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## Endocrine control of cervical patency in canine pyometra: A progesterone-oestrogen perspective

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### Abstract

This study evaluated the relationship between serum progesterone and oestrogen concentrations and cervical patency in canine pyometra. Eighteen dogs were included, comprising twelve pyometric cases—open cervix (n = 6) and closed cervix (n = 6)—and six healthy dioestrous bitches as controls. All cases were presented to the University Veterinary Hospitals, Kokkalai and Mannuthy. Serum progesterone and oestrogen levels were estimated prior to treatment. Although pyometric bitches showed altered hormonal profiles compared to healthy controls, no statistically significant difference in progesterone or oestrogen concentrations was observed between open and closed cervix pyometra. The findings indicate that while hormonal influence is integral to the pathogenesis of pyometra, circulating progesterone and oestrogen levels may not be reliable determinants of cervical patency in affected dogs.

**Keywords:** Canine pyometra, cervical patency, oestrogen, progesterone

### Introduction

The cervix is a specialized anatomical and functional component of the female reproductive tract that serves as a dynamic interface between the vagina and uterus. It plays a crucial role in regulating sperm transport, maintaining uterine defense, and protecting the intrauterine environment. In the bitch, cervical characteristics vary markedly across the oestrous cycle. During proestrus and oestrus, rising oestrogen concentrations promote cervical relaxation and functional opening (Oving *et al.*, 1987) <sup>[15]</sup>. This effect is mediated through stromal remodeling processes, including collagen reorganization, increased glycosaminoglycan content, and enhanced tissue hydration, resulting in reduced cervical rigidity and improved luminal patency. These changes are physiologically essential for mating and sperm passage. In contrast, following ovulation, progesterone becomes the dominant hormone during diestrus and exerts an opposing influence on cervical tissue. Progesterone stabilizes collagen fibers, reduces stromal water content, and increases cervical firmness, thereby inducing closure of the cervical canal and limiting ascending microbial contamination (Vermeirsch *et al.*, 2000) <sup>[22]</sup>.

While this hormonal regulation of cervical function is well recognized under normal physiological conditions, pathological reproductive disorders such as canine pyometra present a more complex scenario. Pyometra is a common and potentially fatal uterine disease of intact bitches, most frequently occurring during the luteal phase when progesterone levels are elevated (Hagman, 2017) <sup>[10]</sup>. Progesterone predisposes the uterus to infection by stimulating endometrial glandular secretion, suppressing myometrial contractility, and attenuating local immune responses (De Bosschere *et al.*, 2001) <sup>[6]</sup>. Despite this progesterone-dominant endocrine environment, pyometra manifests clinically in both open- and closed-cervix forms, indicating that circulating progesterone concentration alone does not determine cervical patency.

Earlier investigations have demonstrated that systemic progesterone concentrations do not differ significantly between bitches with open- and closed-cervix pyometra, suggesting that additional factors regulate cervical status in affected animals (Renton *et al.*, 1993) <sup>[18]</sup>. These findings imply an important role for local regulatory mechanisms, including alterations in steroid hormone receptor expression, inflammatory mediators, and extracellular matrix-modifying enzymes within cervical tissue.

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Supporting this concept, studies in other mammalian species have shown that oestradiol-induced collagen remodeling significantly alters cervical compliance, as demonstrated in pregnant ewes (Oving *et al.*, 1987) <sup>[15]</sup>.

Given the clinical importance of cervical patency in influencing disease severity, systemic illness, and treatment outcomes in canine pyometra, a clearer understanding of the relationship between circulating ovarian steroids and cervical status is warranted. Therefore, the present study aimed to estimate serum progesterone and oestrogen concentrations and to evaluate their association with cervical patency in bitches affected with pyometra.

## Materials and Methods

### Animals and study design

Female dogs of various breeds such as Spitz, Labrador retriever, Basset hound, Lhasapso, Rottweiler, Dachshund, Pomeranian 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. Detailed history, clinico-gynaecological examination and clinical parameters were recorded. Confirmation of pyometra was done by trans-abdominal ultrasonography. Pyometra confirmed bitches were classified into open (Group I, n = 6) and closed (Group II, n = 6) depending on the clinical signs. Blood samples were collected from all the animals before ovariectomy (OHE) and serum separated was stored in deep freezer for sex steroid hormones estimation.

### Hormone Estimation

Serum progesterone and oestrogen concentrations were determined retrospectively using duplicate serum samples by enzyme-linked immunosorbent assay (ELISA) with commercially available kits, following the manufacturer's instructions. Frozen serum samples were completely thawed at room temperature and mixed gently prior to analysis. All reagents, standards, controls, and samples were brought to room temperature before use. For progesterone estimation, 10 µl of standards, controls, and serum samples were dispensed into coated microtiter wells, followed by addition of 200 µl of progesterone enzyme conjugate. The plate was incubated for 60 minutes at room temperature (20-25 °C), washed three times with 1x wash buffer, and developed using tetramethylbenzidine (TMB) substrate. The reaction was stopped using stop solution, and absorbance was measured at 450 nm using an ELISA plate reader.

For oestrogen estimation, 25 µl of standards, controls, and serum samples were added to coated wells, followed by addition of oestradiol biotin reagent and incubation for 45 minutes at room temperature. Subsequently, oestradiol enzyme reagent was added and incubated for an additional 45 minutes. After washing, TMB substrate was added and incubated for 20 minutes before stopping the reaction. Wash buffers and enzyme conjugates were prepared according to kit specifications by appropriate dilution with assay diluent or distilled water. Absorbance was recorded at 450 nm within 15 minutes of adding the stop solution. Hormone concentrations were calculated from standard curves generated for each assay. The data were analysed with one way ANOVA using SPSS software version 24.0 (IBM Corp. 2016) <sup>[12]</sup>.

## Results and Discussion

### Serum progesterone (P<sub>4</sub>) estimation

Mean serum progesterone concentration (ng/ml) among dogs under different groups, on the day of admission were presented in Table 1. Mean serum P<sub>4</sub> concentration (ng/ml) in group I, II and III dogs were 17.19±5.45, 22.38±4.38 and 27.36±4.66 respectively. No significant difference noticed among normal dioestrous, open and closed cervix pyometra bitches. The pyometric dogs had relatively lower concentrations of serum P<sub>4</sub> in comparison to normal dioestrous bitches without significance. Also, serum P<sub>4</sub> values did not vary significantly between open and closed pyometra affected dogs. Similar value was reported by Pineda (1989) <sup>[16]</sup> during dioestrous, CL secreted P<sub>4</sub> approximately 20 ng/mL. Chithra (2016) <sup>[3]</sup> observed similar P<sub>4</sub> level in open pyometric bitches as 17.43±1.23 ng/ml while Volpto *et al.* (2013) <sup>[24]</sup> reported P<sub>4</sub> value in closed cases was 13.2±5.5 ng/ml and they observed no significant variation in the P<sub>4</sub> level between pyometric dog groups. Austad *et al.* (1979) <sup>[1]</sup> reported that in pyometric bitches, prolonged or excessive P<sub>4</sub> production was not noticed. Also they concluded that both pyometra and healthy bitches, blood P<sub>4</sub> level was in normal level. Colombo *et al.* (1988) and Ververidis *et al.* (2004) <sup>[4, 23]</sup> reported that in both pyometric as well as dioestrous dogs during respective stages of oestrous cycle, circulating P<sub>4</sub> levels were same, and also it was low during early anoestrous and high during dioestrous. Blending *et al.* (1997) <sup>[2]</sup> reported that in bitches with pyometra, patency of the cervix was not related to its serum P<sub>4</sub> level. Kunkitti *et al.* (2011) <sup>[14]</sup> reported that in normal healthy bitches, when compared with oestrogen, P<sub>4</sub> played a vital role in controlling functions of cervix. On the contrary to the present study, Dineshkumar (2005) <sup>[7]</sup> reported that P<sub>4</sub> concentration in closed cervix pyometra was significantly different from open pyometra and the values were 18.90±3.41 and 5.63±1.83 ng/mL in closed and open pyometra respectively.

### Serum oestrogen (E<sub>2</sub>) concentration

Mean serum oestrogen (E<sub>2</sub>) concentration (pg/ml) among dogs under three groups, on the day of admission were presented in Table 1. Mean serum E<sub>2</sub> concentration (pg/ml) in group I, II and III dogs were 50.43±14.15, 38.66±13.10 and 56.04±20.95 respectively. No significant difference noticed among normal dioestrous, open and closed cervix pyometra bitches. Mean serum oestradiol-17<sub>β</sub> concentration (pg/ml) in three groups respectively were 40.33±2.49, 35±5.77 and 46.66±6.80 respectively. Though no significant difference was noticed between the groups, pyometric dogs recorded a lower value compared to normal dioestrous dogs, with the lowest value recorded in closed pyometra. Similar observations of no significant difference in the serum oestradiol-17<sub>β</sub> concentration between pyometric and normal bitches (32.26±6.6 and 35.76±27.7) has also been studied by Srisuwanasagul *et al.* (2006) <sup>[21]</sup>. Volpto *et al.* (2013) <sup>[24]</sup> reported that the E<sub>2</sub> concentration of bitches with open and closed pyometra were 43±6.5 pg/ml and 56.3±11.9 pg/ml respectively. Schoon *et al.* (1982) <sup>[20]</sup> reported no significant difference of E<sub>2</sub> concentration in open and closed pyometra dogs. Kida *et al.* (2006) <sup>[13]</sup> reported that hormonal disorders and ovarian abnormality resulted from the prolonged influence of oestrogen (ovarian cysts, prolonged heat, active ovarian neoplasia) and were considered as the prime cause of endometritis-pyometra complex.

Wide variation in the oestradiol-17 $\beta$  concentration during dioestrous in dogs had been reported by many authors. Concannon (2011) <sup>[5]</sup>; Papa and Hoffmann (2011) <sup>[16]</sup> and Fonseca *et al.* (2012) <sup>[9]</sup> measured the oestradiol levels during dioestrous and it was reported to be ranging from 15 to 30 pg/ml. Similar to the observations of this study, Dineshkumar (2005) <sup>[7]</sup> also observed the higher serum oestradiol-17 $\beta$  values in normal dioestrous bitches than pyometric bitches. The author also observed serum E<sub>2</sub> concentration in closed pyometra was slightly higher than open pyometra which was contrary to the present study. Relatively higher values observed in open pyometra than closed pyometra dogs in the study could be attributed to the presence of follicular cystic ovaries recorded in three out of six open pyometra cases which finds agreement with the reports of Hosken *et al.* (1992) <sup>[11]</sup> and Blendinger *et al.* (1997) <sup>[2]</sup>. They stated that high E<sub>2</sub> levels could be due to prolonged oestrous, caused by follicular cystic ovaries or ovarian imbalance.

To the contrary of our study, Ververidis *et al.* (2002) <sup>[23]</sup> stated that in pyometric dogs, serum E<sub>2</sub> concentration was notably higher than that of dioestrous dogs during corresponding phases of oestrous cycle but won't reach levels noticed in proestrus or oestrus. Ververidis *et al.* (2004) <sup>[23]</sup> reported that in CEH-P bitches, serum E<sub>2</sub> concentrations varied significantly higher than dioestrous bitches during corresponding stages of oestrous cycle.

**Table 1:** Serum progesterone (ng/ml) and oestrogen (pg/ml) concentration (Mean  $\pm$  SE) on the day of admission in different groups (n = 18)

| Group (n = 6) | Serum P <sub>4</sub> concentration | Serum E <sub>2</sub> concentration |
|---------------|------------------------------------|------------------------------------|
| I             | 17.19 $\pm$ 5.45 <sup>a</sup>      | 50.43 $\pm$ 14.15 <sup>a</sup>     |
| II            | 22.38 $\pm$ 4.38 <sup>a</sup>      | 38.66 $\pm$ 13.10 <sup>a</sup>     |
| III           | 27.36 $\pm$ 4.66 <sup>a</sup>      | 56.04 $\pm$ 20.95 <sup>a</sup>     |

<sup>a</sup> Similar superscript indicates no significant difference between rows at 5% level ( $p < 0.05$ )-non-significant

## Conclusions

The present study demonstrated that serum progesterone and oestradiol concentrations did not differ significantly among normal dioestrous bitches and those affected with open-or closed-cervix pyometra, indicating that circulating ovarian steroid levels alone do not determine cervical patency in pyometric dogs. These findings suggest that local uterine and cervical factors play a more critical role than systemic endocrine status. Clinically, this implies that serum hormone estimation alone may not reliably predict cervical status or disease severity in canine pyometra, emphasizing the need for comprehensive diagnostic evaluation.

Future research should focus on cervical tissue-level mechanisms, including steroid receptor expression, inflammatory pathways, and extracellular matrix remodeling, to improve understanding and management of this condition.

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