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Effect of Meloxicam in a Protocol of Balanced Anaesthesia with Propofol and Sevoflurane on the α-δ-γ-Tocopherols in Response to Inflammatory Oxidative Stress Induced by Ovariectomy in Dogs

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Abstract: Meloxicam is a drug using in pain management. Surgery causing oxidative stress. The aim of this study was to evaluate effect of meloxicam to determine possible variations in the concentrations of α-tocopherol, δ-tocopherol and γ-tocopherol on the response to inflammatory oxidative stress induced by ovariectomy in dogs. Dogs received meloxicam pre surgery (0,2 $mgkg^{-1}$ SC) and after surgery (0.1 $mgkg^{-1}$ OS, every 24 hours). Physiological, haematological, biochemical parameters intraoperative and postoperative response to surgical stimulus was evaluated. To determine oxidative stress, malondialdehyde (MDA), α- δ γ-tocopherol were evaluated at baseline, 12, and 24 hours after the last administration of meloxicam. The physiological and haematological parameters remaining within the canine normal ranges. Concentration of glycemia increases while albumin level decreases after surgery. No rescue analgesia was required. MDA levels increased from baseline at 12, and 24 hours after meloxicam discontinuation: P=0.000. α- δ γ-Tocopherol concentrations decreased from baseline at 12, and 24 hours after discontinuation of meloxicam: P=0.000. The results showed that ovariectomy induced oxidative stress in the patients, despite the intra and post-operative clinical monitoring showing a good analgesia and the reduced endogenous tocopherol levels could be due to their consumption to cope with the stress induced by surgery.

Keywords: analgesia; dog; meloxicam; oxidative stress; ovariectomy; surgery; tocopherols

1. Introduction

Surgery, induces inflammatory oxidative stress. [1] Inflammation, pain and stress induced by surgery could have a negative influence on the healing of severed tissues and on the evolution of surgical lesions such as cancerous ones [1]. The anesthetic protocol chosen could be decisive on tissue healing and on the evolution of any diseases present. [2] Clinical, experimental and retrospective studies have demonstrated that a balanced anesthesia regimen could reduce the side effects induced by surgical stress [2]. There are different classes of drugs used to control perioperative pain in dogs: opioids, α-2 adrenergic receptor agonists, local anesthetics, non-steroidal anti-inflammatory drugs such as meloxicam that is commonly introduced into balanced anesthetic protocols and can be prescribed for pain control even after the patient is discharged. [3]

Meloxicam is an oxicam-derivative belonging to the class of non-steroidal anti-inflammatory drugs (NSAIDs) with anti-inflammatory and analgesic properties which selectively inhibits the inducible isoform of the enzyme cyclooxygenase (COX)-2. This enzyme has an important role in mediating the inflammatory response, while the synthesis of prostaglandins necessary for the normal physiological functioning of the stomach and kidneys is under the control of the constitutive isoform, COX-1. Clinical studies conducted on human patients have demonstrated an anti-inflammatory, antipyretic and analgesic efficacy comparable to that of traditional NSAIDs and improved gastrointestinal tolerability. The selective inhibitors of COX-2 are also totally devoid of antiplatelet effects being the platelet production of TXA2 dependent on COX-1. All this makes this molecule used on a large scale for the treatment of numerous diseases [4] [5]. Previous studies in dogs have demonstrated that meloxicam provides a good analgesic efficacy perioperatively at doses of 0.2-0.1 $mgkg^{-1}$ does not cause a significant change in physiological parameters [6].

The association between propofol and sevofluorane represents a balanced general anaes-thesia protocol, widely used in humans and in recent times it is also used in dogs [7,8]. Propofol is an injectable anaesthetic commonly used for induction and maintenance of general anaesthesia in dogs [8–10]. The chemical structure of propofol is similar to that of the vitamin E and therefore propofol has antioxidant power [11,12]. Sevoflurane is a halogenated anaesthetic widely used in clinical practice due to its low cardiotoxic effects, low irritant action on the airways and low coefficient of solubility with plasma proteins. This last characteristic allows to rapidly obtain the achievement of the anaesthetic plane suitable for the surgical intervention [13]. Sevoflurane is cleared rapidly through the respiratory system, allowing for rapid recovery of the patient. In addition, sevoflurane has neuroprotective and anti-inflammatory effects [13–15].

Oxidative stress plays an essential role in the stages of many surgical procedures, however the surgery itself can cause significant physiological stress in the body and the relevance of ROS production has also been demonstrated in general surgical procedures [16–18]. The oxidative state can modify the activity of numerous enzymes such as superoxide dismutase (SOD), catalase (CAT), as well as increase the mechanisms of lipid peroxidation and inflammation [18,19]. MDA is used in biomedical research as a marker of lipid peroxidation by reaction with thiobarbituric acid (TBA) and this reaction leads to the formation of MDA-TBA2, a conjugate which absorbs in the visible spectrum at 532 nm and gives a red- rose. Other lipid peroxidation-derived molecules besides MDA can also react with TBA and absorb light at 532 nm, contributing to the overall absorption signal that is measured. The TBARS assay is generally considered to be a good indicator of the overall levels of oxidative stress in a biological sample. The applicability of this TBARS assay is shown in human serum, low density lipoproteins, and cell lysates [20]. To protect the tissue from oxidant damage, antioxidant enzymes are also present in the biological system. When the animals are exposed to surgical oxidative stress, they also react with compensatory induction of endogenous antioxidants. The possible integration of dietary components such as vitamin E (tocopherols and tocotrienols) can reduce lipid peroxidation. Infact α-tocopherol, δ-tocopherol and γ-tocopherol have important qualities thanks to their powerful antioxidant action which allows it to protect cell membranes [21]. But this vitamin also plays an important role in relation to diseases of cardiovascular origin, given that it is able to reduce the aggregation processes of platelets, with a consequent reduction of emboli, plaques and thrombi in the arteries. Alpha-, δ-, and γ-tocopherol are also a valuable anticoagulant because it prevents unwanted clotting of the blood without impeding the normal clotting required in case of wounds, useful for stopping bleeding. The unique antioxidant properties of γ-tocopherol, whereby DNA-damaging nitrogen dioxide is rapidly converted to nitric oxide, suggest a mechanistic justification for a functional role in the prevention of DNA damage over time. Some cell, animal and human studies show that γ-tocopherol can have significant beneficial effects, protecting cells from inflammatory damage; however, γ-tocopherol levels increase in response to known etiologic risk factors. The antioxidant mechanism of action, apparent physiological regulation, and impact on various enzymatic pathways suggests that γ-tocopherol may have a functional role in maintaining the health of an organism [21].

The aim of this study was to evaluate the efficacy of perioperative meloxicam in balanced anesthesia with propofol and sevoflurane and to determine possible variation in the concentration of α- δ- γ-tocopherols in response to inflammatory oxidative stress induced by ovariectomy in dogs.

2. Materials and Methods

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The present clinical study was approved by the Review Board for Animals Care of the University of Parma prot N. 03/CESA /2023, which provided a consent to the clinical study: regulation (EU) no. 536/2014. (22A01712) (GU General Series n.65 of 18-03-2022); European Law (O.J. of E.C. L 358/1 12/18/1986), and USA Laws (Animal Welfare Assurance No A5594-01, Department of Health and Human Services, USA). The owners signed a voluntary informed consent form prior to the dogs' enrollment in the study. Twenty female dogs, aged 1±1.5 months, and weighing 16± 0.5 kg were enrolled in the study. The inclusion criterion of the patients was to undergo ovariectomy surgery; dogs enrolled in this study had normal physiological, haematological and biochemical parameters.

2.1. Treatment Administration and Anaesthesia

Food and water were given up to eight hours before the start of surgery. Dogs has received 0,2 $mgkg^{-1}$ of meloxicam SC (Metacam 2% Boehringer Ingelheim Italia S.p.A.) and 0,03 $mgkg^{-1}$ of atropine sulfate IM (Atropine Sulfate 0,1% A.T.I.) a catheter 20G x 32 mm (DELTA VEN) was placed in in the cephalic vein, through which dogs will receive lactate ringer 5 $mlkgh^{-1}$ for the duration of the surgery. Twenty minutes after premedication the patients were induced under general anaesthesia with propofol administered to effect, intubation was performed with a Magill cuffed tube. Maintenance under general anaesthesia was performed with sevofluorane (Sevoflo Zoetis Italy) delivered in 100% oxygen using a rebreathing circle system. Ventilation was performed using the pressure meter ventilator (SIMV) (GE Datex-Ohmeda Avance Ultramed Italy) was set using the following parameters: respiratory rate 12 breaths min^{-1}, positive pressure at the end of inspiration (PEEP) 4 cmH_2O, inspiratory/expiratory ratio (I:E) 1:7, and airway pressure 12 cm H_2O. After surgery was administered meloxicam 0.1 $mgkg^{-1}$ orally, every 24 hours.

2.2. Physiological and Anaesthesiological Parameters

Heart rate (HR, beats per minute) measured by auscultation using a stethoscope; respiratory rate (RR, breaths per minute) measured by counting thoracic wall excursions; non-invasive blood pressure (mmHg) (systolic, SAP; mean, MAP; diastolic, DAP) by placing a small size cuff around the base of the tail and body temperature(TC°); end-tidal carbon dioxide tension ($EtCO_2$) (mmHg), arterial haemoglobin oxygen saturation (SpO_2) (%) and concentration of inspired and expired isoflurane (CSI/CSE) were measured using a monitor (GE Datex-Ohmeda Avance multiparametric monitor for anaesthesia Ultramed Italy). These parameters were recorded at: T0 baseline; after a 30-minute acclimation period in surgical preparation room and at 20 minutes after premedication (P) (except $EtCO_2$ and concentration of inspired and expired isoflurane); after induction general anaesthesia (A); at skin incision (SI); at laparotomy (L); during traction and removal of first ovarian (TPI;); during traction and removal of second ovarian (TPII;); and at the skin suturing (SC).

2.3. Assessment of Intra and Postoperative Response to Surgical Stimulus

The evaluation of the intraoperative response to the surgical stimulus was evaluated using a cumulative pain scale [22]. A numerical score between 0 and 4 was assigned based on the percent change from RR, HR, and SAP values recorded after induction general anaesthesia (A); throughout surgery. according to the following scheme: 0 ≤ 0%; 1 = variation ≤ 10%; 2 = variation > 10% but ≤ 20%; 3 = variation > 20% but ≤ 30%; 4 = change > 30%. The sum of the scores for the three parameters was the response to the surgical stimulus. Total score 10, corresponding to the 20% increase in (HR, RR, SAP), was considered the cut-of point, for the administration of rescue analgesia: 2 $mcgkg^{-1}$ of fentanyl (Fentadon 50 mcg ml^{-1} Dechra) Postoperative pain score was as-signed, from waking up every 6 hours to 24 hours postoperatively, using Colorado state University Veterinary Medical Center (Canine acute, pain scale). Score 0-4. Score 2, corresponding to moderate to mild pain, was the cut of point for the administration of postoperative rescue analgesia: methadone 0.2 $mgkg^{-1}$ IM (Semfortan 10 $mgml^{-1}$).

2.4. Haematological, Biochemical Parameters and Inflammatory Oxidative Stress

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After carrying out the above measurements, 5 ml of blood was drawn from the cephalic vein. All samples were taken by the same operator. Each sample was divided into two aliquots of which, one was placed in a vacuum serum isolation tube (serum clot activator Z, Vacuet-te®, Greiner Bio-One, Kremsmünster, Austria) used to evaluate biochemical parameters (glycemia; aspartate transaminase (AST); alanine aminotransferase (ALT), total protein, albumin and azotemia. Furthermore, blood sample was used to evaluate the oxidative stress by assessing lipid peroxidation, catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO), butyrylcholinesterase (BuChe) and tocopherols. Another aliquot of blood was placed in a vacuum tube with EDTA (K3-EDTA, Vacuette®, Greiner Bio- One, Kremsmünster, Austria), and was used for the evaluation of the blood count (only at the baseline). Both groups of tubes were immediately refrigerated at 4°C and subsequently (within 3-4 hours) centrifuged (only the sample put in serum tube) for 15 minutes at 1500 x g to obtain serum aliquot.

Complete blood counts were assessed using the IDEXX Italy. Glucose, albumin, and total protein were measured using the glucose oxidase/peroxidase, brom cresol green, and biuret methods, respectively. AST and ALT were measured at 37°C using kinetic methods. To evaluate all the above parameters was used using spectrophotometer UV-Vis (A560, Fulltech, Rome, Italy [23,24].

Oxidative stress was evaluated by assessing lipid peroxidation, catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO), butyrylcholinesterase (BuChe), α-tocopherol, δ-tocopherol, and γ-tocopherol. For biochemical assessment of, sample was collected at baseline and 12 hours after the end of treatment with meloxicam. For oxidative status evaluation, blood samples were collected at baseline 12 and 24 hours after the end of treatment with meloxicam.

2.5. Determination of Malondialdehyde

Phosphoric acid (85%, 15 mol/1), sodium hydroxide, SDS (8,1%) and sodium chloride were purchased from Merck (Darmstadt, Germany). Thiobarbituric acid (TBA) was from Fluka (Buchs, Switzerland). All reagents were of analytical grade or highest grade available. Malondialdehyde (MDA) standard was prepared by hydrolysis of TMP; TBA (25 #1) reagent (0.11 mol/l: 800 mg TBA dissolved in 50 ml 0,1mol/1 NaOH) was prepared for the assay. For a quantitative determination of TBARS, 200 microliters of an MDA standard solution were used instead of plasma. MDA stock solutions were prepared by hydrolysis of 50 microliters of TMP (10 mmol/1) in 10 ml 0,01 M hydrochloric acid for 10 min at room temperature. The MDA stock solution was diluted with ultrapure water to different concentrations of MDA standards. Calibration in plasma was performed by adding 200 #1 phosphoric acid containing different amounts of MDA to pooled samples of plasma. After adding the samples (200 microliters) to the reaction mixture, tubes were then incubated at 90°C in a water bath. Lipid peroxidation in blood serum samples was evaluated by measuring the production of MDA after reacting with glacial acid acetic thiobarbituric acid for 1 h. The tubes were then put on ice to stop the reaction. After cooling to room temperature 100 microliters of standards and samples were placed into a flat-bottom 96-well multititer plate. Absorption was read at 535 nm and 572 nm to correct for baseline absorption in a multititer plate reader. MDA equivalents (TBARS) were calculated using the difference in absorption at the two wavelengths and quantification was made with the aid of calibration curves.

2.6. Determination of α-tocopherol, δ-tocopherol and γ-tocopherol

N-hexane, and ethyl acetate were UHPLC/MS-grade from Optima, Fisher Chemical products (Milan, Italy). All other reactants and solvents, tocopherols (α-tocopherol, δ-tocopherol, and γ-tocopherol) were purchased from Sigma-Aldrich (Milan, Italy). Blood samples were procured from dogs by venipuncture, centrifuged (2000 × g, 8 min, at 4◦C). Serum was separated and aliquoted into amber microcentrifuge tube and stored at −70 ◦C. Samples were thawed at room temperature, gently mixed by inversion and centrifuged at 2000 × g at 4 ◦C for 15 min. Serum aliquots of 200 µL were deproteinated by addition of 200 µL ethyl alcohol containing 0.5 mol/L echinenone and 4 mol/L tocol in an amber glass vials. Samples were vortex-mixed for 3 min followed by addition of 800 L hexane with mixing for 10 min at 1500 rpm by mechanical vortex than samples were centrifuged for 15 min

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at 1600 rpm and supernatants were transferred into amber glass vials. The hexane extraction process was repeated. The supernatants were evaporated under nitrogen stream at room temperature. The residues were reconstituted in 100 L mobile phase containing 30 mg/L BHT without ammonium acetate followed by 2 min vortex and 2 min ultrasonic bath. Twenty microliter samples were injected into HPLC column. The reconstituted residue and injection solvent were stable for a maximum 24 h at 4 ◦C. A Shimadzu (Milan, Italy) LC-20AD HPLC system equipped with an RF-20A fluorescence detector, a CBM-20A controller, a CTO-20A column oven, an LC-20AD pump and a DGU-20A3 degasser were used to perform chromatographic analysis. Analyses were carried out using a LiChrosorb® Si 60 (5 µm) column (4.6 mm I.D. × 250 mm), protected by a guard column with the same stationary phase. The analyzes were run at 50 ◦C under isocratic conditions using a mobile phase composed of n-hexane/ethyl acetate (90:10 v/v). The volume of injection was of 15 µL and the flow rate was 0.9 mL/min. Fluorescent excitation and emission wavelengths were 295 nm and 330 nm, respectively. Alpha-δ- and γ-tocopherol were identified using commercial standards. For the quantitative analysis, the standard external method was used with appropriate calibration curves e any quantification estimated as the mean value of three repeated measurements. The results are expressed as mean ± standard deviation.

2.7. Statistical Analysis

Statistical analysis was performed using SPSS version 27.1 (IBM Italy). Data were an-alyzed for normality using the Shapiro-Wilk test and reported as mean ±SD or median (range) as appropriate. Differences along the time line were analyzed with t-test or wilcoxon test appropriate. Inter-observer agreement for quality of postoperative analgesia were analyzed using the Kendall's coefficient of concordance W. Multiple linear regression between MDA and tocopherols was performed. The differences were considered significant for $p \leq 0.05$. Sample size was calculated using Sample Size Calculator software, confidence level was 80%, margin of error was 5%, population proportion was 50%, population size was 20. Exclusion criterion for alterations of the blood count and of the basal biochemical parameters.

3. Results

The sample size was 18 subjects thus the selected sample was representative of the population. The level of agreement among observers who assigned postoperative pain scores was high W=1.

Physiological parameters remaining within the physiological ranges, of patients under general anesthesia, after induction and throughout surgery showing optimal homeo-stasis. SPO2 was 96/100% showing good tissue oxygenation. $ETCO_2$ decreased along the time line from 45 to 36 /38 mmHg showing a good adaptation of the patient to the pressure metric ventilator and a good anesthetic plan. The inspired and expired concentrations of sevofluorane were 5-3% CSI and 4-7% CSE (Table 1). The CPS scores were 0 – 6 for the entire duration of the surgery (Table 2). Canine acute pain scale score assigned were 0 for the entire postoperative period. No patient required either intraoperative or postoperative salvage analgesia. The blood count was normal for all subjects. Glycemia increased 12 hours after meloxicam discontinuation p=0.007. Albumin decreased 12 hours after discontinuation of meloxicam p=0.008. Aspartate transaminase (AST); Alanine aminotransferase (ALT), total protein, and blood urea nitrogen were normal at baseline and 12 hours after meloxicam discontinuation (Table 3). MDA levels increased from baseline at 12 and 24 hours after meloxicam discontinuation: p=0.000. α-δ γ-tocopherol concentration decreased from baseline at 12 and 24 hours after meloxicam discontinuation: P=0.000. Multiple linear regression between the con-centration of MDA and tocopherols showed a reduction of the latter at 12 and 24 hours after discontinuation of meloxicam p=0.000 (Table 4).

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Table 1. Legend:HR = heart rate; RR = respiratory rate; SAP = systolic blood pressure; DAP = dyastolic blood pressure; MAP = mean blood pressure EtCO2 = end-tidal carbon dioxide tension; SpO2 = arterial hemoglobin oxygen saturation TC° = body temperature; CSI%, CSE% concentration of inspired and expired sevofluorane. B = baseline values; P= 20 minutes after administration of atropine and meloxicam. A= after induction general anaesthesia; (SI = skin incision; L= laparotomy; TPI = traction of the first ovarian pedicle; TPII = traction of the second ovarian pedicle; SC = skin suturing. Values expressed with median and range (HR, RR, SAP, $EtCO_2$, SPO_2) and mean +/- standard deviation (TC°). * Significant difference compared to baseline values.

	B	*P*	*A*	*SI*	*L*	*TPI*	*TPII*	*SC*
HR Beats min	121/104/145	129/98/171	146/136/158	151/130/178	141/130/160	144/130/173	145/130/173	136/123/164
RR Breaths min	39/30/56	47/34/105	12/12/12	12/12/12	12/12/12	12/12/12	12/12/12	12/12/12
SAP mmHg	125/110/137	107/95/130	102/99/109	106/90/131	116/106/122	109/100/132	107/100/135	108/91/125
MAP mmHg	104/92/120	97/80/122	99/95/107	104/88/125	109/107/113	105/98/125	103/95/126	102/88/120
DAP mmHg	63/57/61	54/47/65	51/49/55	54/45/66	58/64/60	57/54/68	55/52/68	56/47/66
$ETCO_2$ mmHg			45/26/52	39/33/44	35/32/42	35/30/42	37/34/42	36/27/38
$SP0_2$%			99/96/99	98/97/100	98/97/100	98/96/100	98/96/100	98/96/100
TC°	39.0±0.1		39.0±01	38.5±0.01	38.5±0.02	38±0	38±0.1	38±0.2
CSI%			5.1/4.5/6.8	5.4/4.9/5.6	4.5/4.2/5.6	4.2/3.1/5	4.2/3.1/5	2.4/0.7/4.90
CSE%			3.9/3.2/4.6	4/3.2/4.6	4/3.2/4.4	4.5/3.2/4.8	4.4/3.2/4.6	2.8/0.7/4.6

Table 2. Legend: CPS, cumulative scale of pain, by giving scores of percent variations, compared with the values recorded after induction general anaesthesia (A) of heart rate, respiratory rate, SNAP = systolic non-invasive blood pressure, according to the following scheme: 0 no change or reduction of the above parameters: 1 increase of 10%, 2 increase from 20% to 30%, 3 increase from 30% to 40%, 4 increase from 40% to 50%. The sum of the scores gives us the total score; IS = skin incision; L= laparotomy; TPI = traction of the first ovarian pedicle; TPII = traction of the second ovarian pedicle; SC = skin suturing.

		CPS		
IS	*L*	*TPI*	*TPII*	*SC*
2/0/5	1.5/0/3	1.8/0/6	1.6/0/6	1.45/0/4

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Table 3. Median and range values of the biochemical parameters: Glycemia; Aspartate Transaminase (AST); Alanine Aminotransferase (ALT), Total Protein, Albumin and Aztemia. At baseline and 12h after the last administration of meloxicam.

	Baseline	12h after meloxicam	p valiu
Glycemia mg/dl	90/84/96	143/105/148	**0.007**
ALT U/l	17/12/25	17/13/24	**1.000**
AST U/l	28/14/39	28/13/39	**1.000**
Total Protein g/dl	8,7/5,3/8,7	8,9/4,34,95	**0.371**
Albumin g/dl	3,8/3,7/5,7	3,7/3,4/4,6	**0.008**
Azotemia mg /dl	18/13/26	18/13/26	**1.000**

Table 4. Median and range values of the Malondialdehyde (MDA), α-tocopherol, δ-tocopherol, and γ-tocopherol. At baseline 12h and 24 h after the last administration of meloxicam.

	Baseline	12h after meloxicam	24h after meloxicam	p valiu
MDA μg\mL	33.18(2.44/72.20)	52.48(15.40/102.10)	52(15.13/98.55)	**0.000**
α- tocopherolsmg/L	13.7(6.48/19.80)	12.8 (6.26 /19.38)	(12.5 6.25/19.35)	**0.000**
γ- tocopherols mg/L	3.0(2.72/ 6.20)	2.73(2.63/3.50)	2.71(2.50/3.47)	**0.000**
δ- tocopherols g/L	.92(.87/1.80)	.85(.80/.92)	.84(.76/.86)	**0.000**

4. Discussion

The anesthetic protocol, consisting of perioperative meloxicam, propofol and sevofluorane, proved effective and free of clinically diagnosable side effects. Physiological parameters remaining within the physiological ranges for dogs, under general anesthesia, showing optimal homeostasis. $ETCO_2$ showing a good adaptation of the patient to the pressure metric ventilator and a good anesthetic plan. This aspect is of fundamental importance for the management of the anesthetized patient, as a rapid achievement of the anesthetic plane suitable for surgery, shortens the surgical times and enhances the analgesic efficacy for an immediate synergistic action between the halogenated and NSAIDs. This aspect is due to the low solubility in the blood of sevofluorane which is also rapidly eliminated from the respiratory system [25]. The CPS scores were 0 – 6 for the entire duration of the surgery while those of the Canine acute, pain scale assigned were 0 for the entire postoperative period. No patient required either intraoperative or postoperative salvage analgesia. However, although perioperative pain management was clinically optimal, surgery resulted in inflammatory oxidative stress, evidenced by decreased albumin, increased glycemia and MDA serum concentration. Multiple linear regression between the concentration of MDA and tocopherols showed a reduction of the latter at 12, 24 hours after discontinuation of meloxicam. Inflammation increases capillary permeability resulting in leakage of serum albumin. Hypoalbuminemia is an indicator of oxidative stress also linked to surgery; the evaluation of the blood albumin concentration is a valid clinical indicator of the state of well-being of the surgical patient. Therefore its determination represents a tool for the evaluation of inflammation during surgery. Hypoalbuminemia rather than corrected in healthy surgical patients should be prevented or managed [26]. Hyperglycemia predisposes to multiple postoperative complications. However, perioperative glycemia assessments is not usual clinical practice. A clinical study in humans has demonstrated that the increase of glycemia in after major surgery in healthy patients did not cause postoperative complications, but only diabetic patients reported complications [27]. Surgery causes inflammation and therefore

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oxidative stress independently of perioperative pain management. The temporary ischemia and reperfusion of an organ during surgery give rise to oxidative reactions. Given the role of oxidative stress in the development of numerous post-surgical pathologies, research in recent years has focused its attention on identifying substances capable of preventing inevitable development of this inflammatory status during surgery [1]. Several studies in women and female laboratory animals have demonstrated that there is an increase in stress after ovariectomy induced in dog and there is few information on the evaluation of ovariectomy inflammatory oxidative stress. In a study found increased plasma concentrations of thiobarbituric acid reactive substances (TBARS) in ovariectomized female dogs on postoperative day 30°. The determination and prevention of this oxidative status certainly represents a method of prevention for various pathologies that could arise from ovariectomy in dogs [28].

Lipids are a major constituent of the erythrocyte membrane and are susceptible to at-tack by free radicals (lipid peroxidation) which promote changes in the shape and integrity of the membrane, compromising the function of these cells. Malondialdehyde (MDA) is a thiobarbituric acid-reactive substance formed as a byproduct of lipid peroxidation and its production is related to the magnitude of oxidative stress [29]. MPO is expressed by activated neutrophils and is capable of initiating lipid peroxidation at sites of inflammation, and its increase must be accompanied by an increase in lipoperoxidation [30]. Moreover, among tocopherols, α-tocopherol is the form of vitamin E that preferentially accumulates in blood and tissues. It is considered the main antioxidant defense against lipid peroxidation in the cell membranes of all mammals and the most important role of α-tocopherol in tissues appears to be PUFA membrane protection against the effects of oxygen radicals. It inhibits the peroxidation of membrane lipids by eliminating lipid peroxyl radicals and is converted into a tocopheroxyl radical consequently [18]. α-tocopherol maintains nearly normal values membrane-bound enzyme activities, thus preserving mitochondrial membrane integrity and protected from some enzymatic activities oxidation by free radicals [31].

In this study we believe that dietary tocopherol supplementation may decrease the susceptibility to lipid peroxidation in the tissues of animals undergoing ovariectomy in dogs [32]. Therefore, the administration of tocopherols could have a protective effect against the oxidative stress with elevation of serum MDA observed in this research. The plasma concentrations of α-tocopherol, δ-tocopherol and γ-tocopherol found were in line with those reported in the literature in humans (5.5 to 17.0 mg/L) both at baseline and after surgery despite the reduction in tocopherol concentration and this could be related to good anaesthesiological management of operated patients [33]. Therefore the dosage of tocopherols could be included as a screening in the evaluation of the surgical patient and as a means of evaluating oxidative stress, thus becoming an integral part of the anaesthesiological perioperative assessment.

Some author reported that in human patients with osteoarthritis, tocopherols have been shown to improve clinical symptoms and reduce free radical and pro-inflammatory cytokine synthesis and also in dogs with osteoarthritis, tocopherols could reduce clinical symptoms and free radical synthesis and the authors demonstrated that tocopherols supplementation in the early stages of surgically induced osteoarthritis in dogs reduces the production of pro-inflammatory markers (PGE_2, NOx) in the synovial fluid and that this vitamin also contributes to the reduction of histological lesions in the articular cartilage and decrease in pain associated with the development of osteoarthritis [21,34,35]. In particular, α-tocopherol appears to modulate a variety of cellular functions that are not necessarily a result of its antioxidant activity [35]. Therefore the administration of tocopherols before or during surgery is recommended.

5. Conclusions

The effect of meloxicam in the balanced anaesthesia protocol with propofol and sevoflurane may be correlated to tocopherol levels which however remained within the concentration range referred in humans and this could be relevant in the control of the inflammatory response accompanying tissue damage during reperfusion.

6. Patents

Author Contributions: Conceptualization: G.L.C. and P.L.; Data curation: F.L, D.I., V.N., F.D.L., P.L., D.M. and I.L.; Investigation: G.L.C., N. I., C.I., V.F., F.M., F.S., V.A., D.F. and D.M.; Writing: P.L., G.L.C. All authors have read and agreed to the published version of the manuscript

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Institutional Review Board Statement: The present clinical study was approved by the Review Board for Animals Care of the University of Parma prot N. 03/CESA /2023, which provided a consent to the clinical study: regulation (EU) no. 536/2014. (22A01712) (GU General Series n.65 of 18-03-2022); European Law (O.J. of E.C. L 358/1 12/18/1986), and USA Laws (Animal Welfare Assurance No A5594-01, Department of Health and Human Services, USA). The owners signed a voluntary informed consent form prior to the dogs' enrollment in the study.

Informed Consent Statement: Prior to the patients' enrolment in the study a written informed consent has been obtained from the owners to perform the clinical investigations and to publish the data.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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