RESEARCH ARTICLE

Efficacy of Progesterone Receptor Antagonist for Treatment of Pyometra in Bitches

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Abstract

The present study aimed to assess the efficacy of Progesterone Receptor Antagonist for Treatment of Pyometra in Bitches. The present research work was carried out on a total of 12 bitches diagnosed with pyometra. The animals were randomly assigned to two treatment protocols, with 6 animals in each group. In Group I, bitches were administered only with supportive therapies, i.e. fluid therapy, cloprostenol, and ceftriaxone. In Group II, bitches were administered with progesterone receptor antagonist (mifepristone) and supportive fluid therapy as in Gr-I. The physiological, hematological, and biochemical parameters were evaluated on days 0, 4^{th,} and 8th of treatment. The recovery rate of treated bitches was assessed by ultrasonography post-treatment. There was a significant increase in the mean PCV and a decrease in leucocyte count in group II on day 8. After treatment, the mean serum globulin and creatinine level reduction was significant ($p < 0.05$) in group II than group I. The mean serum ALT concentration in bitches with pyometra in different groups pre-treatment, during, and post-treatment were within the normal physiological range (10-94 U/L). The mean concentration of serum ALP decreased significantly ($p < 0.05$) in both groups on day 8. The ultrasound examination on day 8 post-treatment revealed a reduction in uterine lumen, the thickness of the uterine wall, and decreased echogenicity indicative of treatment response in both groups. Bitches treated with progesterone receptor antagonist (mifepristone, Group-II) showed higher recovery (100 vs. 50%) and lower recurrence (17 vs. 67%) than Group I. From the present findings, it could be concluded that progesterone receptor antagonist proves to be a safe and efficient drug for the treatment of pyometra in bitches.

Keywords: Bitch, Mifepristone, Prostaglandin, Pyometra.

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Introduction

Pyometra or chronic purulent endometritis is a common metestrual disease mainly affecting bitches over 8 years of age (Hadiya *et al*., 2020; Singh *et al*., 2020[a]). The pathogenesis of pyometra is primarily a hormonal imbalance or abnormal response to normal concentrations of estrogens and progesterone that affects the epithelial cells of the uterus and facilitates bacterial adherence, colonization, and growth (Noakes *et al*., 2001). Progesterone results in endometrial proliferation and uterine glandular secretion, decreased myometrial contraction, closure of the cervix, and support to bacterial infection (Smith, 2006). The effective treatment of pyometra is an ovario-hysterectomy, as it removes discomfort from the distended uterus and sources of endotoxin (Rootwelt-Andersen and Farstad, 2006). In the absence of life-threatening signs, pyometra can be treated medically invaluable bitches to restore fertility (Melandri *et al*., 2019). Repeated administration of $PGF_2\alpha$ is indicated for metritis or open pyometra in healthy young bitches, which causes luteolysis and thus reduces plasma progesterone concentrations. Prostaglandins, however produce substantial adverse effects on the health and life of the animal. Antiprogestins are synthetic steroids that bind with great affinity to progesterone receptors without any effects of progesterone (Hoffmann *et al*., 2000). Progesterone blockers are suggested to be beneficial for the treatment of closed pyometra in canines. Using an antiprogestin (aglepristone) in combination with $PGF_2\alpha$ was highly successful (Fieni, 2006). Mifepristone has five times greater relative binding affinity than progesterone for progesterone receptors thus, mimicking the effects observed during luteolysis, leading to relaxation of the cervix (Verstegen *et al*., 2008). The present study was aimed to evaluate the effect of progesterone receptor antagonists in the treatment of canine pyometra.

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Materials and Methods

The study was conducted on 12 clinical pyometra cases in bitches, irrespective of their age, breed, and parity. They were

presented at the Department of Veterinary Gynaecology and Obstetrics and Veterinary Clinical Complex, College of Veterinary Science & AH, Anjora, Durg, Chhattisgarh. Clinical and ultrasonographic (Prosound 2 ALOKA) examination was carried out for confirmation of pyometra. Ultrasonography revealed an enlarged lumen of both the uterine horns with the hyperechoic bands pocket and the appearance of intraluminal anechoic to hypoechogenecity for the thickened uterine wall. The affected bitches were randomly divided into two equal groups with six animals in each. The bitches in group I (Control or standard treatment) were administered with supportive fluid therapy along with cloprostenol 5 µg/kg b.wt. on day 1, 2 and 6th i/m and ceftriaxone @ 20 mg/kg b.wt. i/v daily for 5 days. Group II (test treatment) bitches were administered with progesterone receptor antagonist (mifepristone) @ 5 mg/kg b.wt. Orally on 1, 2, 3, and on 7th day along with supportive fluid therapy with cloprostenol and ceftriaxone as in Group I.

Bitches with pyometra were subjected to ultrasonography on day 0 (pre-treatment), day 4th (during treatment) and day 8th (post-treatment) using 2.5 to 5 MHz trans-abdominal probes. The clinical parameters, rectal temperature, respiratory rate, and heart rate were recorded in all the animals. 2 mL of blood was collected on days 0, 4^{th,} and 8th from a cephalic vein in EDTA vials for hematology, and 5 mL of blood was collected in vials without anticoagulant for serum biochemical studies. Hemoglobin (Hb g/dL), packed cell volume (PCV %), total leukocyte count (TLC cells/mm^3), differential leukocyte count (DLC %) were recorded using Semi-automatic Haematological Analyser (MS39, Melet Schloesing Laboratories). The serum was collected after centrifugation of blood samples @3000 g for 5 min and stored at -20°C till further analysis. Blood glucose (mg/dL, blood urea nitrogen (BUN, mg/dL), creatinine (mg/dL), alkaline phosphatase (ALP, IU/L), alanine aminotransferase (ALT, IU/L), total protein (g/dL) albumin (g/dL) and globulin (g/dL) were estimated on Semi-automated Biochemistry Analyser (Systronics India Ltd.) following procedures and kits of Biolab Diagnostics.

The data were tabulated, and the mean and standard error were computed for all groups. Data were analyzed by using analysis of variance (ANOVA) as per the standard procedure. Statistically, significant difference was considered at 5 percent level.

Results and Discussion

Effect of Treatment Regimens on Physiological Parameters

On day 0 rectal temperature was slightly elevated in Group II (102.6 ± 0.46) compared to normal physiological limits. The mean rectal temperature reduced gradually and returned to normal on day 8 following treatment protocols (Table 1). Statistically, the elevated temperature did not differ significantly between days and groups. The mean heart rate in treatment groups before, during, and after treatment was within the normal physiological range (60–140 per minute) and did not differ significantly between days and groups. Bitches suffering from pyometra may also exhibit normal, subnormal, or elevated body temperature depending upon the severity of infection in the uterus (Jena *et al*., 2013). Elevated body temperature was reported in bitches with pyometra by Kashi *et al*. (2009). The present findings on heart and the respiratory rate were in close agreement with the findings of Samantha *et al*. (2018).

Effect of Treatment Regimens on Haematological Parameters

The mean values of hemoglobin concentration, packed cell volume (PCV), total leucocyte count (TLC), and differential leucocyte count (DLC) in bitches affected with pyometra in different groups on days 0, 4, and 8 of treatment are presented in Table 1. There was a significant ($p<0.05$) increase in the mean PCV in group II on day 8 over day 0. The hemoglobin level in clinically healthy bitches ranges from 12 to 18 g/dL (Schalm *et al*., 1991 and Kochhar *et al*., 1996). The lower mean hemoglobin values than the normal physiological levels suggest anemia in the pyometra-affected bitches (Greene, 2006 and Hadiya *et al.,* 2020). The post-treatment PCV values observed were within the normal physiological range (37–55%) and agreed with Jena *et al*. (2013).

On days 0 and 4, the mean leucocyte counts were significantly ($p < 0.05$) higher in group II than group I (Table 1). The white blood cell count in clinically healthy and normal bitches varies between 6000–17000 cells/mm^3 (Schalm *et al*., 1991). In the present study a marked leucocytosis recorded on day '0' was in accordance with the findings of Kochhar *et al*. (1996) and Jagyaseni (2016), which decreased significantly on day 8 of the treatment in both the groups. Leucocytosis as the most consistent finding among the bitches affected with pyometra has also been reported by Jena *et al*. (2013), Lakshmikanth *et al*. (2016), and Hadiya *et al*. (2020), suggestive of bone marrow inflammatory response and diffused suppurative inflammation of uterus to combat the infection.

On day 0 and 4 mean neutrophil count was significantly ($p<0.05$) higher yet within the normal physiological range (60–70%) in group II as compared to group I. The present findings were similar to the reports of Ravishankar *et al*. (2004), Jena *et al*. (2013), and Lakshmikanth *et al*. (2016). Neutrophilia observed in 100% of affected bitches might be due to retention of purulent exudates in the uterus, which exerts a chemotactic effect on neutrophils resulting in accelerated granulopoiesis (Mudasir *et al*., 2011). On days 0 and 4, the mean lymphocyte percentage were significantly ($p<0.05$) higher in group I than in group II. Moreover, the pre-treatment lymphocyte count significantly increased on post-treatment days 4 and 8 in group II. The lymphocyte count was below the normal physiological range in the affected bitches.

Table 1: Effect of treatment (standard control and mifepristone) on physiological, hematological, and biochemical parameters of bitches affected with pyometra

Parameter	*Groups*	*Day 0*	*Day 4*	*Day 8*
		Physiological parameters		
Temperature (°F)	Control	100.8 ± 0.13	100.4 ± 0.13	100.4 ± 0.13
	Mifepristone	102.6 ± 0.46	101.2 ± 0.26	101.0 ± 0.20
Heart rate (per min)	Control	78.0 ± 1.93	80.00 ± 1.93	77.0 ± 1.93
	Mifepristone	91.3 ± 8.74	81.50 ± 1.50	77.3 ± 5.33
Respiration rate (per min)	Control	36.0 ± 2.732	32.0 ± 2.732	32.0 ± 2.732
	Mifepristone	38.6 ± 5.86	36.0 ± 3.27	35.6 ± 2.67
		Haematological parameters		
Haemoglobin (gm/dL)	Control	12.45 ± 0.36	12.50 ± 1.01	12.32^{A} ± 0.89
	Mifepristone	12.55 ± 1.07	13.30 ± 0.29	13.63^{B} ± 0.15
Packed Cell Volume (%)	Control	38.81 ± 0.760	39.63 ± 1.02	39.42 ± 0.96
	Mifepristone	33.66^{a} ± 1.73	36.58^{ab} ± 1.64	39.56^{b} ± 1.72
Total Leucocyte Count (Cells/mm^3)	Control	12466.00^{A} ± 114	12183^{A} ± 116	12000 ± 1207
	Mifepristone	37850.00^{Bb} ± 712	24733^{Bab} ± 387	12500^{a} ± 1025
Neutrophils (%)	Control	73.00^{A} ± 1.63	71.50^{A} ± 1.68	71.16 ± 1.70
	Mifepristone	84.66^{Bb} ± 2.71	78.16^{Bab} ± 2.02	70.66^{a} ± 2.800
Lymphocyte (%)	Control	34.33^{B} ± 1.72	35.66^{B} ± 1.58	36.83^{A} ± 0.90
	Mifepristone	11.00^{Aa} ± 2.32	15.50^{Aab} ± 2.23	21.33^{Bb} ± 1.40
Monocyte (%)	Control	1.50 ± 0.22	1.51^{A} ± 0.22	1.16^{A} ± 0.16
	Mifepristone	2.50 ± 0.22	2.33^{B} ± 0.21	1.66^{A} ± 0.33
Eosinophil (%)	Control	1.66^{A} ± 0.33	1.83 ± 0.30	1.33 ± 0.21
	Mifepristone	2.16^{B} ± 0.83	2.16 ± 0.83	3.16 ± 1.47
Biochemical parameters				
Total Protein (g/dL)	Control	5.07^{A} ± 0.36	5.23 ± 0.26	5.02 ± 0.31
	Mifepristone	7.09^{Bb} ± 0.40	6.07^{ab} ± 0.36	5.26^{a} ± 0.35
Albumin (g/dL)	Control	3.78 ± 0.22	3.71 ± 0.23	3.66 ± 0.23
	Mifepristone	3.76 ± 0.46	3.85 ± 0.34	4.07 ± 0.28
Globulin (g/dL)	Control	2.28^{A} ± 0.40	2.30 ± 0.40	2.09 ± 0.36
	Mifepristone	3.32^{Bb} ± 0.50	2.22^{ab} ± 0.31	1.19^{a} ± 0.38
BUN (mg/dL)	Control	32.10^{A} ± 7.17	41.57 ± 4.83	41.57 ± 4.83
	Mifepristone	49.76^{Bb} ± 3.32	43.57^{b} ± 3.66	31.55^{a} ± 1.76
Creatinine (mg/dL)	Control	0.95^{A} ± 0.08	0.94^{A} ± 0.10	0.73^{A} ± 0.10
	Mifepristone	2.66^{Bb} ± 0.29	2.21^{Bab} ± 0.30	1.47^{Ba} ± 0.17
Glucose (mg/dL)	Control	93.44 ± 6.11	91.64 ± 5.52	89.74 ± 4.81
	Mifepristone	150.10 ± 27.49	127.98 ± 15.75	90.21 ± 14.3
ALT (U/L)	Control	41.57 ± 4.83	38.92 ± 3.88	37.28 ± 3.85
	Mifepristone	49.76 ± 3.32	43.57 ± 3.66	31.55 ± 1.76
Alkaline Phosphatase (U/L)	Control	69.19 ± 6.86	68.73 ± 6.59	67.56 ± 6.39
	Mifepristone	86.83^{b} ± 9.48	73.47^{ab} ± 9.41	46.75^{a} ± 7.70

Values bearing different superscripts (upper case between groups and lower case within the group) differ significantly from each other ($p < 0.05$).

Lymphocytopenia was also reported in bitches with pyometra (Shah *et al*., 2017) and may be due to an absolute increase in neutrophil count due to severe suppurative inflammation of the uterus. There was no significant difference between groups in mean monocyte and eosinophil count. The mean monocyte count was elevated than the normal bitches which is in accordance with the reports of Samantha *et al*. (2018). The significant increase in monocyte count may be attributed to chronic inflammatory responses. The eosinophil values were in close agreement with Jena *et al*. (2013). However, lower eosinophils counts were reported by Ravishankar *et al*. (2004) and Lakshmikanth *et al*. (2016).

Effect of Treatment on Serum Biochemical Parameters

The mean serum total protein in the treatment groups varied significantly ($p < 0.05$; Table 1) post-treatment compared to pre-treatment. The mean values recorded in the affected bitches were within the normal physiological range (5.3 to 7.6 mg/dL) and corroborated with the findings of Lakshmikanth *et al*.

(2016). Emanuelli *et al.* (2012) reported higher values of total protein. Hyperproteinaemia may be developed in pyometra-affected bitches due to dehydration (Verstegen *et al*., 2008). Before treatment, the mean albumin level was slightly lower in group II compared to the normal physiological range but increased gradually to post-treatment. Hypoalbuminemia might be due to loss of albumin via the damaged kidneys and/or increased globulin production as a defense mechanism against infection (Singh *et al*., 2020[b]). Ravishankar *et al*. (2004) reported hypoalbuminemia in pyometra cases with unchanged total plasma protein concentration. After treatment, the reduction in mean globulin level was significant ($P< 0.05$; Table 1) in group II than in group I. The present finding of hyperglobulinemia in affected bitches found was in line with the report of Jena *et al*. (2013).

On day 0, the mean BUN value was significantly higher in group II than group I. Increased BUN levels in pyometra cases were observed, which then decreased following treatment. The present findings are in line with the findings of Shah *et al*. (2017) in pyometra-affected bitches. However, Jagyaseni (2016) recorded normal BUN levels in pyometra cases. There was a significantly ($p < 0.05$) lower mean creatinine level in group II than group I. An increased BUN and serum creatinine levels can be associated with prerenal, renal, or post-renal causes (Ravishankar *et al*., 2004). Similar pre-treatment findings were also recorded by Lakshmikanth *et al*. (2016) and Hadiya *et al.* (2020). Elevated serum creatinine might be due to organic tissue destruction or dehydrations (Mudasir *et al*., 2011). Though glucose level was apparently higher in group II on days 0 and 4, there were no significant differences. No pertinent report on the effect of glucose in bitches affected with pyometra was found in the literature.

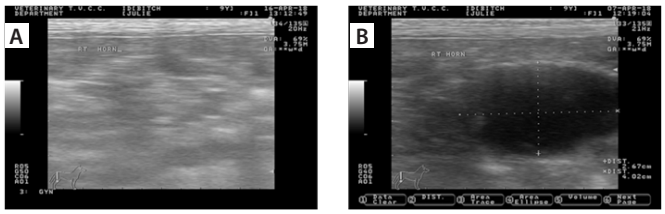

Fig. 1: Ultrasonographic images of the uterine horn of pyometra affected bitch. (A) Pre-treatment, increase echogenicity in the tubular horns, (B) Post-treatment, tubular horns filled with anechoic to hypoechoic area

Table 2: Post-treatment recovery of bitches affected with pyometra

Observations	*Group I (Control)*	*Group II (Mifepristone)*
No. of bitches treated	6	6
No. of bitches recovered	3	6
Recovery rate (%)	50	100
No. of bitches showed estrus	0	5
Recurrence of pyometra in bitches	2	1
Recurrence rate (%)	67	17

No significant difference was observed in the mean activity of ALT between groups on days 0, 4, and 8 (Table 1), and values were within the normal physiological range (10-94 U/L). These findings corroborated with the reports of Samantha *et al*. (2018). In group II, the mean ALP values were significantly ($p < 0.05$) higher and reduced on days 4 and 8 over day 0. Similar findings were reported by Dabhi *et al*. (2007) and Shah *et al*. (2017). Three to four-fold increased plasma ALP activity was found in pyometric bitches due to damage to the vital organs and bone marrow associated with toxemia than healthy bitches (Hagman *et al*., 2009).

Post-Treatment Recovery Response of Bitches

The ultrasound examination post-treatment (Day 8) revealed a reduction in uterine lumen, the thickness of the uterine wall, and decreased echogenicity compared to day 0 indicative of treatment response (Fig. 1). These findings agreed with Samantha *et al*. (2018) and Singh *et al*. (2018). Recovery rate and recurrence rate in bitches of group I were recorded as 50% and 67%, respectively, as against 100% and 17% in group II (Table 2). The bitches ($n = 3$) that did not recover within eight days of treatment were subjected to ovario-hysterectomy.

Bitches treated with progesterone receptor antagonist (mifepristone, group II) were cured entirely with a 100% recovery rate. Only one bitch showed recurrence of pyometra was subjected to ovario-hysterectomy. Fieni (2006) reported 84.4% and 83.33% recovery rates after using cloprostenol, which was higher than the present study. The present finding of recovery rate in the mifepristone-treated group was in close agreement with Shah *et al*. (2016). A lower recovery rate (50 to 83.3%) was also observed by Fieni (2006). Nevertheless, the success of treatment was associated with improved activity and appetite and an initial increase in vulvar discharge followed by diminished and serous discharge. Bitches treated with mifepristone showed some side effects like anorexia, excitation, vomiting, and diarrhea.

From the present findings, it could be concluded that mifepristone (progesterone receptor antagonist) proves to be a safe and efficient drug for opening the cervical canal in bitches. A combination with prostaglandin emerges as a new therapeutic possibility with a high success rate for the treatment of pyometra.

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