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OXIDATIVE STATUS IN FELINE PYOMETRA

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Resumo

O estado oxidativo do organismo depende do equilíbrio entre os agentes oxidantes e os antioxidantes. O stresse oxidativo desenvolve-se quando os agentes oxidantes excedem a capacidade antioxidante do organismo. O stress oxidativo causa danos celulares, bem como alterações no seu metabolismo. A resposta antioxidante pode ser avaliada através da determinação de parâmetros individuais, tais como os tióis séricos, ou através da determinação da capacidade antioxidante total (TAC, do Inglês Total Antioxidant Capacity) do organismo. Diferentes parâmetros de avaliação do estado oxidativo do organismo provaram ser biomarcadores clinicamente úteis em diferentes doenças em cães e gatos. Contudo, existe falta de informação sobre o estado oxidativo em gatas com piómetra.

Desta forma, os objetivos principais deste estudo foram avaliar o estado oxidativo em gatas diagnosticadas com piómetra, através da determinação dos níveis séricos de tióis e TAC (determinados através de quatro métodos diferentes: TEAC1, TEAC2, FRAP E CUPRAC), e avaliar a evolução clínica destes antioxidantes no período pós-ovariohisterectomia.

As concentrações séricas de tióis e TAC foram determinadas em 17 gatas com piómetra (grupo em teste) e em 13 gatas saudáveis submetidas a ovariohisterectomia eletiva (grupo de controlo). Em seis gatas com piómetra foi possível efetuar o doseamento de tióis e TAC nos dias dois e 10 após a cirurgia. Foi realizada histopatologia dos órgãos reprodutores de todas as gatas incluídas no estudo, para comprovar a presença de piómetra nas gatas doentes, e para excluir doenças uterinas no grupo de controlo.

No momento do diagnóstico, as gatas com piómetra apresentaram concentrações séricas de tióis e TAC significativamente inferiores às gatas do grupo de controlo ($P < 0,001$ em ambos os casos). Nas gatas em que foi realizado um seguimento no período pós-cirurgia através de medições seriadas de antioxidantes, as concentrações de tióis e TAC apresentaram-se significativamente superiores no décimo dia pós-ovariohisterectomia que no dia do diagnóstico ($P < 0,05$ em ambos os casos).

Segundo os resultados obtidos neste estudo, em gatas a piómetra está associada ao desenvolvimento de stresse oxidativo. Para além disso, as concentrações séricas dos antioxidantes analisados tenderam a retomar os valores fisiológicos no período pós-ovariohisterectomia. Assim, estes parâmetros indicam utilidade clínica na monitorização do período pós-cirúrgico em gatas com piómetra.

Palavras-Chave: antioxidantes, capacidade antioxidante total, gata, piómetra, stresse oxidativo, tióis,

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List of abbreviations and acronyms

ABTS – 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)

CUPRAC – Cupric ion reducing antioxidant capacity

FRAP – Ferric reducing ability of plasma

ROS – Reactive oxygen species

TAC – Total antioxidant capacity

TEAC1 – Trolox equivalent antioxidant capacity, method 1

TEAC2 – Trolox equivalent antioxidant capacity, method 2

Thiol – Total serum thiols

Oxidative Status in Feline Pyometra

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Abstract

The oxidative status of the organism is dependent of the balance between oxidant reactants and antioxidant defenses. Oxidative stress develops when the oxidant reactants exceed the antioxidant defenses of the organism. The oxidative stress causes alterations in cellular metabolism and cellular damage. The antioxidant response of the organism can be assessed by determination of individual parameters such as total serum thiols (Thiol) and/or by determination of the total antioxidant capacity (TAC) of the organism. Parameters of oxidative status have proved to be useful biomarkers in several canine and feline diseases. Pyometra is considered one of the most important uterine diseases in cats. However, information about the oxidative status in queens with pyometra is lacking.

Therefore, the main objectives of this study were to evaluate the oxidative status of feline pyometra through the determination of serum concentration of Thiol and TAC, and to evaluate the clinical evolution of these antioxidants in the post-ovariohysterectomy period.

Serum concentrations of Thiol and TAC (determined by four different methodologies) were assessed in 17 queens with pyometra and in 13 healthy queens submitted to elective ovariohysterectomy (control group). Serum Thiol and TAC were also evaluated in six queens with pyometra at days two and 10 after surgery. Routine histopathology of the reproductive organs was performed in all animals, to confirm pyometra in the diseased queens, and to exclude uterine pathology in the control cats.

At presentation, diseased queens presented significantly lower serum concentrations of Thiol and TAC than controls ($P < 0.001$ in both cases). In the queens in which serial determinations of antioxidants were performed, serum Thiol and TAC were significantly higher at day 10 post-surgery than at presentation ($P < 0.05$ in both cases).

The results of the present study indicate that pyometra in queens is associated with presence of oxidative stress. Moreover, serum Thiol and TAC tended to evolve to physiologic values after surgery. Therefore, oxidative stress parameters could be useful in assessing the post-operative period in feline pyometra.

Keywords: antioxidants, oxidative stress, pyometra, queen, thiols, total antioxidant capacity

1. Background

Pyometra is considered one of the most important feline uterine pathologies (Agudelo, 2005; Hagman, Holst, Moller & Egenvall, 2014). It is characterized by a uterine infection and inflammation, associated with endometrial cellular infiltration and accumulation of purulent content in the uterine lumen (Pires *et al.*, 2016).

The etiology of pyometra is multifactorial, but the most important factor reported is the exposure of the uterus to endogenous or exogenous progesterone. Repeated exposure to consecutive cycles or the exogenous administration of progestogens for contraception leads to dilation of the endometrial glands, uterine distention and fluid accumulation, causing alterations in the uterine microenvironment which increase the predisposition to infection (Hagman *et al.*, 2014; Pires *et al.*, 2016). When the queens are in estrus or in the peripartum, the cervix is relaxed, therefore predisposing to an ascending bacterial invasion of the endometrium, which increases the risk of pyometra (Lawler, Evans, Reimers, Colby & Monti, 1991).

The most frequent clinical signs presented by queens with pyometra are vaginal discharge, abdominal distention, dehydration, palpable uterus, fever and lethargy (Kenney, Matthiesen, Brown & Bradley, 1987). Queens with pyometra can present an open or a closed uterine cervix. In cases of open-cervix pyometra, the uterine content drains out through the vulva, however, vulvar discharge can be unapparent due to the fastidious grooming habits of queens. In cases of closed-cervix pyometra, the uterine content stays cloistered to the uterine lumen, and queens often manifest abdominal distention and signs of severe illness (Agudelo, 2005; Mitacek *et al.*, 2014)

The most common infectious agent isolated from the feline pyometra is *Escherichia coli*, but other agents of the normal vaginal flora or of suspected fecal contamination have also been reported, namely *Streptococcus* sp., *Klebsiella* sp., *Staphylococcus* sp., *Pasteurella* sp., *Proteus* sp., *Moraxella* sp., *Pseudomonas* sp. and *Tritrichomonas foetus* (Dahlgren, Gjerde & Pettersen, 2007; Johnston, Kustritz & Olson, 2001; Lawler *et al.*, 1991).

Pyometra typically develops one week to two months after estrus. The diagnosis is based on history and clinical signs, and is commonly confirmed by abdominal radiography or ultrasonography (Agudelo, 2005). Pyometra is considered an emergency due to the risk of potentially life-threatening complications, including azotemia, septicemia, endotoxemia, uterine rupture, peritonitis and shock. Surgical treatment (ovariohysterectomy) associated with supportive therapy is considered the treatment of choice (Kenney *et al.* 1987; Wiebe & Howard, 2009). Most cats recover successfully with an appropriate treatment. However, severe complications can develop, with mortality reported to range from 5.7 to 8.0% of cases (Hagman *et al.*, 2014; Kenney *et al.*, 1987), indicating the necessity of new biomarkers for a correct disease evaluation and treatment monitoring.

Development of oxidative stress has been described in several feline and canine diseases, including chronic kidney disease, demodicosis, feline hypertrophic cardiomyopathy, feline infectious peritonitis,

inflammatory bowel disease, among others (Krofič Žel, Tozon & Nemec, 2014; Martínez-Subiela *et al.*, 2014; Christiansen *et al.*, 2015; Tecles *et al.*, 2015, Rubio *et al.*, 2017). Moreover, parameters of oxidant / antioxidant status of the organism have been proved to be clinical useful biomarkers in diagnosis, in monitoring disease progression and response to treatment, and in prognosis of different diseases in dogs and cats (Branter, Drescher, Padilla & Trepanier, 2012; Crnogaj *et al.*, 2017; Rubio *et al.*, 2016a; Viviano *et al.*, 2009). However, information about the oxidative status of queens with pyometra, and about the clinical usefulness of parameters of oxidant / antioxidant status in feline pyometra is lacking.

The oxidative status of the organism is dependent of the balance between oxidant reactants and antioxidant defences (Castillo *et al.*, 2012). The oxidative stress develops when oxygen and nitrogen free radicals exceed the capacity of the antioxidant defences of the organism (Mandelker, 2008). The predominance of oxidant reactants originates alterations in cellular metabolism and cellular damage, which can be in some cases irreversible (Rubio, Hernández-Ruiz, Martínez-Subiela, Tvarijonaviciute & Cerón, 2016b; Frijhoff *et al.*, 2015; Tecles, Caldín, Tvarijonaviciute, Escribano & Martínez-Subiela, 2015). The antioxidant response can be assessed by determination of individual parameters such as total serum thiols (Thiol) and / or by determination of the total antioxidant capacity (TAC) of the organism (Rubio, Tvarijonaviciute, Martinez-Subiela, Hernández-Ruiz & Cerón, 2016c).

In human medicine, Thiol is considered one of the most important biomarkers of protein oxidation (Da Costa, Dos Santos & Lima, 2006) and is used both as an oxidant and as an antioxidant status assay (Jansen & Ruskovska, 2015). Thiol antioxidants interacts with the electrophilic groups of reactive oxygen species (ROS), protecting cells from oxidative injuries. This interaction causes consumption of the Thiol antioxidants, consequently decreasing Thiol concentrations in the presence of oxidative stress (Dickinson & Forman, 2002; Yardim-Akaydin, Y. Ozkan, E. Ozkan, Torun & Simsek, 2003). Serum Thiol have also proved to be useful biomarker of the oxidative status in dogs (Rubio *et al.*, 2016a; Rubio *et al.*, 2017). However, to the author's knowledge, the clinical significance of Thiol in cats have not been evaluated so far.

The TAC represents the sum of the activities of the different antioxidants, and also the antioxidative effects provided by the interactions between antioxidants (Wayner, Burton, Ingold, Barclay & Locke, 1987; Koracevic, Koracevic, Djordjevic, Andrejevic & Cosic, 2001). Different methodologies, evaluating different antioxidants, are described to determination of the TAC of the tissue of interest. For that reason, different assays integrated in a panel should be performed for TAC evaluation until a reference method is described (Rubio *et al.* 2016b).

The main objectives of this study were to evaluate the oxidative status in feline pyometra through the determination of serum concentration of thiols and TAC (by four different spectrophotometric assays), and to evaluate the behavior of these antioxidants after ovariohysterectomy in queens with pyometra. Furthermore, the methodologies used in this study for Thiol and TAC determinations were validated for cats, and correlations between the different antioxidants evaluated were assessed.

2. Materials and Methods

The study was approved by the Scientific Council of Escola Universitária Vasco da Gama as complying with the Portuguese legislation for the protection of animals (Law no. 92/1995).

2.1 Animals and Sample Collection

17 Female cats diagnosed with pyometra that were presented to three veterinary medical centers from Portugal – Baixo Vouga Veterinary Hospital, University Veterinary Hospital of Coimbra and Veterinary Policlinic of Aveiro – between February 2014 and April 2017 were enrolled in the study. Identification and clinical data, including age, breed, weight, reproductive history, history of contraceptives administration, clinical signs and results of complementary diagnostic exams at the time of diagnosis and during the post-ovariohysterectomy period were recorded for all queens. Diagnosis of pyometra was established in all cases based on clinical history, results of physical examination and complementary diagnostic exams, and confirmed by abdominal ultrasonography and routine histopathology of the uterus.

Serum samples from 13 queens presented for elective ovariohysterectomy at the Baixo Vouga Veterinary Hospital, between September 2016 and February 2017 that were considered clinically healthy were used as controls. Clinical history, physical examination, results of complementary diagnostic exams, and hematological analysis required as part of the pre-anesthetic examination were used to assess control animals.

Whole blood samples were collected before surgery from all animals included in the study by venous puncture technique into Ethylene diamine tetra acetic acid (EDTA) tubes for hematology and into dry tubes for serum biochemistry. In six queens of the diseased group, whole blood samples were also collected at days two and 10 post-ovariohysterectomy. Within 15 minutes after collection, blood samples collected to the dry tubes were centrifuged (10 min, 2000 x g, 4°C) and supernatant was used for serum biochemistry. Remaining serum samples were stored immediately after use at -20°C until antioxidant determinations.

All the queens of the diseased and of the control groups were submitted to ovariohysterectomy according with standard surgical procedures (Howe, 2006). Routine histopathology of the reproductive organs was performed in all the queens included in the study, at the Laboratory of Histology and Anatomical Pathology of the University of Trás-os-Montes and Alto Douro, to confirm the pyometra in the diseased cats, and to exclude uterine pathology in the queens of the control group.

2.2 Antioxidants Assays

Determination of serum concentrations of antioxidants was performed at the Interdisciplinary Laboratory of Clinical Analysis Interlab-UMU, University of Murcia, Spain. All analyses were performed on an automated biochemistry analyzer (Olympus AU600, Olympus Diagnostica, GmbH) in one batch.

Serum concentration of Thiol and TAC were determined in samples of all the queens of the diseased and of the control groups before surgery, and in six queens of the diseased group at days two and 10 post-surgery.

Serum Thiol was determined by a spectrophotometric method previously described for dogs, which uses aromatic disulfides as reagents, occurring a thiol-disulfide exchange (Jocelyn, 1987). Total antioxidant capacity was determined by four different spectrophotometric methods, including trolox equivalent antioxidant capacity (TEAC), ferric reducing ability of plasma (FRAP), and cupric ion reducing antioxidant capacity (CUPRAC). Moreover, TEAC was performed in all cases by two different methods, namely the method described by Arnao, Cano, Hernandez-Ruíz, García-Cánovas & Acosta (1996) (TEAC1) and by the method described by Erel (2004) (TEAC2).

Briefly, the FRAP method consists in a reduction of Fe^{3+} to Fe^{2+} by the antioxidants present in the sample, and it reflects mainly the plasma concentrations of uric acid (Benzie & Strain, 1996); the CUPRAC assay uses the reduction of Cu^{2+} into Cu^{1+} , by the action of the non-enzymatic antioxidants in the sample (Campos, Guzmán, Lopez-Fernández & Casado, 2009) and evaluates plasma oxidants such as ascorbic acid, uric acid, bilirubin and albumin (Apak, Güçlü, Özyürek, Esin & Altun, 2005); the TEAC assay reflects plasma concentrations of albumin, ascorbic acid, urate, α -tocopherol and bilirubin, and it can be performed by two different methods: the first one uses 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS), that oxidize in the presence of H_2O_2 in a peroxidative reaction, and generates a radical cation (TEAC1) (Arnao *et al.*, 1996); the other TEAC method (TEAC2) also uses ABTS as substrate, but is based on a decolorization by antioxidants, dependent on their concentrations and antioxidant capacity (Erel, 2004).

2.3 Statistical analysis

Results are shown as medians (with Inter Quartile Range) unless otherwise stated and were calculated using routine descriptive statistical procedures and software (Graph Pad Prism, Version 6). D'Agostino & Pearson omnibus normality test was used to assess normality. As data were not normally distributed, differences in serum concentrations of Thiol and TAC (determined by the different methods) between the groups of diseased and of control cats before surgery were evaluated using Mann-Whitney test. Friedman test followed by the Dunn's multiple comparison test that was used to evaluate differences in analytes between the different sampling time-points in queens submitted to ovariohysterectomy. Correlations between variables were determined using Spearman correlation analysis. Values of $P < 0.05$ for two-sided analysis were considered significant.

3. Results

3.1 Population

A total of 30 queens were enrolled in the study, namely 17 cats with pyometra and 13 healthy controls. Queens of the diseased group were all domestic short-haired, with ages ranging from one to 12 years of age (mean 5.8 years, standard deviation 3.9 years). Queens of the control group were also all of domestic short-hair breed, with ages ranging from six months to eight years of age (mean 1.87 years, standard deviation 2.29 years).

3.2 Method validation

To the author's knowledge, serum thiol and TAC methodologies used in this study were not previously validated for use with feline serum samples. For this reason, analytical validation was performed of all methods in the present study. Inter- and intra-assay imprecision was below 15%, and the dilution of the samples resulted in linear regression equations with correlation coefficients close to 1.

3.3 Antioxidants at diagnosis

At time of diagnosis, queens with pyometra presented lower levels of Thiol, CUPRAC and TEAC2 when compared with the healthy group ($P < 0.0001$ in all cases). The FRAP concentrations were higher on the diseased queens than in controls ($P < 0.05$). No significant differences in concentrations of TEAC1 were found between the two groups (Table1).

Table 1. Median (Inter Quartile Range) data of serum antioxidants in queens with pyometra (n=17) and controls (n=13) at time of diagnosis (Statistically significant differences are highlighted in bold).

	Control group	Pyometra group	<i>P</i>
Thiol (mmol/L)	0.2981 (0.2719-0.3490)	0.1468 (0.1071-0.2168)	<0.0001
TEAC1 (mmol/L)	0.6016 (0.5638-0.6527)	0.5979 (0.5710-0.6155)	0.6950
TEAC2 (mmol/L)	0.4941 (0.4197-0.5413)	0.3567 (0.2835-0.3829)	<0.0001
FRAP (mmol/L)	0.2433 (0.2050-0.3154)	0.3221 (0.2751-0.3700)	0.0301
CUPRAC (mmol/L)	0.4367 (0.4021-0.4917)	0.3219 (0.2794-0.3881)	<0.0001

CUPRAC – Cupric ion reducing antioxidant capacity; FRAP – Ferric reducing ability of plasma; TAC – Total antioxidant capacity; TEAC1 – Trolox equivalent antioxidant capacity, method 1; TEAC2 – Trolox equivalent antioxidant capacity, method 2; Thiol – Total serum thiols.

3.4 Antioxidants in the post-surgery period

Serum concentrations of Thiol, TEAC1, TEAC2 and CUPRAC were significantly higher at day 10 post-surgery than on the day of presentation ($P < 0.05$ in all cases) (Table 2 and Figure 1). No significant differences in concentrations of any of the analyzed antioxidants were detected between day two post-surgery and the day of diagnosis (Figure 1).

Table 2. Serum concentrations of antioxidants in queens with pyometra (n=6) before and 10 days after ovariectomy (Statistically significant differences are highlighted in bold).

	Before surgery	10 days after surgery	<i>P</i>
Thiol (mmol/L)	0.1607 (0.0922-0.2751)	0.2199 (0.1883-0.3113)	0.0391
TEAC1 (mmol/L)	0.5979 (0.5710-0.6372)	0.6563 (0.6325-0.6880)	0.0156
TEAC2 (mmol/L)	0.3567 (0.3061-0.3829)	0.4505 (0.3871-0.4694)	0.0234
FRAP (mmol/L)	0.3350 (0.2303-0.3700)	0.3548 (0.3071-0.4634)	0.2344
CUPRAC (mmol/L)	0.3219 (0.2794-0.4095)	0.3802 (0.3489-0.4773)	0.0391

CUPRAC – Cupric ion reducing antioxidant capacity; FRAP – Ferric reducing ability of plasma; P – Statistical significance; TAC – Total antioxidant capacity; TEAC1 – Trolox equivalent antioxidant capacity, method 1; TEAC2 – Trolox equivalent antioxidant capacity, method 2; Thiol – Total serum thiols.

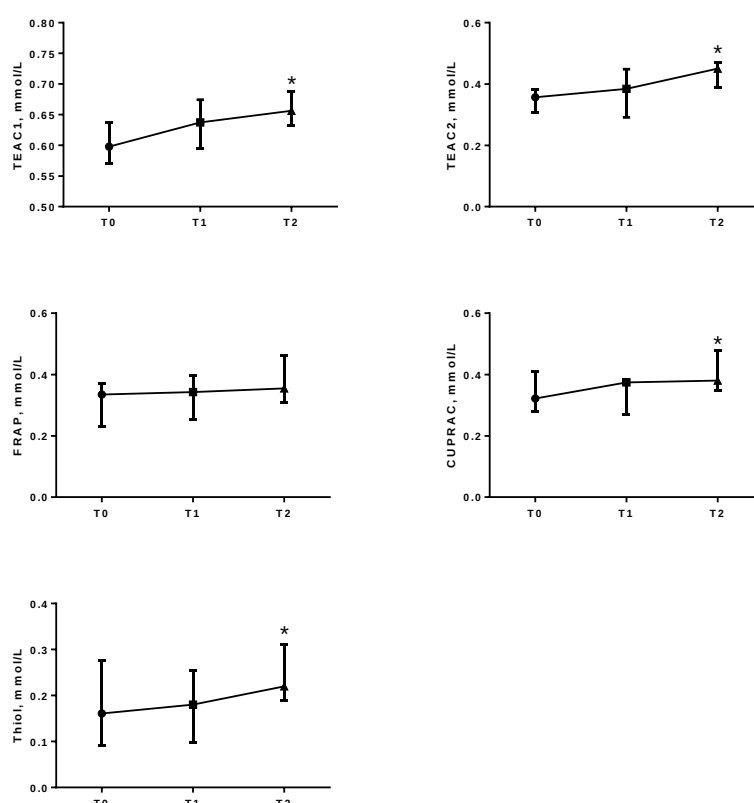


Figure 1. Median and Inter Quartile range data of serum total antioxidant capacity (TEAC1, TEAC2, FRAP and CUPRAC) and Thiol in cats with pyometra before surgery (T0), and two (T1) and 10 (T2) days after ovariectomy. * - $P < 0.05$; CUPRAC – Cupric ion reducing antioxidant capacity; FRAP – Ferric reducing ability of plasma; TAC – Total antioxidant capacity; TEAC1 – Trolox equivalent antioxidant capacity, method 1; TEAC2 – Trolox equivalent antioxidant capacity, method 2; Thiol – Total serum thiols

3.5 Antioxidant Correlations

Correlation coefficients and significance between serum concentrations of all the evaluated analytes in this study are presented in Table 3. Significant negative correlations were detected between concentrations of FRAP and Thiol, FRAP and CUPRAC, and FRAP and TEAC2. Significant positive correlations were detected between the concentrations of all the other antioxidants, except between Thiol and TEAC1.

Table 3. Correlation coefficients and significance between the different assays performed (Significant correlations are presented in bold)

	Thiol (mmol/l)	TEAC1 (mmol/l)	TEAC2 (mmol/l)	FRAP (mmol/l)
TEAC 1 (mmol/l)	r=0.281; P=0.063			
TEAC2 (mmol/l)	r=0.858; P<0.0001	r=0.526; P= 0.001005		
FRAP (mmol/l)	r=-0.516; P=0.001	r=0.345; P=0.027	r=-0.318; P=0.038	
CUPRAC (mmol/l)	r=0.955; P<0.0001	r=0.379; P=0.018	r=0.913; P<0.0001	r=-0.385; P=0.016

CUPRAC – Cupric ion reducing antioxidant capacity; FRAP – Ferric reducing ability of plasma; P – Statistical significance; r – Coefficient of correlation; TAC – Total antioxidant capacity; TEAC1 – Trolox equivalent antioxidant capacity, method 1; TEAC2 – Trolox equivalent antioxidant capacity, method 2; Thiol – Total serum thiol..

4. Discussion

To the author's knowledge, this is the first study to evaluate the oxidative status of queens with pyometra. Moreover, to our knowledge, this is also the first study in feline serum samples to evaluate Thiol and TAC by the methodologies described. The inter- and intra-assay imprecision below 15%, have proven their validation.

The TAC is an analyte frequently used to determine the oxidative status of a biological sample (Ghiselli, Serafini, Natela & Scaccini, 2000; Rubio *et al.*, 2016b). Previous reports shown that TAC values change due to several diseases in humans, dogs and cats (Rubio *et al.*, 2016a; Suresh, Annam, Pratibha & Prasad, 2009; Tecles *et al.*, 2005). Different methods, which evaluate different analytes, are described for determination of the TAC of a sample. For that reason, no single method is considered totally accurate, and determination of TAC through different assays integrated in a panel have been recommended for assessment of TAC (Huang, Ou & Prior, 2005; Rubio *et al.*, 2016b). In the present study, TAC was determined by four different methodologies.

Data of the present study indicate the presence of oxidative stress in feline pyometra, since statistically significant differences were detected in four out of the five evaluated parameters. The CUPRAC, TEAC2 and Thiol assays were lower and FRAP was higher in queens with pyometra than in healthy animals. Higher FRAP levels on diseased animals were previously observed in dogs with cardiac disease (Heteyey *et al.*, 2007). Discrepancies between results in FRAP and the other TAC assays could be attributed to the fact that different methods evaluate different analytes. All the antioxidants evolved in the same direction (P<0.0001), except from the FRAP (P<0.05) and TEAC 1 values (that had no

significant differences between the two groups).

Serum concentration of Thiol was also evaluated in this study, showing that before surgery, Thiol concentrations were significantly lower in queens with pyometra than in control queens. Serum thiols are components of CUPRAC. In the present study, the results obtained from those assays were very similar, both at the moment of diagnosis and in the postoperative period. However, these results were not obtained in dogs with leishmaniosis, probably because of the influence of analytes other than Thiol in the CUPRAC concentrations (Rubio et al. 2016a).

In queens in which serial determinations were performed, serum Thiol and TAC were significantly higher at day 10 post-surgery than before surgery. After ovariohysterectomy, concentrations of these antioxidants returned to physiologic values, suggesting that pyometra was the cause of the oxidative stress, and that ovariohysterectomy was an efficient treatment method in this cases.

The main limitations of this study were the low number of animals used in both groups due to the absence of adult unsprayed queens who could develop pyometra, the low number of queens that showed up to elective ovariohysterectomy, the difference of ages between both groups, the exclusion of two lipemic samples and the lack of information about this subject.

5. Conclusions

According with the obtained results, pyometra in queens is associated with development of oxidative stress. Moreover, serum Thiol and TAC tended to evolve to physiologic values after surgery. Therefore, oxidative stress parameters could be useful in assessing the post-operative period in feline pyometra. Further studies should be performed to increase knowledge and expand clinical usefulness of oxidative stress biomarkers in this important feline uterine disease.

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