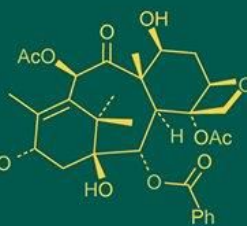
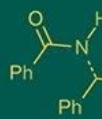


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## Microscopic insights into cervical pathology of canine pyometra

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### Abstract

Cervical patency, in particular, plays a crucial role in determining whether the condition manifests as open or closed cervix pyometra, thereby affecting the severity of clinical signs, risk of uterine rupture, and treatment strategies. The present study was designed to evaluate and compare the histopathological features of the cervix in pyometric bitches with those of normal dioestrous controls. Eighteen bitches were included, comprising twelve cases diagnosed with pyometra, with six exhibiting open-cervix and six exhibiting closed-cervix pyometra, and six healthy bitches in the dioestrous stage serving as controls. Cervical tissue samples were collected during routine ovariohysterectomy procedures. The specimens were processed using standard histological techniques. Microscopic evaluation focused on multiple aspects of cervical histology, including epithelial integrity, glandular structure and activity, stromal organization, inflammatory cell infiltration, vascular changes, and connective tissue remodeling. Differences in these parameters were analyzed to determine the relationship between histopathological patterns and cervical patency in pyometra. The number of neutrophils infiltrated in the stromal layer of the cervix in pyometric dogs and dioestrous dogs were counted. Significant difference was noticed between three groups. This approach allowed for a detailed comparison between pathological and normal cervical tissues, providing insights into the structural changes associated with disease progression and clinical presentation.

**Keywords:** Cervical patency, open and closed pyometra, neutrophils infiltration

### Introduction

Although hormonal influences, particularly progesterone and oestrogen, play a central role in the pathogenesis of pyometra, structural and cellular changes in the cervix and uterus determine disease severity and clinical presentation. Histopathological evaluation provides critical insights into these tissue-level alterations, including epithelial integrity, glandular proliferation, stromal remodeling, vascular changes, and inflammatory cell infiltration, which have been reported in canine pyometra lesions (Prasad *et al.*, 2017 and Xavier *et al.*, 2024)<sup>[8, 11]</sup>. Such microscopic examination is essential not only for understanding disease progression but also for identifying potential correlations between cervical patency, uterine pathology, and systemic hormonal status. The disease typically occurs during the luteal phase of the oestrous cycle, when prolonged progesterone exposure predisposes the endometrium to cystic hyperplasia and secondary bacterial infection (Bosschere *et al.*, 2001)<sup>[4]</sup>. Clinical manifestations depend largely on cervical patency: open-cervix pyometra often presents with vulvar discharge, whereas closed-cervix pyometra may lead to more severe systemic illness due to retained uterine contents (Renton *et al.*, 1993)<sup>[9]</sup>.

Though ovarian steroid hormones, particularly progesterone and oestrogen, play a role in disease susceptibility, structural and cellular changes within the cervix and uterus determine clinical outcome (Blendinger *et al.*, 1997; Kunkitti *et al.*, 2011)<sup>[2, 7]</sup>. Differences between open-and closed-cervix pyometra include variations in epithelial disruption, stromal density, and inflammatory response (Blendinger *et al.*, 1997; Schoon *et al.*, 1992)<sup>[2, 10]</sup>. Despite its clinical importance, comprehensive cervical histopathology in pyometra remains inadequately characterized. This study aimed to evaluate and compare histopathological features of the cervix in open-and closed-cervix pyometra and contrast them with normal dioestrous bitches to elucidate tissue-level determinants of cervical function.

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## Materials and Methods

Female dogs of various breeds with clinical signs suggestive of pyometra and six healthy dogs in dioestrous phase (Group III, n = 6) presented to University Veterinary Hospitals, Kokkalai and Mannuthy were utilized for the study. Pyometra confirmed bitches were classified into open cervix (Group I, n = 6) and closed cervix (Group II, n = 6) depending on the clinical signs. Laparotomy was performed with the animal positioned in its dorsal recumbence. The samples were collected and preserved in 10 per cent neutral buffered formalin. The tissues were processed by routine paraffin embedding techniques (Sheehan and Hrapchak, 1980) [11]. The paraffin embedded tissues were sectioned at four-micron thickness and stained with haematoxylin and eosin stain (Bancroft and cook, 1995) [11]. The stained sections were subjected to detailed examination under light microscope. The number of neutrophils in the stroma of mucosa was examined microscopically (400x). For each uterine cervix, 3 sections and 5 fields were investigated. The data were analyzed using suitable statistical software (IBM Corp. 2016) [6] and comparisons between groups were performed using a one-way ANOVA model.

## Results and Discussion

From the study, it revealed that in normal dioestrous bitches, infiltration of very few inflammatory cells could be noticed. Cervical glands were noticed which had apparently normal lining epithelium (Fig. 1). In open cervix pyometra, the blood vessels were dilated with pooling of blood characterized by presence of large number of leucocytes within the blood vessels and also paving of leucocytes on the wall of the blood vessels. These are the changes characteristic of an inflammatory process in that area. Massive infiltration of the inflammatory cells was also observed. The cervical glands were dilated, hyperplastic and cystic changes noticed. Also, hypertrophy of the muscle fibers were observed. In closed cervix pyometra, scanty infiltration of the inflammatory cells in the sub-epithelial layer could be visualized in the Fig. 2 and 3. The cervical glands were apparently normal. Hypertrophy of the muscle fibers were also noticed. The number of neutrophils infiltrated in the stromal layer of the cervix in pyometric dogs and dioestrous dogs were counted, (Table 1). Significant difference was noticed between three groups,

Group I was significantly different from Group II & Group III, Group II was significantly different from Group I and Group III, Group III was significantly different from Group I and Group II at five per cent level.

The research works pertaining to the histopathological evaluation in the cervix of pyometra affected dogs are scarce. A pioneering work by Kunkitti *et al.* (2011) [7] reported the histopathological changes pertaining to the infiltration of leucocytes in normal and pyometra affected dogs. They reported that neutrophils were the most commonly observed inflammatory cells in the cervix of pyometra affected dogs and subepithelium contained more leukocytes than other layers. The observations of this study correlate with reports of Kunkitti *et al.* (2011) [7] who observed more neutrophil infiltration in open pyometric dogs when compared to closed pyometric ones, which might have triggered structural changes in cervix and influenced its patency. (Cawston, 1996; Winkler *et al.*, 1999) [5, 13] stated that the degree of cervical relaxation in women, was linked with the level of neutrophil infiltration, which could generate collagenolytic enzymes viz., proteases and metalloproteinases. The rise in collagenase enzyme would lead to huge turnover of collagen and deprivation of recently synthesized collagen, which resulted in decreased collagen content in the cervical area of remodeling.

Corroborative to the observations of this study, indicating increased neutrophil infiltration in the cervix of open pyometra dogs in comparison to closed pyometra dogs, Kunkitti *et al.* (2011) [7] also reported that, in open-cervix pyometric dogs more neutrophil infiltration was noticed than closed pyometric dogs, both during anoestrous and dioestrous stages. However, in pyometric dogs, they observed more neutrophils and plasma cells in the vaginal part of the cervix than uterine part which had more inflammatory cells. Also, Tamada *et al.* (2012) [12] stated that on histopathological examination of pyometric cervix, patency of cervix was directly proportional to neutrophilic count infiltrated in the uterine cervical tissue. Histopathological observations of hyperplastic changes of the cervical glands in cervix of both open and closed cervix pyometra dogs also finds agreement with Bosschere *et al.* (2001) [4] who opined that in pyometric bitches, histopathology of the uterine cervix was similar to that of ones with severe Cystic endometrial hyperplasia.

**Table 1:** Mean±SE of values of neutrophil infiltration in the cervical stromal layer in different groups (n = 18)

| Sl. No | Groups    | Number of neutrophils in the cervical stromal layer |          |           |          |         | Average Total | Mean ± SE               |
|--------|-----------|-----------------------------------------------------|----------|-----------|----------|---------|---------------|-------------------------|
|        |           | Field I                                             | Field II | Field III | Field IV | Field V |               |                         |
|        | Group I   |                                                     |          |           |          |         |               | 23.67±2.29 <sup>a</sup> |
| 1.     | Case 1    | 35                                                  | 15       | 25        | 15       | 20      | 22            |                         |
| 2.     | Case 2    | 25                                                  | 26       | 19        | 20       | 10      | 20            |                         |
| 3.     | Case 3    | 24                                                  | 12       | 35        | 14       | 15      | 20            |                         |
| 4.     | Case 4    | 27                                                  | 29       | 31        | 29       | 37      | 31            |                         |
| 5.     | Case 5    | 12                                                  | 20       | 18        | 30       | 6       | 17            |                         |
| 6.     | Case 6    | 34                                                  | 14       | 23        | 12       | 18      | 20            |                         |
|        | Group II  |                                                     |          |           |          |         |               | 7.17±1.01 <sup>b</sup>  |
| 7.     | Case 1    | 9                                                   | 8        | 6         | 11       | 5       | 8             |                         |
| 8.     | Case 2    | 4                                                   | 6        | 7         | 2        | 11      | 6             |                         |
| 9.     | Case 3    | 8                                                   | 10       | 13        | 4        | 15      | 10            |                         |
| 10.    | Case 4    | 3                                                   | 5        | 6         | 1        | 9       | 5             |                         |
| 11.    | Case 5    | 7                                                   | 8        | 12        | 7        | 4       | 8             |                         |
| 12.    | Case 6    | 11                                                  | 2        | 5         | 9        | 10      | 8             |                         |
|        | Group III |                                                     |          |           |          |         |               | 1.17±0.30 <sup>c</sup>  |
| 13.    | Case 1    | -                                                   | 1        | -         | 1        | -       | 2             |                         |
| 14.    | Case 2    | -                                                   | -        | 1         | -        | -       | 1             |                         |
| 15.    | Case 3    | 1                                                   | -        | -         | -        | 2       | 3             |                         |
| 16.    | Case 4    | -                                                   | 2        | 1         | -        | -       | 3             |                         |
| 17.    | Case 5    | 1                                                   | -        | -         | -        | 1       | 2             |                         |
| 18.    | Case 6    | -                                                   | 1        | -         | 1        | -       | 2             |                         |

<sup>a,b,c</sup>Means with different superscript between rows differ significantly at 5% level ( $p < 0.05$ )-Significant

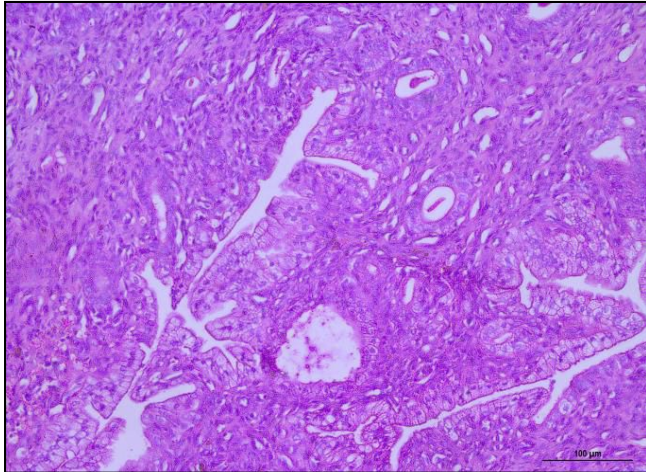


**Fig 1:** Cervical glands-Dioestrous dog (40X)

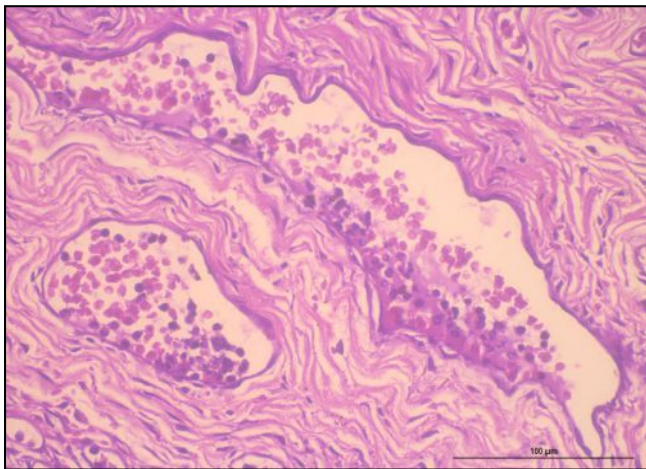


**Fig 2:** Neutrophil infiltration in open cervix pyometra (40X)

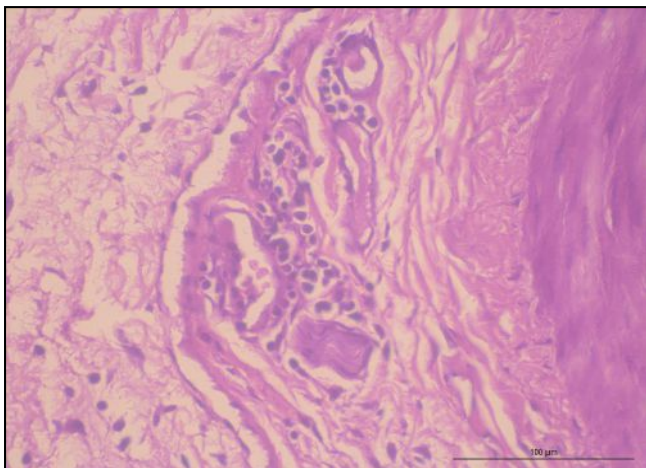


**Fig 3:** Neutrophil infiltration in closed cervix pyometra (40X)

### Conclusions

The study revealed distinct histopathological variations in the uterine cervix of normal dioestrous bitches and those affected with open and closed-cervix pyometra. Normal bitches exhibited minimal inflammatory infiltration and intact cervical architecture, whereas open-cervix pyometra showed marked vascular dilation, extensive neutrophilic infiltration, glandular hyperplasia with cystic changes, and muscular hypertrophy, indicating active inflammatory remodeling. Closed-cervix pyometra demonstrated comparatively scant inflammatory infiltration with relatively preserved glandular structure. A significant difference in

neutrophil infiltration among the groups highlights the close association between inflammatory cell infiltration and cervical patency. These findings suggest that cervical patency in canine pyometra is primarily influenced by local inflammatory-mediated tissue remodeling rather than hormonal factors alone, underscoring the importance of histopathological evaluation in understanding disease progression and clinical manifestation.

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