Benha Veterinary Medical Journal 42 (2022) 170-175

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Predictive markers and risk factors in canine and feline pyometra under Egyptian conditions

Zienab Abdelbaset *, Gamal A. Sosa, Alaa Abdel-Ghaffar, Mohamed M.M. Kandiel

Department of Theriogenology, Faculty of Veterinary Medicine, Benha University, Egypt

ARTICLE INFO

Keywords

Bitch, Histopathology
Pyometra, Queen
Ultrasound

Received 05/04/2022
Accepted 17/04/2022
Available On-Line 01/10/2022

ABSTRACT

Pyometra is the most prevalent reproductive disorder threatens dogs and cats' life. This study aimed to address the relation between pyometra, and oxidative stress and hormonal milieu. Bitches (n= 200) and queen (n= 281) admitted to veterinary clinics from which ten animals per each were pyometric. Health examination, ultrasound investigation and blood sampling were accomplished, besides histopathology of uterus in animals underwent elective ovariohysterectomy. The separated sera were assayed for inflammatory marker (C-reactive protein; CRP), oxidative stress biomarkers (glutathione peroxidase (GPX), catalase (CAT), total antioxidant capacity (TAC), nitric oxide (NO), superoxide dismutase (SOD), lipid peroxidation (MDA)), and hormones (progesterone (P4), estrogen (E2)). Animals with pyometra showed fever, polydipsia, polyuria, vomiting and purulent vulvar discharge. Ultrasound examination revealed the presence of anechoic to hypoechoic uterine fluid with an increased wall thickness. There was a significant increase in CRP (P< 0.01 and 0.05), NO (P< 0.05), MDA (P<0.01 and 0.005), P4 (P< 0.05 and P< 0.0001), and a decrease in SOD (P< 0.0005 and 0.005), CAT (P< 0.05), and TAC (P< 0.05), in bitches and queens, respectively. Histopathology revealed a suppurative inflammation and heavy leucocytic infiltration in all uterine layers, and severe degenerative changes in endometrial glands with neutrophils-infiltrated homogeneous eosinophilic substances filled the lumen. The ultrasound morphometric measures (uterine diameter, and wall thickness, and lumen and wall echo-pattern) were positively correlated with CRP and NO, and negatively correlated with TAC. In conclusion, pyometra greatly influenced the health status and reproductive efficiency of bitches and queens through altering oxidative defense mechanism.

1. INTRODUCTION

Pyometra in pet animals (dogs and cats) is a serious problem affects their life. About 20% of intact dogs experience pyometra by the age of ten years (Hagman et al., 2014). Pyometra is less common in cats than bitches since they are an induced ovulatory, however, cats whose owners do not usually go for elective neutering commonly suffer from this disorder at an age beyond 5-7 years of age (Hollinshead and Krekeler, 2016). They also recorded a high incidence of pyometra in cats is observed at diestrus or pseudopregnancy, during progesterone dominance that lasts approximately 40 days. There are similarities in clinical symptoms, etiology and diagnostic methods of pyometra in dogs and cats, with progesterone exposure predisposing to uterine bacterial infection (Hagman et al., 2014). Progesterone causes hyperplasia of the endometrium increase the secretory activity of the endometrial glands especially of the endometrial glands leading to and (Lawler and Johnston, 1995). Bacterial invasion of the uterus generates systemic inflammatory response syndrome (an exaggerated body defense response to harmful stressor) in nearly 60% of cases (Karlsson et al., 2012). C-reactive protein (CRP) is a classical acute phase protein appears in high concentration during early stage of any infection, so its level can be used as a prognostic marker for pyometra (Hillström et al., 2014).

The clinical signs of pyometra are often vague and differ between cases. Animals suffer from pyometra show signs of health disturbance (e.g., lethargy, vomiting, polydipsia) and even animal death may occur (Hagman, 2018). Lacking vulvar discharge represents a great challenge in pyometra diagnosis. Ultrasound provides useful information aid in the diagnosis/assessment of the condition non-invasively (Rautela and Katiyar, 2019). Oxidative stress is a usual phenomenon in the body. Many disturbances/diseases occur due to oxidative stress in association with the exhaustion of antioxidant system (Amin et al., 2021). One of the most important antioxidant enzymes in the biological system is superoxide dismutase (SOD) which converts superoxide anion into hydrogen peroxide that is transformed to water by catalase and glutathione peroxidase (Rahman et al., 2006). Superoxide radicals are potent inhibitors for nitric oxide creates peroxynitrite which is a strong reactive oxygen species (ROS) resulting in hydrogen peroxide and oxygen production (Grieve and Shah, 2003). MDA level is a mirror to lipid peroxidation status in the body, and therefore it is a common marker of antioxidant status and oxidative stress (El-Bahr and El-Deeb, 2016). For authors knowledge, scarce research concerned with the inter-relationship between pyometra, oxidative stress, and the occurrence of the problem in dogs and cats. Therefore, the present study aimed to focus the lights on the alteration of oxidative status and

Correspondence to: zienbabdelbaset@yahoo.com

steroid hormones (progesterone (P4) and estrogen (E2)) in pyometra (diagnosis by clinical and ultrasound examination) and the histopathological changes in bitch and queen.

2. MATERIAL AND METHODS

Research ethics
All animal manipulations and samplings were approved by the Ethics Committee of Faculty of Veterinary Medicine Benha University (BUFVTM06-02-22)

2.1. Animals and clinical examination
In the present study about 481 of pet animals (200 bitches and 281 queens) of mixed breeds and age (1-9 years of bitches and 1.5-8 years of queens) were examined, 20 of them showed health distress assumed pyometra (10 bitches and 10 queens). Animals were admitted to the clinic at theriogenology Dept., Fac. Vet. Med., Cairo University as well as private pet clinics in Cairo during period from June 2020 to Dec. 2021, animals were examined for health status and symptoms of pyometra include body temperature, general inspection including animal demnour, body condition according to Misk and EL-sherry (2020).

2.2. Ultrasound examination
Ultrasound (US) examination was conducted while the animal in dorsal recumbent position using a real-time B-mode ultrasound scanner (Akai AK-30 Ultrasound, China) equipped with a multifrequency micro convex transducer according to Kähn (2004) in brief, a carboxymethyl-cellulose gel was applied in the area between the two rows of mammary glands from the pubis to umbilicus. The ultrasound probe was firmly positioned against the skin of the abdominal wall to direct the ultrasound beam ventro-dorsally to locate the bladder. Urinary bladder (UB) was identified in the pelvis as a hollow anechoic organ and served as a land mark for uterine examination. The US probe proceeded cranially to visualize the uterus at the fundus of UB with probe rotation from side to side. Ultrasound was performed to evaluate uterine size, uterine wall thickness and fluid accumulation within the lumen of the uterus (Misk and EL-sherry, 2020).

2.3. Blood sampling and assessment
Non-hemolyzed blood samples were collected from animals through cephalic venipuncture into plain vacutainer tubes under aseptic precautions according to Sharif et al. (2013). Blood samples were centrifuged at 3000 rpm for 15 min and the harvested sera were stored at -20 °C according to Amin, et al. (2014), until being assayed.

2.3.1. Measurement of C-reactive protein
C-reactive protein (CRP) level was measured using the ELISA method using a commercial kit (Ichroma CRP-25, Tridelta, Ireland) according to Pepys and Hirschfield, (2003)

2.3.2. Measurement of antioxidants biomarkers
superoxide dismutase (SOD), Glutathione peroxidase (GPx) and catalase enzyme activity, total antioxidant capacity (TAC), nitric oxide (NO) and malondialdehyde (MDA) were measured colorimetrically using commercial kits (SD 25 21, GP25 24, CA 25 17, TA 25 13, NO 25 33 and MD 25 29 supplied by BIO-DIAGNOSTIC, Giza, Egypt) (Nishikimi et al., 1972; Paglia and Valentine ,1967; Aebi, 1984; Koracevic et al., 2001; Montgomery and Dymock, 1961; Ohkawa et al., 1979, respectively).

2.3.3. Steroid hormone assessment:
Serum progesterone (P4, ng/ml) and estradiol (E2, pg./ml) concentrations were quantitatively estimated by ELISA method according to Hanita and Hanisah (2012) and Goldstein et al. (1982), respectively, using commercial ELISA kits (EIA-1561and EL1-1254, respectively) supplied by DRG Diagnostics GmbH, Germany and Chemux BioScience, South San Francisco, USA

2.4. Histopathological examination
Small tissue specimens (1cm³) cut from uterine tissues after ovariohysterectomy were processed for histopathological examination (Maya-Pulgarin et al., 2017). Samples were fixed in 10% in neutral formalin for 72 hours, dehydrated in ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin wax. The paraffin tissue block was sectioned at 5 µm thickness, stained with Hematoxylin-Eosin (H&E) and examined under a Nikon Eclipse E800 light microscope according to Anderson and Bancroft, (2002). The OMAX 9.0 MP USB digital camera (China) was used for image capture. Quantitative analysis of histopathological changes (epithelial thickness (ET), endometrial glands diameter (EGD), endometrial glands wall thickness (EGWT) and myometrium thickness (MT)) according to Floyd (2002) and uterine ultrasound parameters (uterine horn diameter (UHD), uterine wall thickness (UWT), Lumen mean gray value (LMGV) and wall mean gray value (WMGV)) according to Chou et al. (2010) were accomplished using NIH Image J (National Institutes of Health, Bethesda, Maryland, USA).

2.5. Statistical analysis
Data were computed and expressed as mean ± SEM using SPSS software (IBM® SPSS® statistics, version 23) according to Ho (2013). The incidence of pyometra in bitches and queens was calculated by chi-square ($\chi 2$) analysis. Independent-samples t- test compared between pyometric and non-pyometric animals. The correlation between CRP, TAC and NO and ultrasound parameters was verified by Spearman's correlation coefficient. Significance level for all statistical tests was $P<0.05$.

3. RESULTS

3.1. Clinical examination
Animal admitted with pyometra showed signs of fever, anorexia, lethargy, vomiting and abdominal distention (Fig. 1A). In the meantime, vulvar discharge although was obvious (Fig. 1B, 1C), it was absent in some cases, especially cats. Pyometra was recorded in bitches (n=10/200, 5.0 %) and queens (n=10/281, 3.6%) of an age 5.24±1.17 and 3.8±1.21 years, respectively. The incidence rate of pyometra cases in bitches was not significantly varied than that in queens ($\chi 2= 0.61$, $P= 0.44$).

Fig (1): Clinical symptoms of pyometra. A: Abdominal distention (arrow) in Lolo bitch. B: Vomiting (curved arrow) and purulent vulvar discharge (notched arrow) in pit-bull bitch. C: Wet hair (arrowhead) in perineal region and around vulva in Persian queen

3.2. Ultrasound measures
In dogs and cats, uteri with pyometra appeared highly convoluted and distended with anechoic to hypoechoic fluid

and variable wall thickness (Ultrasonogram 1). The ultrasound quantified changes in the uterus of bitches and queens suffered from pyometra is presented in table (1). There was a significant increase in UHD (P< 0.0001), UWT (P< 0.001 and 0.0001), LMGV (P< 0.0001) and WMGV (P<0.0001) in pyometric bitches and queens, respectively, compared to non pyometric group.

Table (1): Uterine quantitative ultrasound measures in bitches and queens with pyometra

Parameter	Bitches		Queens	
	Non-pyometric group	Pyometra group	Non-pyometric group	Pyometra group
Uterine horn diameter (cm)	1.15±0.26	5.29±0.10****	1.01±0.47	2.80±0.21****
Uterine wall thickness (cm)	0.37±0.04	1.41±0.15***	0.46±0.04	0.76±0.02****
Lumen mean-value (pixels)	29.72±1.29	46.85±1.67****	26.77±0.70	61.74±4.37****
Wall mean gray value (pixels)	47.48±4.79	119.62±9.14****	33.34±1.15	157.02±9.13****

Data (mean ± SE, n=10). *** and **** indicated significant differences at P<0.001, 0.0001

3.3. Oxidative stress and hormonal changes

Data regarding the changes in biochemical and hormonal parameters in bitches and queens showed pyometra is presented in table (2). There was significant increase in CRP (P< 0.01, 0.05), NO (P< 0.05), MDA (P< 0.01, 0.005), P4 (P< 0.05, 0.0001), but a decrease in SOD (P<0.0005, 0.005), CAT (P<0.05), TAC (P<0.05), in pyometric bitch and queen, respectively. There were no marked variations in E2, GPX enzyme activity levels compared to non-pyometric group. There was a slight (P= 0.09) decrease in E2 and E2/P4 ratio in queens showed pyometra. The quantified ultrasound measures (UHD, UWT, LMGV and WMGV) were positively correlated with CRP (R= 0.98, 0.94, 0.93 and 0.95, respectively in bitches, and R= 0.79, 0.77, 0.78 and 0.77, respectively, in queens), NO (R= 0.97, 0.93, 0.92 and 0.91, respectively, in bitches, and R= 0.97, 0.97, 0.96 and 0.98, respectively, in queens), and negatively correlated with TAC (R= 0.92, 0.87, 0.85 and 0.84, respectively, in bitches, and R= 0.77, 0.78, 0.79 and 0.77, respectively, in queens) (Table 3).

3.4. Histopathological findings

In comparison with the normal uteri (Fig. 2A& 3A) of bitches and queens, respectively, the uterus in pyometra showed disturbed histological architecture characterized by severe suppurative inflammation in all layers of the uterus with heavy polymorphonuclear cellular infiltration in tunica mucosa, submucosa and muscularis. The uterine lumen was filled with pus in the form of finely granular eosinophilic and basophilic substances infiltrated with neutrophil (Fig. 2E). Moreover, there was severe epithelial desquamation in the endometrium (Fig. 2B& 3B). The uterine submucosa showed severe degenerative changes in most of endometrial glands. The affected glands showed hyperplasia of lining epithelium with few leucocytic infiltration (Fig. 2C& 3C), and filling of the lumen with neutrophils-infiltrated homogeneous structureless eosinophilic substances (Fig. 2D). The queen's myometrium showed severe hyaline degeneration with mild leucocytic infiltration (Fig. 3D). Quantitative analysis of the histopathological changes (Table 4) in bitches and queens showed a significant decrease in ET (P< 0.0001, 0.001), and an increase in EGWT (P< 0.01, 0.0001), EGD (P< 0.005, 0.0001), while no marked variations were noted in MT.

Table (2): Effect of pyometra on the hormonal and biochemical parameters in bitches and queens

Biochemical parameter	Bitches			Queens		
	Non pyometric group	Pyometric group	P value	Non pyometric group	Pyometric group	P value
CRP (mg/dl)	1.45±0.26	5.33±0.67**	0.01	0.88±0.26	2.00±0.32*	0.05
SOD (U/ml)	447.56±2.07	402.38±0.90****	0.0005	426.98±17.79	371.26±6.56**	0.005
CAT (U/l)	258.69±23.03	158.73±9.55*	0.05	208.30±20.38	134.28±2.61*	0.05
GPX (mU/ml)	19.30±4.09	32.91±9.38	0.28	18.20±2.59	20.97±2.44	0.47
TAC (mmol/l)	5.33±0.29	2.28±0.59*	0.05	5.37±0.63	2.61±0.73*	0.05
NO (µmol/l)	168.35±4.42*	124.71±12.23	0.05	246.16±18.61*	119.17±3.55	0.05
MDA (nmol/l)	6.04±0.53	9.12±0.58**	0.01	5.52±0.13	10.59±0.40**	0.005
P4 (ng/ml)	0.83±0.42	10.67±2.05*	0.03	0.31±0.15	15.13±0.07****	0.00001
E2 (pg/ml)	20.05±4.13	22.80±3.06	0.62	14.20±2.29	20.30±1.48	0.09
P4/ E2 ratio	0.03±0.01	0.47±0.12*	0.05	0.02±.01	0.57±0.20*	0.05

CRP: C-reactive protein, SOD: superoxide dismutase, CAT: Catalase, GPX: glutathione peroxidase, TAC: total antioxidant capacity, NO: nitric oxide, MDA: malondialdehyde, P4: progesterone and E2: estrogen. Data (mean ± SE, n=10 per each species). *, **, ***, **** indicated significant differences at P< 0.05, 0.01, 0.001, 0.0001, respectively.

Table (3): Correlation between inflammatory markers (CRP), oxidative stress markers (TAC and NO) and uterine measures in pyometric bitches and queens

Parameter	Bitches				Queens			
	UHD	UWT	ULMGV	UWMGV	UHD	UWT	ULMGV	UWMGV
CRP (mg/dl)	0.981**	0.94**	0.93**	0.95**	0.79*	0.77*	0.78*	0.77*
TAC (mmol/l)	-0.923**	-0.86*	-0.85*	-0.84*	-0.77*	-0.78*	-0.79*	-0.77*
NO (µmol/l)	0.965**	0.93**	0.92*	0.91*	0.97**	0.97**	0.96**	0.98**

UHD: Uterine horn diameter, UWT: Uterine wall thickness, ULMGV: Uterine lumen mean-gray value, UWMGV: Uterine wall mean-gray value, CRP: C-reactive protein, TAC: Total antioxidant capacity, and NO: Nitric oxide. * and ** indicated significant correlation at the P< 0.05 and 0.01, respectively

Table (4): Effect of pyometra on histopathological structure of uteri of bitches and queens

	Bitches			Queens		
	Non pyometric group	Pyometra group	P value	Non pyometric group	Pyometra group	P value
1-Endometrial measures:						
a)Epithelial thickness (nm)	0.5±0.09****	0.18±0.05	0.0001	0.19±0.01***	0.13±0.01	0.001
b) Endometrial gland diameter (nm)	1.33±0.34	3.12±0.98***	0.005	0.45±0.06	1.79±0.16****	0.0001
c) Endometrial glands wall thickness (nm)	0.39±0.10	0.72±0.26**	0.01	0.16±0.02	0.45±0.04***	0.001
2-Myometrial thickness (nm)	4.72±0.49	4.62±0.92	0.83	3.74±0.53	3.75±0.46	0.981

Data (mean ± SE.) **, ***, **** indicated significant differences at P< 0.01, 0.005, 0.0001 respectively.

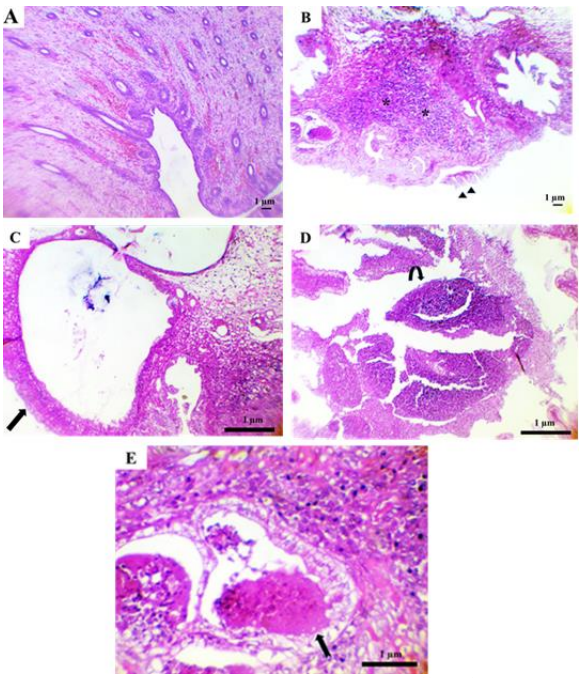

Fig (2): Histopathological changes in uteri of bitches suffered from pyometra (B, C, D and E) as compared to uterus from non-pyometric one (A). There was epithelial desquamation (arrow heads) and heavy polymorphonuclear cellular infiltration in uterine layers (*). Cystic hyperplasia of endometrial glands (arrow) with homogenous structureless eosinophilic substances infiltrated with neutrophil in their lumniae (notched arrow). Finely granular eosinophilic and basophilic substances infiltrated with neutrophil (curved arrows). Scale bar equal 1µm

4. DISCUSSION

Pyometra in dogs and cats is a dangerous uterine disorder affects their reproduction and life-as well (Hagman et al., 2014)). In the current study, there was a profound increase in CRP, NO and MDA, and a depression in SOD and CAT activities accused for the decrease in TAC. These change coincident with a disturbance in the hormonal milieu characterized by an increase in P4 levels and a decrease in P4/E2 ratio. It could be postulated that pyometra induces serious inflammatory changes and interferes with body antioxidant mechanisms which affects animals' life.

In the current study, 5.0% (10 out 200) bitches and 3.5% (10 out of 281) queens of an age 5.24±1.17 and 3.8±1.21 years, respectively brought to gynecological clinics for examination suffered from pyometra. The incidence of dogs and cats pyometra is still not well recorded and probably under-estimated. A higher incidence (19%) of pyometra in bitch was reported by Jitpean (2015). Limited data availability and breed differences may blame for this difference (Antonov et al., 2015). On the other hand, Hagman (2018) found that pyometra was not common in queens as bitches, this might be explained by queens are induced ovulator so less progesterone dominance (Hollinshead and Krekeler, 2016). The incidence of pyometra raises in bitches > 4 years of age (Smith, 2006) and cats > 5 years (Hagman, 2018) due to repeated estrous cycles without pregnancy with changes occur in uterine wall under progesterone effect (Hagman et al., 2014). Clinical symptoms related to pyometra are not specific, except for vulvar discharge which is not usually present in dogs and very scarce in cats. Several clinical signs were observed in cases of pyometra include fever, anorexia, depression, vomiting, abdominal distention, polyuria, polydipsia, weight loss. These findings indicated the local and systemic influences of the disease. Likewise, similar findings were reported formerly (Hagman, 2018). In dogs, the presence of vulvar discharge varied from 65-77% and it may be absent in closed pyometra (Jitpean, 2015). Nevertheless, vulvar discharge can also be absent in queens due to the self-care habits (Von Reitzenstein et al., 2000). The presence of fever in pyometra may be attributed to septicemia and toxemia which causes anorexia and depression (Rautela and Katiyar, 2019). Abdominal distention was a marked sign observed in all cases suffered from pyometra due to fluid accumulation in the uterus (Von Reitzeinstei et al., 2000). Polyuria and polydipsia may be attributed to endotoxins release following bacterial invasion which interfere with renal Na+ and Cl absorption leading water absorption impairment. E. coli endotoxins also block receptors of antidiuretic hormone on the collecting ducts, leading to obligatory polyuria with compensatory polydipsia (Macintire, 1994). Ultrasonography is proved as a helpful non-invasive mean in identifying uterine enlargement and differentiating pyometra from a normal pregnancy (Mattei et al., 2018). The uterus of bitches and queens suffered from pyometra was characterized by an accumulation of anechoic to hyperechoic fluid with significant increase in the value of UWD, UWT, LMGV and WMGV. This agreed with Misk and EL-sherry (2020), who noticed that uteri of pyometric pet animals appeared distended with anechoic to hypoechoic fluid in associated with uteri wall thickness due to the presence of uterine exudates or pus (Rautela and Katiyar, 2019). C-reactive protein (CRP) is the 1st acute-phase proteins rapidly elevated at the onset of any infection or inflammation (Du Clos and Mold, 2004). It binds to several intra-cellular antigens of damages cells and pathogenic micro-organisms (Murata et al., 2004). In the present study, CRP markedly elevated in pyometric animals, and its levels were positively correlated with the ultrasound detectable changes, indicating its diagnostic value for pyometra in pet animals. This finding agreed with Hillström et al. (2014), who noticed that CRP is an inflammatory marker, increases in cases of inflammation including pyometra cases. An inequality between the reactive oxygen species (ROS) production and their elimination is responsible for the oxidative stress (Hussain et al., 2016). Such status leads to chronic inflammation occurrence. In the present study, TAC significantly decreased in pyometra cases coincident with a decrease in SOD and CAT activities and an increase in NO and MDA levels. Former studies indicated a decrease in serum TAC levels (Amin et al., 2021) in pyometric dairy cows, and SOD and catalase in pyometric bitches (Rautela et al., 2018), perhaps due to an exhaustion of antioxidant system (Amin et al., 2021). SOD is considered as the 1st line of defense against ROS where it removes both intra- and extracellular superoxide radicals and decreases the danger of these radicals (Grieve and Shah, 2003). SOD protects cells during oxidative stress by breaking superoxide radicals forming hydrogen peroxide, which later dissociates to water (Powers and Jackson, 2008). NO is pro-inflammatory mediator, however, the increment in NO in pyometra dogs and cats here might indicate its role in the pathogenesis of pyometra as the overproduction of NO induces inflammation through tissue damage and vasodilatation (Sharma et al., 2007). They also observed a significant increase in the circulating MDA in pyometra cases. MDA is a degradation byproduct from lipid peroxidation (oxidation of membrane phospholipids Similarly, Rautela and Katiyar (2019) noticed elevated MDA level in pyometra, due to the imbalance between body pro-oxidants and antioxidants mechanisms causing cellular structure damage (Nishikimi et al., 1972). Pyometra is a foremost uterine disorder mediated by prolonged exposure to P4. In the present study, P4 level

increased and the E2/P4 ratio was altered. Similarly, a substantial increase in P4 hormone in the condition of pet animals suffered from pyometra was noticed (Hollinshead and Krekeler, 2016). This might explain the occurrence of this condition in the diestrus phase due to progesterone dominance from an increased corpora lutea activity (Hollinshead and Krekeler, 2016). Quantifiable histopathological changes revealed a significant decrease in ET, and an increase in EGWT and EGD ($P< 0.005, 0.0001$), while no marked variations were noted in MT. The increase in the subepithelial connective tissue zone containing endometrial glands accused to the main changes in the uterine wall found here. These results agreed with Kempisty et al. (2013), who found an increase in endometrial glands size and number. Nevertheless, there was a significant decrease in epithelial thickness in pyometra cases (Rautela and Katiyar, 2019). They also detected epithelial damage due to high ROS production in association with inflammatory conditions that damages epithelial cells barrier resulting in tissue injury and severe epithelial desquamation.

5. CONCLUSION

The health status and reproductive efficiency of pyometric dogs and cats were greatly compromised by the inflammatory condition and oxidative stress. This is inter-related with the altered endocrine milieu which predisposes to the chronicity of the inflammation. This study is a step to expand the field of future diagnosis of pathophysiology of pyometra in bitches and queens.

ACKNOWLEDGMENT

The authors would like to thank Prof. Dr. Abdel-Baset I. El-Mashad, Emeritus Prof. of Pathology, Faculty of Veterinary Medicine, Benha University, for histopathology examinations, and Prof. Dr. Khaled E. E. Attia, Head of Department of Theriogenology, Cairo university, for allowance some clinical cases at Theriogenology Clinic, Cairo University

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest for current data

6. REFERENCES

1. Aebi, H., 1984. [13] Catalase in vitro. In Methods in enzymology. 105: 121-126.
2. Amin, Y.A., Ali, R.A., Fouad, S.S. and Ibrahim, R.M., 2021. The deleterious effect of postpartum pyometra on the reproductive indices, the metabolic profile, and oxidant/antioxidant parameters of dairy cows. Veterinary World. 14(2): 329.
3. Anderson, G. and Bancroft, J, 2002. Tissue processing and microtomy. Chapter 6. In: Theory and Practice of Histological Techniques. Bancroft, J.D. and Gamble, M (Eds.). Edition 5th. Elsevier Sciences Limited, UK. pp. 85-108.
4. Antonov, A.L., Atanasov, A.S., Fasulkov, I.R., Georgiev, P.I., Yotov, S., Karadaev, M. and Vasilev, N., 2015. Influence of some factors on the incidence of pyometra in the bitch. Bulgarian Journal of Veterinary Medicine, 18(4): 367-372.
5. Chou, S.Y., Chen, C.Y., Chow, P.K., Hsu, C.S., Hsu, M.I. and Chiang, H.K., 2010. Ultrasonographic evaluation of endometrial changes using computer assisted image analysis. Journal of Obstetrics and Gynecology Research. 36(3): 634-638.
6. Du Clos TW and Mold C., 2004. C-reactive protein: An activator of innate immunity and a modulator of adaptive immunity. Immunol Res. 30: 261-278.
7. El-Bahr SM, El-Deeb WM., 2016. Acute-phase proteins, oxidative stress biomarkers, proinflammatory cytokines, and cardiac troponin in Arabian mares affected with pyometra. Theriogenology. 86: 1132-1136.
8. Floyd, A. D., 2002. Quantitative Data from Microscopic Specimens. Chapter 6. In: Theory and Practice of Histological Techniques. Bancroft, J.D. and Gamble, M (Eds.). Edition 5th. Elsevier Sciences Limited, UK. pp. 729-748.
9. Goldstein, D., Zuckerman, H., Harpaz, S., Barkai, J., Geva, A., Gordon, S., Shalev, E. and Schwartz, M., 1982. Correlation between estradiol and progesterone in cycles with luteal phase deficiency. Fertility and Sterility. 37(3): pp.348-354.
10. Grieve, D.J. and Shah, A.M., 2003. Oxidative stress in heart failure: more than just damage. European heart journal. 24(24): 2161-2163.
11. Hagman, R., 2018. Pyometra in small animals. Veterinary Clinics: Small Animal Practice. 48(4): 639-661.
12. Hagman, R., Holst, B. S., Moller, L., & Egenvall, A., 2014. Incidence of pyometra in Swedish insured cats. Theriogenology. 82(1): 114-120.
13. Hanita, O. and Hanisah, A.H., 2012. Potential use of single measurement of serum progesterone in detecting early pregnancy failure. Malays J Pathology. 34(1): 41-6.
14. Hillström, A., Hagman, R., Tvedten, H. and Kjelgaard-Hansen, M., 2014. Validation of a commercially available automated canine-specific immunoturbidimetric method for measuring canine C-reactive protein. Veterinary Clinical Pathology. 43(2): 235-243.
15. Ho, R., 2013. Handbook of univariate and multivariate data analysis with IBM SPSS. 2nd edition. CRC press. Taylor and Francis Group.
16. Hollinshead, F. and Krekeler, N., 2016. Pyometra in the queen: to spay or not to spay? Journal of Feline Medicine and Surgery. 18(1): 21-33.
17. Hussain, T., Tan, B., Yin, Y., Blachier, F., Tossou, M.C. and Rahu, N., 2016. Oxidative stress and inflammation: what polyphenols can do for us?. Oxidative medicine and cellular longevity, 2016 (9 pages).
18. Jitpean, S., 2015. Predictive markers and risk factors in canine pyometra. 85: 11-15.
19. Kähn, W., 2004. Ultrasonography in dogs and cats. Chapter 5. In: Veterinary Reproductive Ultrasonography. Schlütersche Verlagsgesellschaft mbh and Co.KG, Hans-Böckler-Allee 7,30173 Hannover, Germany. pp. 227-249.
20. Karlsson, I., Hagman, R., Johannisson, A., Wang, L., Karlstam, E. and Wernersson, S., 2012. Cytokines as immunological markers for systemic inflammation in dogs with pyometra. Reproduction in domestic animals. 47: 337-341.
21. Kempisty, B., Bukowska, D., Wozna, M., Piotrowska, H., Jackowska, M., Zuraw, A., Ciesiolka, S., Antosik, P., Maryniak, H., Ociepa, E. and Porowski, S., 2013. Endometritis and pyometra in bitches: a review. Veterinarni medicina.58(6): 289–297.
22. Koracevic, D., Koracevic, G., Djordjevic, V., Andrejevic, S., & Cosic, V., 2001. Method for the measurement of antioxidant activity in human fluids. Journal of clinical pathology. 54(5): 356-361.
23. Lawler D.F. and Johnston S.D., 1995. Complications of the Non Ovulatory Copulation in the Queen. In: Current Veterinary Therapy. Bonagura, J.D. and Kirk, R.W (Eds.). Edition 6th. Saunders Co, Philadelphia. pp. 1166-1167.
24. Macintire, D.K., 1994. Emergencies of the female reproductive tract. Veterinary Clinics of North America: Small Animal Practice. 24(6): 1173-1188.
25. Mattei, C., Fabbi, M. and Hansson, K., 2018. Radiographic and ultrasonographic findings in a dog with emphysematous pyometra. Acta Veterinaria Scandinavica. 60: 1-5.
26. Maya-Pulgarin, D., Gonzalez-Dominguez, M.S., Aranzazu-Taborda, D., Mendoza, N. and Maldonado-Estrada, J.G., 2017. Histopathologic findings in uteri and ovaries collected from clinically healthy dogs at elective ovariohysterectomy: a cross-sectional study. Journal of Veterinary Science. 18(3): 407-414.

27. Misk, T.N. and EL-Sherry, T.M., 2020 Pyometra in cats: Medical versus surgical treatment. Journal of Current Veterinary Research. 2(1): 86-92.
28. Montgomery, H.A. and Dymock, J.F., 1961. Determination of nitrite in water. Analyst. 86(102): 414.
29. Murata, H., Shimada, N. and Yoshioka, M., 2004. Current research on acute phase proteins in veterinary diagnosis: an overview. The Veterinary Journal. 168(1): 28-40.
30. Nishikimi, M., Roa, N.A. and Yogi, K., 1972. Measurement of superoxide dismutase. Biochem. Biophys. Res. Commun. 46: 849-854.
31. Ohkawa, H., Ohishi, N., and Yagi, K., 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical biochemistry. 95(2): 351-358.
32. Paglia, D. E. and Valentine, W. N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of laboratory and clinical medicine. 70(1): 158-169.
33. Pepys, M.B. and Hirschfield, G.M., 2003. C-reactive protein: a critical update. The Journal of clinical investigation, 111(12): 1805-1812.
34. Powers, S.K and Jackson, M.J., 2008. Exercise-induced oxidative stress: Cellular mechanisms and impact on muscle force production. Physiol Rev. 88: 1243-1276.
35. Rahman, I., Biswas, S.K. and Kode, A., 2006. Oxidant and antioxidant balance in the airways and airway diseases. European journal of pharmacology. 533(1-3): 222-239.
36. Rautela R, Das GK, Khan FA, Prasad S, Kumar A, Prasad JK., 2018. Antibacterial, anti-inflammatory and antioxidant effects of Aegle marmelos and Murraya koenigii in dairy cows with endometritis. Livest Sci. 214: 142-148.
37. Rautela, R. and Katiyar, R., 2019. Review on canine pyometra, oxidative stress and current trends in diagnostics. Asian Pacific Journal of Reproduction. 8(2): 45-55.
38. Sharif, H., Hagman, R., Wang, L. and Eriksson, S., 2013. Elevation of serum thymidine kinase 1 in a bacterial infection: canine pyometra. Theriogenology. 79(1): 17-23.
39. Sharma, J.N., Al-Omran, A. and Parvathy, S.S., 2007. Role of nitric oxide in inflammatory diseases. Inflammo-pharmacology. 15(6): 252-259.
40. Von Reitzenstein, M., Archbald, L.F. and Newell, S.M., 2000. Theriogenology question of the month. Journal of the American veterinary medical association. 216(8): 1221-1223