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Optimum timing for operation in bitches with pyometra related to endotoxemia

Melih UÇMAK[1,*], Çağatay TEK[1], Mehmet C. GÜNDÜZ[1], Ahmet SABUNCU[1], Adem ŞENÜNVER[1], Funda A. BAĞCIGİL[2], Tülay BAKIREL[3]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, İstanbul University, 34320 Avcılar, İstanbul - TURKEY

[2]Department of Microbiology, Faculty of Veterinary Medicine, İstanbul University, 34320 Avcılar, İstanbul - TURKEY

[3]Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, İstanbul University, 34320 Avcılar, İstanbul - TURKEY

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Abstract: The aim of this study was to evaluate the plasma levels of endotoxin in bitches with pyometra and to investigate the effects of preoperative supportive therapy on plasma endotoxin levels (LPS), the complete blood count, serum biochemistry parameters (alanine amino transferase (ALT), alkaline phosphatase (ALP), total protein, albumin, urea, and creatinine), clinical health status at the 12th, 24th, and 48th h following presentation, and to explore if they were correlated. Randomly allocated into 2 equivalent groups were 20 bitches with pyometra, aged from 3 to 16 years (mean 9.15 ± 3.65 years). After collection of the samples, bitches in ovariohysterectomy without premedical supportive therapy (group OH) were operated on without delay. Bitches in ovariohysterectomy with premedical supportive therapy (group M) were operated on with a 48 h delay. Bitches with pyometra receiving supportive therapy had no significant difference in endotoxin levels ($P < 0.001$), total blood counts, or serum biochemical parameters, which were evaluated along with food intake and activity scores during the 2 day period. There was a moderate positive correlation between LPS (endotoxin) and WBC (white blood cell), ALP (alkaline phosphatase), and creatinine levels and a moderate negative correlation between LPS, activity, and food intake scores at the 12th h evaluation. In conclusion, against the probability of high levels of circulating endotoxins in pyometra cases, bitches must undergo surgery at an optimum time, which is thought to be within 12 h of supportive therapy.

Key words: Pyometra, bitches, endotoxin, supportive therapy

Pyometralı köpeklerde en uygun operasyon zamanının endotoksemi ile ilişkilendirilerek belirlenmesi

Özet: Bu çalışmanın amacı; pyometralı köpeklerde plazma endotoksin seviyelerini değerlendirmek, operasyon öncesi destekleyici tedavinin, kliniğe getirilmelerini izleyen 12, 24, 48. saatlerde plazma endotoksin seviyeleri (LPS), total kan sayımı, bazı biyokimyasal parametreler (alanin amino transferaz (ALT), alkalin fosfataz (ALP), total protein, albumin, üre, kreatinin) ve klinik sağlık durumu üzerine etkilerini araştırmak ve varsa korelasyonlarını açığa çıkarmaktı. Yaşları 3

* E-mail: dr_veterinarian@yahoo.com

ile 16 arasında değişen (ortalama 9,15 ± 3,65 yaş) pyometralı 20 adet dişi köpek rastgele iki eşit gruba ayrıldı. Örneklerin toplanmasından sonra, operasyon öncesi medikal destek tedavisi uygulanmayan grup (OH), gecikme olmaksızın ameliyat edildi. Operasyon öncesi medikal destek tedavisi uygulanan gruptaki (M) dişi köpekler 48 saatlik bir gecikme ile ameliyat edildi. Destek tedavisi alan pyometralı dişi köpeklerde iki gün boyunca endotoksin seviyeleri, total kan sayımı, değerlendirmeye aldığımız biyokimyasal parametreler ve gıda alımı ile aktivite skorlarında önemli fark bulunmamıştır ($P < 0,001$). Onikinci saatteki değerlendirmede LPS ile WBC (lökosit), ALP, kreatinin seviyeleri arasında orta derecede pozitif korelasyon ve LPS ile aktivite, gıda alımı skorları arasında orta derecede negatif korelasyon bulundu. Sonuç olarak, pyometra vakalarında dişi köpekler sirkülasyonlarındaki muhtemel yüksek seviyedeki endotoksinlere rağmen 12 saat destekleyici tedavi süresi içinde olan optimum bir zamanda ameliyata alınmalıdır.

Anahtar sözcükler: Pyometra, dişi köpek, endotoksin, destekleyici tedavi

Introduction

Pyometra, inflammation of the uterus with accumulation of pus, usually occurs because of a bacterial interaction with an endometrium that has undergone pathologic changes by hormonal stimulation (1,2). Besides the well described clinical symptoms, hematological symptoms and systemic effects (1-5), little is known about the relation between pyometra and endotoxin (6). Predominantly gram-negative bacterial infection is responsible for the formation of the disease. Of these, *Escherichia coli* is the major causative agent in canine pyometra (1,3,6).

Endotoxin (LPS) is a lipopolysaccharide part of the outer cell wall of gram-negative bacteria (7). Circulation LPS is extremely toxic to host cells, is both chemical and heat stable, and its toxicity is not reduced by chemotherapeutical agents. Endotoxin in blood is mostly disposed of by the hepatic reticuloendothelial system, macrophage phagocytosis, and specific anti-LPS antibodies (8,9). After the depression of the reticuloendothelial system or the insufficiency of these mechanisms, endotoxin levels increase. Elevated blood endotoxin concentrations in pyometra (5,6) have many pathophysiologically destructive pathways. Affected haemopoietic tissue causes left shift leucocytosis; an affected liver causes hepatocyte degeneration, cholestasis, and depression of the hepatic reticuloendothelial system; affected lungs cause pulmonary edema and congestion; and affected kidneys cause glomerulonephritis, renal tubular damage, polyuria, polydipsia, electrolite loss, and dehydration. Additionally, the endotoxin causes low cardiac output (CO), arterial blood pressure (BP), hypotension, lactic acidosis, and eventually shock and death (7,9-13).

Although there are some medical therapy options using aglepristone and $PGF_{2\alpha}$ (14-16), ovariohysterectomy still maintains its success as a radical and effective solution for the treatment of pyometra complex in bitches (1,4,16). However, higher anesthesia risks and lower success rates may result due to the abnormalities caused by septicemia and toxemia (1,17). The ability of the animal to detoxify anesthetics is depressed by toxemia. Toxins cause hepatocellular and renal damage, influence drug protein binding, increase the pharmacologic activity of anesthetics and change their excretion (18). A significant rise in venous PCO_2 production is also reported in early endotoxemia along with the expected rise in lactic acid production (19). The ideal situation is to stabilize the patient before surgery, but this is not always possible (1,12,17).

The aims of the research presented were: (a) to evaluate the plasma levels of endotoxin in bitches with pyometra and to explore if they are correlated with complete blood count or serum biochemistry parameters, (b) to investigate the effects of preoperative supportive therapy with antibiotics (enrofloxacin, Baytril-K 5%, Bayer) and intravenous fluids on the plasma toxin levels on some blood parameters and clinical health status at the 12th, 24th, and 48th h following presentation. By evaluating these data, we aimed to determine an optimum time for operating (the ideal amount of time to stabilize a bitch before the operation) on pyometric bitches, which is thought to reduce the risks of anesthesia. Our further aim was to evaluate the plasma levels of endotoxin, complete blood count, serum biochemistry parameters, and the clinical health status of the bitches during the week following the operation and to compare these data to the data collected on the day of presentation.

Materials and methods

We randomly allocated 20 bitches with pyometra into 2 equivalent groups: ovariohysterectomy without premedical supportive therapy (OH) and ovariohysterectomy after premedical supportive therapy (M). In order to assess surgery risks, activity, food and water intake parameters, complete blood count analysis, biochemical blood parameters (urea, creatinine, total protein, albumin, ALT, and ALP), and plasma endotoxin concentrations were evaluated. Activity and food intake were evaluated by the following scores: 1 = very bad and 5 = very good.

Blood samples were taken from the cephalic vein in K3 EDTA tubes (FL Medical, Italy) for complete blood count analysis and in plain tubes (BD Vacutainer®, UK) for biochemical parameters. For endotoxin analysis, blood samples were first taken in pyrogen-free lithium heparinized test tubes (Venosafe, Terumo Europe) and, after being centrifuged for 5 min at 3000 × *g*, plasma samples were transferred into endotoxin-free Eppendorf tubes (Eppendorf, Biopur, USA). Plasma samples were frozen and stored at –20 °C until the analysis.

Complete blood count and biochemical analysis were performed using routine laboratory methods.

Concentrations of endotoxin in blood plasma of the bitches were analyzed using a kinetic turbidimetric Limulus amoebocyte lysate (LAL) assay. After bringing the samples to room temperature, each were mixed for approximately 30 s on a vortex mixer and then were diluted with LRW (Acila®, AG, Marfelden) at the rate of 1/50 in order to minimize the interference factors and prevent coagulation upon heating. Subsequent procedures against interferences were done by heating the diluted samples for 5 min in boiling water (Benmari Nüve BN402). Standard endotoxin concentrations were prepared by serial dilution starting with the highest or "stock" concentration, CSE (E. coli 0113:H10, Associates of Cape Cod, Inc., East Falmouth, MA, USA). The negative control was the LRW used to dilute the samples for the test. The assay sensitivity range was designed between 0.005 and 50 EU/mL. Samples and controls were transferred to the microplate and 100 µL of LAL reagent (Pryotell®-T, Associates of Cape Cod, Inc., East Falmouth) was added to each as rapidly as possible using a repetitive pipette. The microplate (Becton Dickinson Labware, USA) was placed in the microplate reader (Biotek®, ELX-808), which had been set to read at 405 nm and incubate at 37 ± 1 °C. Start was given by kinetic software (Gen5, Biotek®). Results taken as EU/mL were converted to pg/mL by the equation 1 EU/mL = 100 pg/mL (20).

Uterine swabs (Cultiplast®, LP Italiana, Italy) were cultured onto nutrient agar supplemented with 7% sheep blood and MacConkey agar plates, and incubated at 37 °C for 24-48 h under microaerobic and aerobic conditions, respectively. Isolated bacterial strains were identified by standard techniques.

After the examination and collection of the samples mentioned, bitches in ovariohysterectomy without premedical supportive therapy (group OH) were operated on without delay. The anesthesia protocol consisted of SC atropine (Atropin, Vetaş, Turkey) as premedication (0.005 mg/kg), followed by the induction of the anaesthesia 15 min later with IV propofol (Propofol 1%, Fresenius-Kabi, Turkey) (6 mg/kg), and the intubation and maintenance with isoflurane (Forane® likid, Abbott, England).

Bitches in ovariohysterectomy after premedical supportive therapy (group M) received supportive SC antibiotics (Enrofloxasin, Baytril-K 5%, Bayer, Turkey) at a dosage of 0.1 mL/kg, an IM vitamin combination (Epargriseovit, Deva, Turkey), and intravenous fluid therapy (5% dextrose + ringer lactate solution, Eczacıbaşı, Baxter, Turkey) at a dosage of 20 mL/kg once a day. Bitches were examined and the samples mentioned were collected at the 1st h of presentation and at the 12th, 24th, and 48th h after presentation. Bitches were operated on after a 48 h delay with the same anesthesia protocol mentioned before.

Antibiotic and vitamin injections were carried on for 7 days following the operation in both groups. The last examinations and sample collections for both groups were performed on the 7th day after the operation.

Statistical analyses were performed by the use of SPSS 10.0. Repeated measures ANOVA and the contrast test method were used for comparison of plasma toxin levels, complete blood count, biochemical blood parameters, activity, and food intake scores in the study groups (M and OH). In

the statistical model, the study groups appeared as between-subject factor, and measuring time (1st h and 7th day) appeared as within-subject factor. Repeated measures ANOVA and the contrast test method, which measures time (1st, 12th, 24th, and 48th h and the 7th day.), appeared as within-subject factor and were used for analyzing the time related changes of the signed parameters in group M. The coefficients of correlations between various parameters in group M were calculated by the Pearson correlation method. The mean values of the results were given as X ± SD. A coefficient of correlation below 0.200 was evaluated as low, between 0.200 and 0.600 as moderate, and above 0.600 as high.

Results

The 20 dogs diagnosed with pyometra ranged in age from 3 to 16 years (mean 9.15 ± 3.65 years). Vaginal discharge was present in 15 of the bitches (75%) with open cervix pyometra and 5 of the bitches (25%) had closed cervix pyometra. Among the 20 bitches with pyometra, 18 had polyuria (90%), 18 had polydipsia (90%), 6 had vomitus (30%), 6 had dehydration (30%), 6 had abdominal extension (30%), 4 had diarrhea (20%), and 4 had elevated body temperatures >39.2 °C (20%). Heart rates (70-120 beats/min) and respiratory rates (10-30 breaths/min) were detected as being in the normal range. A bacteriological examination showed a predominantly gram-negative bacterial infection. *Escherichia coli* was isolated from 12 dogs (60%), *Streptococcus* was isolated from 3 dogs (15%), *Bacillus* spp. were isolated from 2 dogs (10%), *Staphylococcus* was isolated from 1 dog (5%), and bacteria were not isolated from 2 dogs (10%).

Mean values of endotoxin concentrations, complete blood count and biochemical parameters, and the mean scores of activity and food intake parameters for group M (on the 1st, 12th, 24th, and 48th h and 7th day postoperative) and for group OH (on the 1st h and 7th day postoperative) were given in Tables 1 and 2, respectively.

Endotoxin concentrations at the 1st h and 7th day postoperative for group M were compared with those

Table 1. Mean values of endotoxin concentrations, complete-blood-count and biochemical parameters and the mean scores of activity and food intake parameters belonging to the group M.

Group M	1st h	12th h	24th h	48th h	7th day
Endotoxin (pg/mL)	125.560 ± 11.165 a	100.330 ± 13.11 a	119.380 ± 29.109 a	126.700 ± 14.674 a	21.960 ± 4.026 b
WBC (× 103/μL)	38.531 ± 7.283 a	35.370 ± 6.233 a	38.120 ± 6.452 a	36.250 ± 5.975 a	19.270 ± 1.882 b
RBC (× 106/μL)	5.002 ± 0.426	4.862 ± 0.398	4.704 ± 0.359	4.767 ± 0.414	5.031 ± 0.341
HGB (g/dL)	10.930 ± 1.069	10.670 ± 1.008	10.400 ± 0.946	10.400 ± 1.052	11.170 ± 0.961
HCT (%)	32.015 ± 2.702	31.150 ± 2.586	29.990 ± 2.294	30.060 ± 2.673	32.390 ± 2.606
PLT (× 103/μL)	321.000 ± 56.880 a	276.700 ± 50.670 a	263.000 ± 46.480 a	295.800 ± 48.757 a	541.500 ± 43.707 b
MCV	63.680 ± 1.263	63.640 ± 0.809	63.940 ± 1.125	63.410 ± 1.005	64.370 ± 1.260
MCH (pg)	21.280 ± 0.515	21.200 ± 0.430	21.380 ± 0.549	21.160 ± 0.578	21.600 ± 0.512
MCHC (%)	33.780 ± 0.505	33.600 ± 0.554	33.780 ± 0.752	33.710 ± 0.647	33.910 ± 0.660
ALT (IU/L)	27.900 ± 2.927	27.400 ± 5.332	27.500 ± 5.799	26.200 ± 4.052 c	37.100 ± 4.598 d
ALP (IU/L)	203.100 ± 39.675 a	201.900 ± 39.989 a	202.600 ± 39.521 a	200.900 ± 37.682 a	132.600 ± 31.166 b
T.Protein (g/dL)	8.500 ± 0.301 a	8.480 ± 0.148 a	8.500 ± 0.132 a	8.500 ± 0.171 a	7.800 ± 0.175 b
Albumin (g/dL)	2.680 ± 0.109	2.660 ± 0.121	2.500 ± 0.075 a	2.560 ± 0.069 a	2.880 ± 0.129 b
Urea (mg/dL)	27.600 ± 6.110	19.000 ± 1.506	19.100 ± 2.268	19.770 ± 2.487	22.000 ± 3.225
Creatinine (mg/dL)	0.998 ± 0.040	0.970 ± 0.033	0.990 ± 0.041	1.010 ± 0.038	1.010 ± 0.059
Activity	3.0 ± 0.211 a	3.2 ± 0.200 a	2.9 ± 0.233 a	3.0 ± 0.211 a	4.7 ± 0.213 b
Food intake	c 2.1 ± 0.314 a	1.9 ± 0.277 a	d 1.6 ± 0.306 a	d 1.7 ± 0.335 a	4.3 ± 0.213 b

There is a statistical importance between the values shown with different letters in the same line (a/b $P < 0.001$), (c/d $P < 0.05$).

Table 2. Mean values of endotoxin concentrations, complete-blood-count and biochemical parameters and the mean scores of activity and food intake parameters belonging to the group OH.

Group OH	1st h	7th day
Endotoxin (pg/mL)	153.890 ± 31.379 a	23.500 ± 8.518 b
WBC (× 103/μL)	39.290 ± 6.835 a	16.440 ± 2.028 b
RBC (× 106/μL)	5.634 ± 0.389	5.061 ± 0.306
HGB (g/dL)	12.570 ± 0.957	11.450 ± 0.927
HCT (%)	36.080 ± 2.391	33.270 ± 2.189
PLT (× 103/μL)	212.300 ± 48.640 a	408.800 ± 52.608 b
MCV	63.780 ± 1.151	65.950 ± 1.096
MCH (pg)	22.240 ± 0.587	22.100 ± 0.478
MCHC (%)	35.050 ± 0.860	33.440 ± 0.648
ALT (IU/L)	22.100 ± 3.624	36.400 ± 4.763
ALP (IU/L)	228.600 ± 41.304 a	147.400 ± 25.736 b
T.Protein (g/dL)	8.330 ± 0.274	8.000 ± 0.208
Albumin (g/dL)	2.840 ± 0.112	2.940 ± 0.107
Urea (mg/dL)	34.600 ± 7.277	28.900 ± 4.214
Creatinine (mg/dL)	1.280 ± 0.054	1.100 ± 0.069
Activity	3.2 ± 0.205 a	4.9 ± 0.167 b
Food intake	2.9 ± 0.296 a	4.8 ± 0.178 b

There is a statistical importance between the values shown with different letters in the same line (a/b $P < 0.001$), (c/d $P < 0.05$).

belonging to group OH. No significant difference in the endotoxin concentrations were observed between the 2 groups, but the change connected with time was similar and the effect of time was important. Endotoxin concentrations were significantly higher for the 1st h ($P < 0.001$) compared with those on 7th day postoperative. Mean endotoxin concentration for the 1st h in group M was detected as 125.560 ± 11.165, with a lower bound of 100.303 and upper bound of 150.817. The mean endotoxin concentration for the 7th day postoperative in group M was detected as 21.960 ± 4.026, with a lower bound of 12.852 and upper bound of 31.068. When endotoxin concentrations on the 1st, 12th, 24th, and 48th h and 7th day postoperative for group M were compared among themselves, no significant difference was observed between the concentrations of the 1st, 12th, 24th, and 48th h, but the concentration on 7th day postoperative was significantly lower than those on the 1st, 12th, 24th, and 48th h ($P < 0.001$).

There was a moderate positive correlation between LPS and WBC ($r = 0.418$), LPS and ALP ($r = 0.532$), and LPS and creatinine ($r = 0.330$) levels and a moderate negative correlation between LPS and activity ($r = -0.543$) and LPS and food intake scores ($r = –0.489$) at the 12th h evaluation (Table 3). There was also a moderate negative correlation between WBC and activity ($r = –0.461$) and WBC and food intake scores ($r = –0.348$) and a high positive correlation between RBC and HGB ($r = 0.990$) and RBC and HCT ($r = 0.983$) levels.

Discussion and conclusion

Escherichia coli dominance detected as 60% of the uterine swabs was in accordance with the consensus that the most frequently isolated bacteria in pyometra cases is *Escherichia coli* (1,3,12). The vaginal discharge rate found in our study (75%) showed compatibility with other studies (2,3,21). Observed clinical symptoms and rates in our study (polyuria 90%, polydipsia 90%, vomitus 30%, dehydration 30%, diarrhea 20%, and elevated body temperature 20%) are in line with the other studies (2,3,21).

Table 3. Coefficient of correlation between the endotoxin and WBC, ALP, creatinine, activity, and food intake on the 1st, 12th, 24th, and 48th h and the 7th day.

Endotoxin	WBC	ALP	Creatinine	Activity	Food
1st h	0.109	0.382	0.464	–0.382	–0.709
12th h	0.418	0.532	0.330	–0.543	–0.489
24th h	0.147	0.533	0.642	–0.139	–0.340
48th h	0.460	0.669	0.437	–0.533	–0.537
7th day	0.167	0.497	0.490	–0.334	–0.565

In pyometra cases, bitches have higher total white blood cell counts (2,3,21). In this study the mean value of WBC counts at the 1st h was 38.531 ± 7.283 ($\times 10^3/\mu L$) in group M and 39.290 ± 6.835 ($\times 10^3/\mu L$) in group OH, with a leucocytosis rate of 90% for each. The incidence of anemia in this study was 60% in both groups, with a mild to marked reduction in the concentrations of RBC, HGB, and HCT (1st h values), which was in line with the other studies (2,22).

In pyometra cases, alanine aminotransferase (ALT) is usually within normal limits, whereas alkaline phosphatase (ALP) is often elevated (1-3). Hyperproteinemia and hypoalbuminemia is common, while serum urea and creatinine levels are not among the consistent findings (2,3,12,21). In this study, bitches with pyometra had a marked elevation of ALP and total protein, while no consistent change occurred in the concentrations of ALT, albumin, urea, and creatinine levels. A decrease in albumin concentrations may occur during the later stages of hepatic changes following hyperproteinemia. The lack of severe hepatic disease or renal failure in our study can explain the normal values of the serum albumin, urea, and creatinine concentrations.

Reported blood levels of endotoxin in bitches with pyometra and bitches that are healthy differ depending on the researcher. Okano et al. (6) found that the endotoxin concentrations of bitches with pyometra were: poor prognosis; 74.2 ± 18.3 pg/mL, good prognosis; 9.5± 11.3 pg/mL, and healthy prognosis; 3.4 ± 2.8 pg/mL. Hagman et al. (5) reported that the mean endotoxin levels were 49 pg/mL (a range of 20 to 123 pg/mL) in the pyometra group and 28 pg/mL (a range of 14 to 52 pg/mL) in the control group. Wessels and Wells (23) measured endotoxin levels in bitches with pyometra at a mean value of 438 pg/mL. The values we found in pyometric bitches (both in the M and OH group) were higher than those reported by Okano et al. (6) for pyometra good prognosis but are close to the values that they reported for pyometra poor prognosis. In contrast to their assessment that these values are in the poor prognosis category, none of the bitches in our study died. While our results in pyometric bitches were close to the upper bound levels obtained by Hagman et al. (5), they are much lower than those reported by Wessels and Wells (23). In this study the endotoxin values of healthy dogs are in line with the value reported by Hagman et al. (5). The difference between the detected endotoxin values may be caused by sampling procedures, quantitation of endotoxin, or plasma pretreatment. There are 3 basic LAL test methodologies: gel-clot, chromogenic, and turbidimetric. While Wessels and Wells (23) used the chromogenic LAL test method, we used the kinetic-turbidimetric LAL test. Our choice of using a high dilution rate (1/50) and heating the diluted samples for 5 min in boiling water in order to minimize the interference factors may also have created differences.

Bitches with pyometra that are toxemic have lower success rates (17). Okano et al. (6) state that it is necessary to initiate effective treatment in the early stages of pyometra for bitches with high blood endotoxin concentrations. Preoperative supportive treatment can include antibiotics and intravenous fluid therapy, but reports of endotoxin concentration change associated with antibiotic treatment are distinct. Shenep and Mogan (24) treated *Escherichia coli* sepsis in rabbits with either antibiotics or a placebo and measured the increase of free endotoxin plasma levels at 10-2000 fold, in spite of decreasing levels of bacteremia. They found this increase relevant to the disintegration of circulating dead bacterial cells, as no change occurred in the total endotoxin content of the plasma; however, the circulating free endotoxin had a greater effect on the host compared to the circulating cell bound endotoxin. In a study conducted by Tsumuro et al. (13), in vitro cultured *Escherichia coli*, with the antimicrobial agent flomoxef, showed an increased endotoxin concentration without bacterial growth. In contrast, an in vivo rabbit model of experimental peritonitis showed no significant increase in the plasma endotoxin levels, confirming the existence of an endotoxin rapid clearance mechanism in vivo. Wessels and Wells (23) reported that the circulating plasma endotoxin concentration of bitches with pyometra at a mean value of 559 ± 112 pg/mL was insignificantly reduced to 437 ± 136 pg/mL, following the conventional therapy with antibiotics and supportive ionic fluids after 24 h. In this study, the plasma endotoxin concentration at the 1st h was 125.560 ± 11.165 pg/mL, which was insignificantly reduced to 100.330 ± 13.11 pg/mL at the 12th h with preoperative supportive antibiotic and intravenous fluid therapy; however,

at 24th and 48th h, the endotoxin values began to increase insignificantly, close to the value detected at the 1st h. By acting in the same direction with the hepatic reticuloendothelial system, macrophage phagocytosis, and specific anti-LPS antibodies, intravenous fluid therapy may dilute the circulation endotoxin concentration insignificantly rather than the release from the pus filled uterus and release by the lysis of bacterial cells affected by the antimicrobial agents at the 12th h. However, the unremoved infected uterus is a major source of endotoxin release and endotoxins depress reticuloendothelial function by their direct action, production of reticuloendothelial depressing substance (RDS), and by the reduction of the activity of hepatic Kupffer cells, increasing their own circulating concentration. It can be hypothesized that in such a disease the circulating endotoxins cannot be fully erased, and as their existence above the normal values damage multiple organs at gradually increasing levels, even carrying to an irreversible state, there is no reason for prolonging the preoperative supportive therapy through the high levels of circulating endotoxins.

In conclusion, bitches with pyometra receiving supportive therapy have no significant ($P < 0.001$) difference in endotoxin levels, total blood counts, or serum biochemical parameters, which we evaluated along with food intake and activity scores during the 2 day period. Against the probability of high levels of circulating endotoxins in pyometra cases, bitches must undergo surgery at an optimum time which is thought to be within 12 h of supportive therapy. The disturbed endotoxin concentrations, leucocyte counts, ALP and total protein levels, activity, and food intake scores will come to the normal levels on the 7th day postoperative.

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