since those were the only females showing an evident CL in ovarian histology. This finding agrees with that previously described by Piseddu et al. [16] in dog ovaries.

Mesenchymal cells were observed in the ovarian cytology in all the groups. However, lower numbers were detected in the animals belonging to the follicular phase. The presence of mesenchymal cells in ovarian cytology has been previously described in the canine ovary [16, 18], although they were not associated with any specific estrous stage. In the canine ovary, the exact origin of these cells was not determined, but they were classified as probable fibrocytes and fibroblasts.

In addition, recent publications support the presence of ovarian-derived mesenchymal stem cells (MSCs) in the canine ovary [26]. Those cells appear morphologically as mesenchymal cells and express mesenchymal markers. The ovary is a constantly changing organ. Injury occurs during follicular rupture and ovulation, leading to a constant remodeling process. The presence of MSCs in the ovary has been suggested to be involved in tissue repair after ovulation. Furthermore, these cells are suspected to contribute to form granulosa cell-like epithelial cells [26]. Thus, the presence of mesenchymal cells in the feline ovary may also be associated with the reparation processes.

The presence of mitotic figures in cytology also showed significant differences among the evaluated groups. Mitoses were only present in the follicular and anestrus groups. The transformation of primordial follicles into secondary and further antral follicles involves the proliferation of the granulosa cells [27]. In women's ovaries, the mitotic index of granulosa cells increases along with the diameter increase in the ovarian follicles [28], thus being related to folliculogenesis. In our samples, the exact origin of the cells undergoing mitosis was not determined; however, based on the cytologic features, granulosa cells were suspected. However, other similar-appearing cells, such as ovarian germinal cells, cannot be excluded [16]. Likewise, in the present study, the highest percentage of mitotic figures was observed in queens from the follicular group, which is expected.

Large, multinucleated structures were also present in all the groups, with no association with any specific sexual stage. According to the authors' knowledge, the structures have not been previously described, and their exact origin is undetermined. These structures may correspond to pyknotic granulosa cells due to their large size and the presence of a multilobulated condensed nucleus, or multinucleated giant cells. Multinucleated giant cells have been previously described in histopathology studies of mouse ovaries and have been associated with aging [29]. Such cells have not been observed in the histopathology samples from our study. Therefore, we cannot conclude the exact origin of these structures. No correlation between these cells and the reproductive stage was observed.

Finally, variable numbers of leukocytes were present, predominantly neutrophils and lymphocytes, with few eosinophils. The presence of leukocytes was associated with blood contamination in most samples. However, in four smears from the luteal phase group, a higher proportion of leukocytes were admixed with the luteal cells and, sporadically, in their cytoplasm, suggesting that leukocytes were truly present in the ovary. The presence of leukocytes in the cytoplasm of other cell types is known as emperipolesis. Luteal emperipolesis has been previously reported as a novel feature in normal canine ovaries [16] and other species, including humans, pigs, and rats [30–32].

Leukocytes play a crucial role in reproduction as they are considered potent *in situ* modulators of ovarian function. Their infiltration and distribution in the ovary are orchestrated by changes in reproductive hormones, such as steroids and gonadotropins, throughout the estrous cycle. The main functions related to the presence of leukocytes in the ovary are the loosening of the follicular wall to facilitate follicular growth and ovulation, tissue repair after the follicle rupture and contribution to the luteal formation and regression [30, 31].

Ovarian cytology may be a powerful technique to diagnose pathologies, and the knowledge of the specific physiologic features is mandatory to distinguish a pathologic statement. Cytology has been demonstrated to be a reliable tool to diagnose certain types of canine ovarian neoplasia. In cats, the information on ovarian cytology is scarce and is limited to neoplastic processes [18]. However, the small size of the queen ovaries impairs the sampling obtention, limiting its usefulness to cases where the organ is remarkably increased. In that scenario, and as similarly described in other species, it should also be taken into consideration that cytologic investigations of ovarian masses may induce tumor spillage, and their use should be limited to patients with evident metastatic disease or with a low body condition that does not allow the possibility of exploratory laparotomy [17]. Another limitation of the present study is that it uses one ovary for cytologic purposes and the contralateral one for histologic purposes. Since ovaries are synchronized structures, sampling both would provide a more reliable and robust diagnosis. However, the FNA technique may alter the ovarian structure and, hence, impair the histologic assessment. Therefore, one of the ovaries was used for cytology purposes, and the other was used for histologic evaluation. Of course, some variability between ovaries may be missed. Furthermore, in the present study, the groups were not evenly represented, and they included a low number of individuals. An additional limitation of the present study is that the leukocyte scoring system applied was designed as a semi-quantitative descriptive tool rather than a true measure of leukocyte infiltration within the ovarian parenchyma. Peripheral blood leukocyte counts were not available for the included animals, precluding comparison between circulating and tissue leukocyte populations. Furthermore, leukocytes were not quantified relative to parenchymal ovarian cells, as the cytologic nature of the samples and the variable cellularity did not allow for reliable normalization. Consequently, leukocyte evaluation was restricted to assessing whether their presence was suggestive of being proportional to blood contamination or suggestive of representing true tissue-associated leukocytes. Future studies incorporating paired peripheral blood analyses and quantitative approaches would be valuable to better characterize leukocyte infiltration in ovarian tissue. Lastly, the authors acknowledge that evaluating certain parameters on a per-cell basis, rather than grading them as a percentage per field of view, may be more accurate for future studies in this field. However, the methods used for evaluation remain heterogeneous across the existing literature.

5 | Conclusions

The cytologic description of healthy cat ovaries is a necessary preliminary step for the correct interpretation of the cytology of this organ. Ovarian cytology varies according to the

reproductive cycle. Luteal cells are present in the corpus luteum (CL) during the luteal phase. In contrast, mesenchymal cells are more common in the follicular and luteal phases, and mitotic figures are found in the anestrus and follicular phases.

Acknowledgments

We would like to extend our thanks to Roser Velarde for kindly providing the equipment that made the cytologic pictures possible.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. P. L. Senger, "The Organization and Function of the Female Reproductive Tract," in *Pathways to Pregnancy and Parturition*, 2nd ed. (Current Conceptions, 1999), 24–26.
2. J. C. Haveloc, W. E. Rainey, and B. R. Carr, "Ovarian Granulosa Cell Lines," *Molecular and Cellular Endocrinology* 228 (2004): 67–78, <https://doi.org/10.1016/j.mce.2004.04.018>.
3. D. A. Magoffin, "Ovarian Theca Cell," *International Journal of Biochemistry & Cell Biology* 37 (2005): 1344–1349, <https://doi.org/10.1016/j.biocel.2005.01.016>.
4. C. Stocco, C. Telleria, and G. Gibori, "The Molecular Control of Corpus Luteum Formation, Function, and Regression," *Endocrine Reviews* 28 (2007): 117–149, <https://doi.org/10.1210/er.2006-0022>.
5. H. W. Mossman and I. Judas, "Accessory Corpora Lutea, Lutein Cell Origin, and the Ovarian Cycle in the Canadian Porcupine," *American Journal of Anatomy* 85 (1949): 1–39, <https://doi.org/10.1002/aja.1000850103>.
6. C. J. Thwaites and T. N. Edey, "Histology of the Corpus Luteum in the Ewe: Changes During the Estrous Cycle and Early Pregnancy, and in Response to Some Experimental Treatments," *American Journal of Anatomy* 129 (1970): 439–447, <https://doi.org/10.1002/aja.1001290405>.
7. T. A. Fitz, M. H. Mayan, H. R. Sawyer, and G. D. Niswender, "Characterization of Two Steroidogenic Cell Types in the Ovine Corpus Luteum," *Biology of Reproduction* 27 (1982): 703–711, <https://doi.org/10.1095/biolreprod27.3.703>.
8. K. E. Hehnke, L. K. Christenson, S. P. Ford, and M. Taylor, "Macrophage Infiltration Into the Porcine Corpus Luteum During Prostaglandin F₂ Alpha-Induced Luteolysis," *Biology of Reproduction* 50 (1994): 10–15, <https://doi.org/10.1095/biolreprod50.1.10>.
9. M. J. Fields and P. A. Fields, "Morphological Characteristics of the Bovine Corpus Luteum During the Estrous Cycle and Pregnancy," *Theriogenology* 45 (1996): 1295–1325, [https://doi.org/10.1016/0093-691x\(96\)00099-4](https://doi.org/10.1016/0093-691x(96)00099-4).
10. W. C. Duncan, "The Human Corpus Luteum: Remodeling During Luteolysis and Maternal Recognition of Pregnancy," *Reviews of Reproduction* 5 (2000): 12–17, <https://doi.org/10.1530/ror.0.0050012>.
11. L. Gatel, D. N. Rault, K. Chalvet-Monfray, et al., "Ultrasonography of the Normal Reproductive Tract of the Female Domestic Cat," *Theriogenology* 142 (2020): 328–337, <https://doi.org/10.1016/j.theriogenology.2019.10.015>.
12. K. Jewgenow, O. Amelika, J. Painer, F. Göritz, and M. Dehnhard, "Life Cycle of Feline Corpora Lutea: Histological and Intraluteal Hormone Analysis," *Reproduction in Domestic Animals* 47 (2012): 25–29, <https://doi.org/10.1111/rda.12033>.
13. O. Amelkina, B. C. Braun, M. Dehnhard, and K. Jewgenow, "The Corpus Luteum of the Domestic Cat: Histologic Classification and Intraluteal Hormone Profile," *Theriogenology* 83 (2015): 711–720, <https://doi.org/10.1016/j.theriogenology.2014.11.008>.
14. S. Pal, S. Chakrabarti, D. Deuoghuria, J. P. Phukan, A. Sinha, and P. K. Mondal, "Evaluation of Ultrasound-Guided Fine-Needle Aspiration Cytology of Ovarian Masses With Histopathological Correlation," *Acta Cytologica* 59 (2015): 149–155, <https://doi.org/10.1159/000380937>.
15. R. R. Wilborn and S. H. Maxwell, "Clinical Approaches to Infertility in the Bitch," *Veterinary Clinics of North America: Small Animal Practice* 42 (2012): 457–468, <https://doi.org/10.1016/j.cvsm.2012.01.016>.
16. E. Pisceddu, C. Masserdotti, C. Milesi, and L. Solano-Gallego, "Cytological Features of Normal Canine Ovaries in Different Stages 632 of Estrus With Histologic Comparison," *Veterinary Clinical Pathology* 41, no. 3 (2012): 396–404, <https://doi.org/10.1111/j.1939-165X.2012.00453.x>.
17. W. Bertazzolo, M. Dell'Orco, U. Bonfanti, et al., "Cytological Features of Canine Ovarian Tumours: A Retrospective Study of 19 Cases," *Journal of Small Animal Practice* 45 (2004): 539–545, <https://doi.org/10.1111/j.1748-5827.2004.tb.00200.x>.
18. L. Solano-Gallego, "Reproductive System," in *Canine and Feline Cytology. A Color Atlas and Interpretation Guide*, 4th ed., ed. R. Raskin, D. Meyer, and K. Boes (Elsevier, 2023), 451–458.
19. M. Fuertes-Recuero, A. González-Gil, J. C. Fontanillas Pérez, I. García-Cuenca Ariati, and R. A. Picazo, "Determination of the Appropriate Concentration of Sodium Alginate Used for In Vitro Culture of Cat Preantral Follicles in a Serum-Free Medium Containing FSH, EGF and IGF-1," *Reproduction in Domestic Animals* 58 (2023): 670–678, <https://doi.org/10.1111/rda.14336>.
20. V. M. Shille, K. E. Lundström, and G. H. Stabenfeldt, "Follicular Function in the Domestic Cat as Determined by Estradiol-17b Concentrations in Plasma: Relation to Estrous Behavior and Cornification of Exfoliated Vaginal Epithelium," *Biology of Reproduction* 21 (1979): 953–963, <https://doi.org/10.1095/biolreprod21.4.953>.
21. S. R. Paape, V. M. Shille, H. Seto, and G. H. Stabenfeldt, "Luteal Activity in the Pseudopregnant Cat," *Biology of Reproduction* 13 (1975): 470–474, <https://doi.org/10.1095/biolreprod13.4.470>.
22. H. G. Verhage, N. B. Beamer, and R. M. Brenner, "Plasma Levels of Estradiol and Progesterone in the Cat During Polyestrus, Pregnancy and Pseudopregnancy," *Biology of Reproduction* 14 (1976): 579–585, <https://doi.org/10.1095/biolreprod14.5.579>.
23. D. E. Wildt, S. Y. Chan, S. W. Seager, and P. K. Chakraborty, "Ovarian Activity, Circulating Hormones, and Sexual Behavior in the Cat. I. Relationships During the Coitus-Induced Luteal Phase and the Estrous Period Without Mating," *Biology of Reproduction* 25 (1981): 15–28, <https://doi.org/10.1095/biolreprod25.1.15>.
24. P. M. Schmidt, P. K. Chakraborty, and D. E. Wildt, "Ovarian Activity, Circulating Hormones and Sexual Behavior in the Cat. II. Relationships During Pregnancy, Parturition, Lactation and the Postpartum Estrus," *Biology of Reproduction* 28 (1983): 657–671, <https://doi.org/10.1095/biolreprod28.3.657>.
25. C. Marrinhas, F. Malhão, C. Lopes, et al., "Doing More With Less: Multiple Uses of a Single Slide in Veterinary Cytology. A Practical Approach," *Veterinary Research Communications* 46 (2022): 641–654, <https://doi.org/10.1007/s11259-022-09953-0>.
26. A. B. Trindade, J. Therrien, J. M. Garcia, and L. C. Smith, "Mesenchymal-Like Stem Cells in Canine Ovary Show High Differentiation Potential," *Cell Proliferation* 50 (2017): 1–10, <https://doi.org/10.1111/cpr.12391>.

27. E. G. Kosebent, F. Uysal, and S. Ozturk, "Telomere Length and Telomerase Activity During Folliculogenesis in Mammals," *Journal of Reproduction and Development* 61 (2018): 477–484, <https://doi.org/10.1262/jrd.2018-076>.
28. A. Gougeon, "Ovarian Follicular Growth in Humans: Ovarian Ageing and Population of Growing Follicles," *Maturitas* 30 (1998): 137–142, [https://doi.org/10.1016/s0378-5122\(98\)00069-3](https://doi.org/10.1016/s0378-5122(98)00069-3).
29. A. Converse, M. J. Perry, S. S. Dipali, et al., "Multinucleated Giant Cells Are Hallmarks of Ovarian Aging With Unique Immune and Degradation-Associated Molecular Signatures," *PLoS Biology* 23, no. 6 (2025): e3003204, <https://doi.org/10.1101/2024.12.03.626649>.
30. O. Bukulmez and A. Arici, "Leukocytes in Ovarian Function," *Human Reproduction Update* 6 (2000): 1–15, <https://doi.org/10.1093/humupd/6.1.1>.
31. O. R. Oakley, M. L. Frazer, and C. Ko, "Pituitary-Ovarian-Splenic Axis in Ovulation," *Trends in Endocrinology and Metabolism* 22 (2011): 345–352, <https://doi.org/10.1016/j.tem.2011.04.005>.
32. O. R. Oakley, H. Kim, I. El-Amouri, et al., "Periovulatory Leukocyte Infiltration in the Rat Ovary," *Endocrinology* 151 (2010): 4551–4559, <https://doi.org/10.1210/en.2009-1444>.