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Oxidative stress in relation to serotonin under general anaesthesia in dogs undergoing ovariectomy

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ABSTRACT

Abdominal surgery such as ovariectomy is a traumatic event that can cause oxidative stress. The aim of the present study was to evaluate the concentration of serotonin in relation to ovariectomy-induced oxidative stress in dogs undergoing general anesthesia. Thirty-two female dogs, under general anesthesia, received meloxicam before surgery (0.2 $mgkg^{-1}$ SC) and after surgery (0.1 $mgkg^{-1}$ OS every 24 h). The physiological, hematological, and biochemical parameters: glycemia, aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, albumin and BUN were evaluated. Oxidative stress was determined by malondialdehyde (MDA) assay, catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO) and butyrylcholinesterase (BuChe) at baseline, 36 and 48 h after the last administration of meloxicam. Serotonin (5-HT) concentration was also evaluated at baseline, 36 and 48 h after the last administration of meloxicam. Responses to surgical stimulus were evaluated. Physiological and hematological parameters they fell within the normal ranges for anesthetized dogs. Glycemia increased, albumin levels decreased after surgery. No rescue analgesia was required. MDA and 5-HT concentrations significantly increased from the baseline at 36 and 48 h after surgery ($p < .001$). 5-HT levels could be used as an indicator for oxidative stress induced by surgery and it might be employed for objectively quantifying the well-being of the surgical patient.

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1. Introduction

Many environmental, social and clinical conditions can increase oxidative stress in animals, especially when occur pathological and painful diseases. Trauma and surgical injury can also delay recovery after surgery, through the induction of cell apoptosis and tissue necrosis (Senoner et al. 2019). Surgical stress in animals can be reported using a variety of markers, including cortisol and serotonin hormones, oxidative stress markers and behavioral analysis (Szczubial et al. 2015; Srithunyarat et al. 2016; Bruschetta et al. 2024). The use of a balanced anesthetic protocol could reduce the anesthetics dose with an effective reduction in the side effects induced by surgical stress (Beloeil and Nouette-Gaulain 2012). Drugs useful in the management of perioperative pain in dogs include opioids, α2-adrenergic receptor agonists, local anesthetics, and non-steroidal anti-inflammatory drugs (NSAID) (Mwangi et al. 2018). Propofol was an anesthetic with a chemical structure similar to tocopherol with high antioxidant power and it has low side effects. It was administered intravenously to induce and maintain general anesthesia in dogs (Interlandi et al. 2022; Costa et al. 2023; Costa et al. 2023). Sevoflurane was a halogenated anesthetic that was rapidly removed from the respiratory system and has low side cardiorespiratory effects, low solubility and it was widely used in clinical practice, especially for its neuroprotective and anti-inflammatory effects, that promote rapid recovery of the patient (Tsuchiya et al. 2010; Lee 2012; Liang et al. 2021).

The combination of the two anesthetics allows to obtain a protocol of general anesthesia, already used in humans and in dogs undergoing surgery (Lee 2012; Interlandi et al. 2022).

The use of Meloxicam, a non-steroidal anti-inflammatory drug (NSAID), combined with propofol and sevoflurane enhances the anti-inflammatory and analgesic properties of the anesthetic protocol. Meloxicam selectively inhibits the inducible cyclooxygenase 2 (COX-2), which mediates the inflammatory response as a function of the inflammatory stimulus induced by an external or internal stimulus. Furthermore, trauma or surgical injury also induce the release of the same inflammatory mediators produced by the constitutive COX-1 isoform located in every cell. Like traditional NSAIDs used in humans, Meloxicam also has antipyretic activity and improved gastrointestinal tolerability, making it suitable for the

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management of various pathologies (Xu et al. 2014; Farmacologia Veterinaria - II Edizione 2021).

Serotonin or 5-hydroxytryptamine (5-HT) was a biogenic monoamine known as the 'happiness hormone'. The 5-hydroxytryptamine was a neurotransmitter derived from the amino acid tryptophan, which at the level of the Central Nervous System was responsible for regulating mood and temperature, as well as appetite and sleep (Nichols and Nichols 2008). In the peripheral nervous system, serotonin was involved in vasoconstriction, gastrointestinal motility, bone homeostasis, inflammation, and lactation (Matsuda et al. 2004; Bertrand 2006).

Serotonin was synthesized from the amino acid L-tryptophan by initial hydroxylation to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase (TPH), which limits the rate of serotonin synthesis. There were two genetically distinct expressed isoforms of this enzyme: TPH1 was peripherally expressed into the pineal body and the digestive tract, whereas TPH2 was centrally localized in the brain (Grahame-Smith 1964; Walther et al. 2003).

The produced serotonin was released as a neurotransmitter from the presynaptic neurons into the synaptic space under an appropriate stimulus to ensure interaction with the specific receptor on the plasma membrane of the postsynaptic neuron. The released serotonin was taken back into the synaptic cleft by the presynaptic neuron through a reuptake system operated by the serotonin transporter (SERT).

Surgical oxidative stress can cause an increase in serotonin and enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GSR), which were involved in the antioxidant process (Balaban et al. 2005; Szczubial et al. 2015; Srithunyarat et al. 2016; Hydbring-Sandberg et al. 2021). Salavati et al. revealed that serotonin levels decreased in castrated dogs (Salavati et al. 2018). However, acute stress can induce increases or decreases in serotonin concentrations (Bruschetta et al. 2024).

The oxidative imbalance resulting from surgery determines the production of peroxides and free radicals, which can cause damage to all cellular components such as proteins, lipids, and DNA. The damage was mostly indirect, caused by the reactive oxygen species generated, such as superoxide anion (O_2^-) and hydroxyl radical (OH) (Giles et al. 2017; Ali et al. 2020). Lipid peroxidation processes affecting biological membranes and inflammatory processes determine an increase in malondialdehyde (MDA), which it was used in biomedical research as a marker of lipid peroxidation. Its quantification by the TBARS assay were demonstrated in human serum, low-density lipoprotein, and other cell lysates (Ayala et al. 2014).

General anesthesia allows surgery to be performed without the perception of pain, awareness and memory, movement, while maintaining the vital parameters of the subject involved both the spinal cord with analgesia and suppression of movement (Yang et al. 2009) and the brain with amnesia and loss of consciousness (LOC) (Franks 2008). In line with the common perception that general anesthesia induces sleep, it was useful to establish a correlation between the awakening from sleep, which was determined by hormonal circadian rhythms or by stimuli, and the awakening from anesthesia, which was a recovery from a drug-induced coma. Natural sleep and general anesthesia may function similarly after sleep deprivation (Tung et al. 2002; Tung et al. 2004).

According to previous studies in rats, propofol-induced anesthesia leads to natural sleep, such as deep sleep, which results in increased slow-wave sleep time (SWS) and rapid eye movement sleep (REMS) (Tung et al. 2004). On the other hand, sevoflurane appears to reduce SWS only, but even with other volatile anesthetics such as isoflurane, REMS has not been reduced by anesthesia (Mashour et al. 2010; Pal et al. 2011; Pick et al. 2011).

The aim of this study was to evaluate the oxidative stress in relation to serotonin during ovariectomy in dogs. The hypothesis of the study was to identify a positive or negative correlation between the malondialdehyde concentration (MDA) and serotonin during surgery.

2. Materials and methods

The clinical study was approved by the Review Board for Animals Care of the University of Parma, project No. 03/CESA/2023, according to 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 US laws (Animal Welfare Assurance No. A5594-01, Department of Health and Human Services, USA). G Power 3.1 software was used, with an effect size (f) of 0.5, a significance level (α) of 0.05, a power (1-β) of 0.80 and only one group, in order to adequately determine the sample size for 'a priori' X^2 tests (goodness-of-fit tests: contingency tables). The owners signed a voluntary informed consent form prior to the dogs' enrolment in the study. Thirty-two female dogs, aged 2 ± 0.5 years and weighing 18 ± 0.7 kg were enrolled for bilateral ovariectomy. The inclusion criterion of the patients was necessary ovariectomy. The exclusion criterion was not belonging to the ASA I class.

2.1. Drug administration and anesthesia

Preoperative oral intake of fluids and water can be safely consumed up to 2 h while the food was allowed up to 6 h prior to anaesthesia administration. The dogs also received 0.2 mg kg^{-1} meloxicam subcutaneous (SC) (Metacam 2% Boehringer Ingelheim, Italy) and 0.03 mg kg^{-1} atropine sulphate intramuscular (IM) (atropine sulphate 0.1% A.T.I., Italy). A 20 G × 32 mm catheter (DELTA VEN) was placed in the cephalic vein for the administration of 5 $mlkgh^{-1}$ of Ringer's lactate for the whole procedure. Twenty minutes after premedication, general

anesthesia was induced with propofol (Proposure 1% Merial, Italy) and endotracheal intubation was performed with a Magill cuff tube. Sevoflurane (Sevoflo, Zoetis Italy) in 100% oxygen *via* a rebreathing circuit was administred for general anesthesia. Ventilation was performed using a pressurimeter ventilator (SIMV) (GE Datex Ohmeda Avance Ultramed, Italy) with the following parameters: respiratory rate 12 breaths min^{-1}, positive end-inspiratory pressure (PEEP) 4 cm H_2O, inspiration/expiration ratio (I:E) 1:7 and airway pressure 12 cm H_2O. Meloxicam 0.1 mg kg^{-1} was administered orally every 24 h after surgery.

2.2. Physiological and anesthetic variables

After a 30-minute acclimatization period in the surgical preparation room: heart rate (HR, beats per minute) was taken over using a stethoscope. Respiratory rate (RR, breaths per minute) was taken over by counting thoracic wall excursions, non-invasive arterial pressure (mmHg) (systolic, SAP; mean, MAP; diastolic, DAP) measured using a cuff (10-18cm in circumference) placed at arm level. Body temperature (TC°) was detected placing the thermometer in the rectum, end-tidal carbon dioxide tension ($EtCO_2$, mmHg), arterial hemoglobin oxygen saturation (SpO_2, %) and the concentration of inspired and expired isoflurane (CSI/CSE) were measured using a monitor (GE Datex-Ohmeda Avance multiparametric monitor for anesthesia Ultramed, Italy). These parameters were recorded at the T0 baseline, at 20 min after premedication (P) (except $EtCO_2$ and the concentration of inspired and expired isoflurane), after the induction of general anesthesia (A), at skin incision (SI), at laparotomy (L), during traction and removal of the first ovarian and second ovarian (TPI, TPII), and at skin suturing (SC).

2.3. Assessment of intra- and post-operative response to surgical stimulus

The intraoperative response to the surgical stimulus was assessed using a cumulative pain scale (CPS). A score from 0 to 4 was assigned to the percentage change in RR, HR and SAP values recorded after anesthesia (A), according to the following scheme: 0 ≤ 0%; 1 = variation ≤ 10%; 2 = variation > 10% but ≤ 20%; 3 = variation > 20% but ≤ 30%; and 4 = variation > 30%. A total 10 score, corresponding to the 20% increase of the three parameters considered was the cut-off point for the rescue analgesia: 2 mcg kg^{-1} of fentanyl (Fentadon, Dechra, Italy) (Costa et al. 2019). A canine acute pain scale (Colorado State University Veterinary Medical Center), was used to evaluate postoperative pain by assigning a 0-4 score, from waking up every 6 h–24 h post-surgery. Score 2, corresponding to moderate to mild pain, was the cut-off point for the administration of the rescue analgesia: methadone 0.2 mg kg^{-1} IM (Semfortan, Dechra, Italy).

2.4. Hematological, biochemical parameters, oxidative parameters and 5-hydroxytryptamine assay

The same operator collected five ml blood samples from the cephalic vein, after the previous measurements. Each sample was divided into aliquots, one of which was placed in a vacuum serum isolation tube (serum clot activator Z, Vacuette®, Greiner Bio-One, Kremsmünster, Austria) and used to assess biochemical parameters such as glycemia, aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, albumin, and urea. Another aliquot was used to assess oxidative stress levels by measuring lipid peroxidation, catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO) and butyrylcholinesterase (BuChe).

An additional volume of blood was placed in a vacuum tube containing K3-EDTA (Vacuette®, Greiner Bio-One, Kremsmünster, Austria) for assessment of the blood hemogram at baseline only. Both sets of tubes were immediately refrigerated at 4 °C, and after 3 h only the sample in the serum tube was centrifuged at 1500 rpg for 15 min to obtain the serum aliquot.

An UV-Vis spectrophotometer (A560, Fulltech, Italy) was used to evaluate AST and ALT parameters at 37 °C using kinetic methods (Medica et al., 2018a, 2018b). Glucose, albumin, total protein and blood urea were measured by glucose oxidase/peroxidase, bromocresol green, biuret, and peroxidase/phenylalanine methods, respectively.

Biochemical data were obtained from samples taken at baseline and 12 h after the end of surgery. Oxidative status was measured at baseline, 36 and 48 h after the end of surgery.

The malondialdehyde (MDA) assay required thiobarbituric acid (TBA) from Fluka (Buchs, Switzerland), phosphoric acid (85%, 15 mol/1), sodium hydroxide, SDS (8.1%) and sodium chloride from Merck (Darmstadt, Germany). All reagents were of the highest available grade. The MDA standard was prepared by hydrolysis of TMB. TBA reagent used for the 0.11 mol/l assay: 800 mg TBA dissolved in 50 ml NaOH 0.1 mol/1. Plasma was replaced with 200 µl of MDA standard solution to detect the amount of TBARS. MDA stock solutions were prepared by hydrolyzing 50 µl of TMP (10 mmol/l) in 10 ml of 0.01 M hydrochloric acid for 10 min at room temperature. To obtain different concentrations of MDA standards, the MDA stock solution was diluted with ultrapure water. Plasma calibration was performed by adding 200 µl of phosphoric acid containing different amounts of MDA to the pooled plasma samples. The tubes were incubated at 90 °C in a water bath after the samples were added to the reaction mixture. Lipid peroxidation in blood serum samples was assessed by measuring MDA production after reaction with thiobarbituric glacial acetic acid for 1 h. The tubes were placed on ice to stop the reaction and, after cooling, 100 µl of standards and samples were added to a 96-well microplate. Absorbance was read

at 535 nm and 572 nm to adjust baseline absorbance using a multititer plate reader. TBARS were quantified by measuring the optical density difference between the two wavelengths using appropriate calibration curves. The determination of catalase (CAT) activity was achieved by an enzymatic H_2O_2 reaction. All reagents were allowed to equilibrate at room temperature prior the assay. Each well contained a final test volume of 240 µL. The Catalase sample buffer (1X) and the diluted test buffer were used to assay samples and standards in duplicate. Absorbance was measured at 540 nm using a BIORAD 680 microplate reader (BIORAD Laboratories, Italy) (Wheeler et al. 1990). The superoxide dismutases (SODs) were metalloenzymes which catalyse the dismutation of superoxide anion to molecular oxygen and hydrogen peroxide. The final volume for each duplicated well was 230 µL and all reagents, were used at 25 °C prior to the start of the assay. The absorbance was monitored between 440 and 460 nm using the BIORAD 680 plate reader (BIORAD Laboratories, Italy) (Maier and Chan 2002). MPO activity was measured using the dianisidine-H70 method on a 96-well plate. Samples were added in triplicate to a mixture containing 0.53 mM o-dianisidine hydrochloride, 0.15 mM hydrogen peroxide, and 50 mM potassium phosphate buffer (pH 6.0). The reaction was incubated for 5 min at 25 °C, blocked with 30% sodium azide and the absorbance measured at 460 nm (BIORAD 680 plate reader, BIORAD Laboratories, Italy) (Dhiman et al. 2009). In the BuChE assay, the substrate was hydrolysed to form thiocholine, which reacts with 2-nitrobenzoic acid dissolved in 625 µl of the BuChE assay buffer. The absorbance was measured at 412 nm and 25 °C using a BIORAD 680 plate reader (BIORAD Laboratories, Italy) (Jasiecki et al. 2021). To assess plasma 5-hydroxytryptamine (5-HT) levels, 2 ml of each 5 ml aliquot was filled into an EDTA Vacutainer tube (K3-EDTA, Vacuette, Greiner Bio-One, Kremsmünster, Austria), immediately stored at 4 °C and then (within 2-3 h) centrifuged at 4500 Rpm, 4 °C for 10 min to obtain a 98% free platelet-poor plasma (PPP) fraction (Bruschetta et al. 2017; Bruschetta et al. 2018). PPP samples were stored at −20 °C for 5-HT analysis.

The concentration of 5-HT in the platelet-poor plasma (PPP) fraction was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (DEE5900, Demeditec Diagnostic GmbH, Germany). Serotonin standards and plasma samples were processed according to the kit protocol (Medica et al. 2020). The absorption values were determined at the wavelength of 450 nm (reference wavelength at 630 nm) using a microplate reader BIO-RAD 680 (BIO-RAD Laboratories, Italy). Standard absorbance values were used to obtain the calibration curve, from which the plasma 5-HT concentrations of the samples could be estimated. 5-HT concentration values were expressed in ng/ml. The sensitivity of the assay was 0.5 ng/ml. The average intra- and inter-assay coefficients of variation were 6.2% and 9.1%, respectively.

2.5. Statistical analysis

Statistical analysis was performed using SPSS version 27.1 (IBM, Italy). The data were analyzed for normality using the Shapiro-Wilk test and reported as the mean ± SD or median (range) as appropriate. Differences along the timeline were analyzed with a t-test or the Wilcoxon test as appropriate. Inter-observer agreement for the quality of postoperative analgesia was analyzed using Kendall's coefficient of concordance W. The correlation between MDA and serotonin levels were evaluated by Pearson's correlation. The differences were considered significant at $p \leq .05$.

3. Results

The total number of subjects involved in the research study was 32, with an effective power of 0.80 and a high intra-observer agreement of $W = 1$. Heart rate increases, compared to baseline, along the time line after general anesthesia $p = .000$. Systolic arterial pressure decreases along the entire time line $p = .000$. Median and diastolic arterial pressure showed no significant differences from baseline SPO_2 was 96/100%, $ETCO_2$ showed a significant reduction during anesthesia $p = .000$.

Body temperature was 39/37.9 C°, showing a non-statistically significant reduction.

Inspired concentrations of sevoflurane decreased along the time line after skin incision $p = .001$. Expired concentrations of sevoflurane was reduced compared to induction under general anesthesia during skin suturing $p = .001$ (Table 1). The CPS scores were 0–6 (Table 2).

The canine acute pain scale score assigned was zero. Glycemia significantly increased at 36 h after surgery ($p = .000$). Albumin significantly decreased at 36 h after surgery ($p = .000$). Aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, and blood urea nitrogen were normal at baseline and at 36 h after meloxicam discontinuation (Table 3). MDA levels significantly increased at 36 and 48 h after surgery ($p < .000$). Serotonin levels significantly increased at 36 and 48 h after surgery ($p < .001$). The Pearson's correlation between the MDA and the 5-HT concentration showed a perfect positive linear relationship of 1 ($p = .005$) (Table 4). The levels of SOD, CAT, BuChE and MPO showed no significant changes ($p = .05$) (Table 4).

4. Discussion

The results of the study confirmed a perfect positive linear correlation between the concentrations of 5-HT and MDA ($p = .005$). Therefore, it was likely that a painful stimulus induced by surgery stimulates the body to release serotonin to counteract inflammatory oxidative stress. The present anesthetic protocol with meloxicam, propofol and sevoflurane was effective and free of clinically diagnosable side effects. Optimal homeostasis was observed because vital

Table 1. Physiological and anesthetic variables measured and expressed with median include HR = heart rate; RR: respiratory rate; SAP: systolic arterial pressure; DAP: diastolic arteria pressure; MAP: mean arterial pressure; $EtCO_2$: end-tidal carbon dioxide tension; SpO_2: arterial hemoglobin oxygen saturation.

	B	P	A	SI	L	TPI	TPII	SC
HR (Beats min)	130(106/135)	132(97/170)	148(137/156)*	150(132/180)*	140(132/160)*	145(130/175)*	147(132/174)*	134(125/166)*
RR (Breaths min)	40(32/58)	48(36/107)*	12(12/12)*	12(12/12)*	12(12/12)*	12(12/12)*	12(12/12)*	12(12/12)*
SAP (mmHg)	126(112/138)	108(96/132)*	104(98/110)*	108(90/132)*	118(108/120)*	110(102/134)*	108(102/136)*	109(92/126)*
MAP (mmHg)	105(93/122)	98(82/124)	100(96/108)	105(90/126)	110(106/112)	106(97/126)	104(96/128)	103(90/122)
DAP (mmHg)	62(58/60)	52(46/64)	53(50/54)	56(46/68)	62(65/60)	58(55/70)	56(50/66)	58(46/67)
$EtCO_2$ (mmHg)			42(27/54)	40(32/44)*	35(36/45)*	36(32/44)*	34(32/45)*	36(27/39)*
SpO_2 (%)			99(97/99)	98(96/100)	98(97/100)	98(97/100)	98(98/100)	98(97/100)
T (C°)	39.0 ± 0.1		39.0 ± 01	38.5 ± 0	38.5 ± 0.02	38 ± 0	38 ± 0.1	38 ± 0.1
CSI (%)			5.2(4.6/6.8)	5.5(4.9/5.6)	4.6(4.3/5.6)*	4.2(3.1/5.8)*	4.2(3/5)*	2.3(0.8/5)*
CSE (%)			4(3.2/4.5)	4(3/4.6)	4(3/4.5)	4.6(3.2/4.9)	4.5 (3.2/4.7)	3(0.8/4.3)*

Body temperature (TC°) is expressed as mean +/− standard deviation. Inspired (CSI) and expired (CSE) sevoflurane concentrations are expressed with percentage variation. B: baseline values; $p = 20$ min after administration of atropine and meloxicam; A: after induction of general anesthesia; SI: skin incision; L: laparotomy; TPI: traction of the first ovarian pedicle; TPII: traction of the second ovarian pedicle; SC: skin suturing. * significant difference from baseline.

Table 2. Cumulative pain score (CPS): score 0/4; 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*
1 (1/4)	1 (1/1)	0 (1/5)	1 (1/6)	1 (0/3)

Data are expressed with the median and range.

Table 3. Biochemical parameters: Blood glucose, aspartate transaminase (AST), alanine aminotransferase (ALT), total protein, albumin and blood urea nitrogen measured at 12 h after the last meloxicam dose.

	Baseline	12h after surgery	*p* value
Glycemia mg/dl	95 (87–98)	145 (108–150)	**.000**
ALT U/l	17 (14–26)	17 (15–24)	**1.000**
AST U/l	26 (14–40)	26 (13–40)	**1.000**
Total Protein g/dl	9 (5,5–9)	9 (4,4–5)	**.371**
Albumin g/dl	4 (3,8–6)	3,5 (3,2–5)	**.000**
BUN mg /dl	17(13–25)	18 (14–25)	**1.000**

Data are expressed with the median and range.

Table 4. Malondialdehyde (MDA), serotonin (5-HT), catalase (CAT), superoxide dismutase (SOD), myeloperoxidase (MPO), butyrylcholinesterase (BuChE) levels at baseline and at 36 and 48 h after the last meloxicam administration.

	Baseline	36 h after surgery	48 h after surgery	*p* value
MDA µg\ mL	35 (3–74)	53 (16–105)	51 (14–99)	***p* = .000**
5-HT ng/mL	20.9 (18.7–22.30)	27.01 (19.38–42.00)	26.8 (20.15–45.23)	***p* = .000**
CAT (U/mL)	6.01 ± 0.041	4.82 ± 0.094	7.41 ± 0.18	***p* = .7**
SOD (U/mL)	6.42 ± 0.116	5.31 ± 0.135	5.92 ± 0.123	***p* > .05**
MPO (U/ mL)	1.37 ± 0.081	1.41 ± 0.089	1.32 ± 0.148	***p* > .05**
BuChE (U/ mL)	0.504 ± 0.075	0.641 ± 0.129	0.547 ± 0.139	***p* > .05**

Data are expressed with the median and range and mean ± SD.

signs remaining within physiological ranges for dogs under general anesthesia.

In fact, SPO_2 was 96/100%; $ETCO_2$ decreased along the timeline from 45 to 36/38 mmHg, showing good adaptation of the patient to the pressurimetric ventilator and a good anesthetic plan. This was essential for the anesthetic management of the patient and the rapid attainment of the surgical level of anesthesia. Furthermore, it reduces operating times and improves analgesic efficacy, with immediate synergistic effects between halogenated drugs and NSAIDs due to the poor solubility of sevoflurane in the blood, which was also rapidly eliminated by the respiratory system (Lu et al. 2014).

The pain score was 0-6 throughout surgery and 0 for the entire post-operative period. However, even though the perioperative management of pain was clinically adequate, the surgery resulted in a pro-inflammatory oxidative stress, as indicated by decreased level of albumin ($p = .000$), increased concentration of blood glucose ($p = .000$) and serum MDA ($p = .000$). This was confirmed by the perfect positive correlation between serotonin and MDA after meloxicam withdrawal. Unfortunately, most of the studies present in the literature limit themselves to evaluating and comparing different surgical techniques, for the execution of ovariectomy without carrying out objective evaluations such as toxicological and biochemical studies (Leonardi et al. 2020). The inflammation enhances capillary permeability with consequent leakage of serum albumin, promoting the onset of hypoalbuminemic conditions, as a biomarker of oxidative stress or wellness associated with surgery (Soeters et al. 2019).

Glycemic evaluation of animals during the perioperative period does not appear to be a common practice; however hyperglycemic conditions can lead to numerous postoperative complications. Human clinical studies have shown that increasing glucose levels after surgery did not result in a lower rate of complications in healthy people, unlike people with diabetes. In fact, the incidence of postoperative complications in patients with hyperglycemia was 26% and 14% in normoglycemic patients (Kalogeris et al. 2016). Independently of perioperative pain management, oxidative stress plays a fundamental role in the development of many postoperative pathologies since the surgical procedure itself determines an

oxidative burst that leads to a transient organ ischemia and reperfusion (Kalogeris et al. 2016). An increase in stress following ovariectomy was shown in women and female laboratory animals. However, there was limited information available to assess the inflammatory and oxidative stress consequent to ovariectomy in dog. In a study, the evaluation of oxidative stress induced by ovariectomy in female dogs was performed with the determination of plasma concentrations of glutathione peroxidase (GPX), superoxide dismutase (SOD), thiobarbituric acid reactive substance (TBARS), cationic radicals N, N-diethylparaphenylene diamine (RC-DEPPD) and on protein peroxidation (SH sulphide groups, bityrosine and formylkynurenine). The results of the study showed that there was a significant increase in GPX at 14 days after surgery, a significant increase in TBARS of bityrosine, formylkynurenine and a significant reduction in SH groups at 30 days after surgery. This shows that there may be late post-operative oxidative stress. However, in our study, we found an increase in MDA that indicates oxidative stress at 36 and 48 h after surgery ($p = .000$). Similar results were obtained in dogs undergoing ovariectomy and anesthetized with ketamine and xylazine, in fact, a significant increase in MDA was observed at 24 h after the end of surgery (Prajwalita et al. 2013). Therefore, a method of preventing pathology associated with ovariectomy in the dog was certainly the identification of oxidative status (Szczubial et al. 2015).

Serotonin was produced independently in the mammalian brain and peripheral areas since it does not cross the blood-brain barrier. Outside the Central Nervous System (CNS), most of the serotonin synthesis was largely confined to intestinal enterocromaffin cells and platelets, the latter with a smaller percentage of production. Platelets were an important storage site for 5-HT, and the most notable 5-HT concentration was found in the peripheral circulation, mainly in the platelets, and less in the blood. On the contrary, in the CNS serotonin was synthesized and stored in presynaptic cells (Mohammad-Zadeh et al. 2008). The function of each specific 5-HT receptor was related to its morphology, which was essential for the signaling pathway modulation involved. As a hormone, 5-HT has many different functions, sometimes with opposite effects, and this was due to the presence of seven different heterogeneous receptor classes for 5-HT, which were widely expressed throughout the body. Each receptor class has further subclasses, making a total of 13 (Hoyer et al. 1994). Six of these were G protein-coupled receptors, but only the 5-HT3 receptor involves a ligand-gated Na^+/K^+ ion channel). In the CNS, the cell bodies of serotonergic neurons were in the nine raphe nuclei, which were in the brainstem to develop extensive projections to the forebrain, hindbrain, and spinal cord (Gaspar and Lillesaar 2012). The molecules of interest, such as opioids (e.g. tramadol and butorphanol) or alpha-2 agonists (e.g. medetomidine, dexmedetomidine, romifidine and xylazine), act precisely on G-membrane proteins. Therefore, the combination of these drugs in an anesthetic protocol allows to reduce the relative doses of each drug and above all their side effects (Interlandi et al. 2017).

The large number of receptor types suggests that 5-HT has clinically relevant functions in the CNS in the control of psychological disorders such as depression and anxiety, targeted by many classes of drugs, including antidepressants and antipsychotics, designed to the serotonergic system (Marin et al. 2012). In several body areas, 5-HT was involved in the modulation of intestinal and gastric motility, vasoconstriction, enhancement of platelet aggregation and, above all, in wound healing (Sarrouilhe and Mesnil 2019). In fact, this point was essential for perioperative pain management carried out by the analgesic administration, and it was closely linked to the possibility that pain may have multiple effects on the organism's homeostasis. Stress increases the release of vasoactive catecholamines, which induce tachycardia and hypertension, coupled with cortisol, which leads to an increase in glucagon synthesis. The anabolic effect of pain was accompanied by catabolic effects resulting in delayed healing, pulmonary atelectasis, hypotrophy of the intestinal villi with consequent activation of lipolysis and production of ketone bodies (Viñuela-Fernández et al. 2007).

The future perspective is to evaluate surgical stress with lipid peroxidation and serotonin concentrations in order to compare surgical techniques and anesthetic protocols.

5. Conclusions

In this study, the increase in MDA level was related to the increase in serotonin level. It was likely that the body, subjected to a painful stimulus induced by surgery, released serotonin to counteract inflammatory oxidative stress. Therefore, the determination of serotonin levels may represent a method for objectively quantifying the well-being of the surgical patient, and could be a biomarker for oxidative stress induced by surgery.

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Ethics statement

The present clinical study was approved by the Review Board for Animals Care of the University of Parma, project No. 03/CF.B., L.ESA /2023, which provided consent for 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 US laws (Animal Welfare Assurance No. A5594-01, Department of Health and Human Services, USA). 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.

Authors contributions

Conceptualization: G. B., P.L., G. L. C.; Data curation: F.B.; Investigation: L.M., F. L.; Methodology: N.M.I., R.F.P.; Writing: G. B., P.L., G.L.C. All authors have read and agreed to the published version of the manuscript.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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Data availability statement

The data presented in this study are available on request from the corresponding author.

References

Ali SS, Ahsan H, Zia MK, Siddiqui T, Khan FH. 2020. Understanding oxidants and antioxidants: classical team with new players. J Food Biochem. 44(3):e13145. doi: 10.1111/jfbc.13145.

Amorena M, Anania V. et al. 2021. Farmacologia Veterinaria. Belolli C, Carli S, Ormas P. II Edizione, Idelson-Gnocchi.

Ayala A, Muñoz MF, Argüelles S. 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev. 2014:360438–360431. doi: 10.1155/2014/360438.

Balaban RS, Nemoto S, Finkel T. 2005. Mitochondria, oxidants, and aging. Cell. 120(4):483–495. doi: 10.1016/j.cell.2005.02.001.

Beloeil H, Nouette-Gaulain K. 2012. The perioperative period in cancer surgery: a critical moment! Is there a role for regional anesthesia in preventing cancer recurrence? Ann Fr Anesth Reanim. 31(6):528–536.

Bertrand PP. 2006. Real-time measurement of serotonin release and motility in guinea pig ileum. J Physiol. 577(Pt 2):689–704. doi: 10.1113/jphysiol.2006.117804.

Bruschetta G, Fazio E, Cravana C, Ferlazzo A. 2017. Effects of partial versus complete separation after weaning on plasma serotonin, tryptophan and pituitary-adrenal pattern of Anglo-Arabian foals. Livestock Sci. 198:157–161. doi: 10.1016/j.livsci.2017.02.025.

Bruschetta G, Medica P, Fazio E, Cravana C, Ferlazzo A. 2018. The effect of training sessions and feeding regimes on neuromodulator role of serotonin, tryptophan, and β-endorphin of horses. J Vet Behav. 23:82–86.

Bruschetta G, Zanghì G, Giunta RP, Ferlazzo AM, Satué K, D'Ascola A, Fazio E. 2024. Short road transport and slaughter stress affects the expression profile of serotonin receptors, adrenocortical, and hematochemical responses in horses. Vet Sci. 11(3):113. doi: 10.3390/vetsci11030113.

Costa GL, Di Pietro S, Interlandi C, Leonardi F, Macrì D, Ferrantelli V, Macrì F. 2023. Effect on physiological parameters and anaesthetic dose requirement of isoflurane when tramadol given as a continuous rate infusion vs a single intravenous bolus injection during ovariohysterectomy in dogs. PLOS One. 18(2):e0281602. doi: 10.1371/journal.pone.0281602.

Costa GL, Leonardi F, Interlandi C, Spadola F, Fisichella S, Macrì F, Nastasi B, Macrì D, Ferrantelli V, Di Pietro S, et al. 2023. Levobupivacaine combined with cisatracurium in peribulbar anaesthesia in cats undergoing corneal and lens surgery. Animals. 13(1):170. doi: 10.3390/ani13010170.

Costa GL, Nastasi B, Spadola F, Leonardi F, Interlandi C. 2019. Effect of levobupivacaine, administered intraperitoneally, on physiological variables and on intrasurgery and postsurgery pain in dogs undergoing ovariohysterectomy. J Vet Behav. 30:33–36. doi: 10.1016/j.jveb.2018.11.003.

Dhiman M, Estrada-Franco JG, Pando JM, Ramirez-Aguilar FJ, Spratt H, Vazquez-Corzo S, Perez-Molina G, Gallegos-Sandoval R, Moreno R, Garg NJ, et al. 2009. Increased myeloperoxidase activity and protein nitration are indicators of inflammation in patients with Chagas' disease. Clin Vaccine Immunol. 16(5):660–666. doi: 10.1128/CVI.00019-09.

Franks NP. 2008. General anaesthesia: from molecular targets to neuronal pathways of sleep and arousal. Nat Rev Neurosci. 9(5):370–386. doi: 10.1038/nrn2372.

Gaspar P, Lillesaar C. 2012. Probing the diversity of serotonin neurons. Philos Trans R Soc Lond B Biol Sci. 367(1601):2382–2394. doi: 10.1098/rstb.2011.0378.

Giles GI, Nasim MJ, Ali W, Jacob C. 2017. The reactive sulfur species concept: 15 years on. Antioxidants. 6(2):38. doi: 10.3390/antiox6020038.

Grahame-Smith DG. 1964. Tryptophan hydroxylation in brain. Biochem Biophys Res Commun. 16(6):586–592. doi: 10.1016/0006-291x(64)90197-4.

Hoyer D, Clarke DE, Fozard JR, Hartig PR, Martin GR, Mylecharane EJ, Saxena PR, Humphrey PP. 1994. International union of pharmacology classification of receptors for 5-hydroxytryptamine (Serotonin). Pharmacol Rev. 46(2):157–203.

Hydbring-Sandberg E, Larsson E, Madej A, Höglund OV. 2021. Short-term effect of ovariohysterectomy on urine serotonin, cortisol, testosterone and progesterone in bitches. BMC Res Notes. 14(1):265. doi: 10.1186/s13104-021-05680-y.

Interlandi C, Calapai G, Nastasi B, Mannucci C, Morici M, Costa GL. 2017. Effects of atipamezole on the analgesic activity of butorphanol in rats. J Exotic Pet Med. 26(4):290–293. doi: 10.1053/j.jepm.2017.07.001.

Interlandi C, Di Pietro S, Costa GL, Spadola F, Iannelli NM, Macrì D, Ferrantelli V, Macrì F. 2022. Effects of cisatracurium in sevoflurane and propofol requirements in dog-undergoing-mastectomy surgery. Animals. 12(22):3134. doi: 10.3390/ani12223134.

Jasiecki J, Szczoczarz A, Cysewski D, Lewandowski K, Skowron P, Waleron K, Wasąg B. 2021. Butyrylcholinesterase-protein interactions in human serum. Int J Mol Sci. 22(19):10662. doi: 10.3390/ijms221910662.

Kalogeris T, Baines C, Krenz M, Korthuis R. 2016. Ischemia/reperfusion. Compr Physiol. 7(1):113–170. doi: 10.1002/cphy.c160006.

Lee JY. 2012. Oxidative stress due to anesthesia and surgical trauma and comparison of the effects of propofol and thiopental in dogs. J Vet Med Sci. 74(5):663–665. doi: 10.1292/jvms.11-0221.

Leonardi F, Properzi R, Rosa J, Boschi P, Paviolo S, Costa GL, Bendinelli C. 2020. Combined laparoscopic ovariectomy and laparoscopic-assisted gastropexy versus combined laparoscopic ovariectomy and total laparoscopic gastropexy: a comparison of surgical time, complications and postoperative pain in dogs. Vet Med Sci. 6(3):321–329. doi: 10.1002/vms3.249.

Liang T-Y, Peng S-Y, Ma M, Li H-Y, Wang Z, Chen G. 2021. Protective effects of sevoflurane in cerebral ischemia reperfusion injury: a narrative review. Med Gas Res. 11(4):152–154. doi: 10.4103/2045-9912.318860.

Lu C-C, Tso-Chou L, Hsu C-H, Tsai C-S, Sheen MJ, Hu OY-P, Ho S-T. 2014. Pharmacokinetics of sevoflurane elimination from respiratory gas and blood after coronary artery bypass grafting surgery. J Anesth. 28(6):873–879. doi: 10.1007/s00540-014-1841-7.

Maier CM, Chan PH. 2002. Role of superoxide dismutases in oxidative damage and neurodegenerative disorders. Neuroscientist. 8(4):323–334. doi: 10.1177/107385840200800408.

Marin P, Becamel C, Dumuis A, Bockaert J. 2012. 5-HT receptor-associated protein networks: new targets for drug discovery in psychiatric disorders? Curr Drug Targets. 13(1):28–52. doi: 10.2174/138945012798868498.

Mashour GA, Lipinski WJ, Matlen LB, Walker AJ, Turner AM, Schoen W, Lee U, Poe GR. 2010. Isoflurane anesthesia does not satisfy the homeostatic need for rapid eye movement sleep. Anesth Analg. 110(5):1283–1289. doi: 10.1213/ANE.0b013e3181d3e861.

Matsuda M, Imaoka T, Vomachka AJ, Gudelsky GA, Hou Z, Mistry M, Bailey JP, Nieport KM, Walther DJ, Bader M, et al. 2004. Serotonin regulates mammary gland development via an autocrine-paracrine loop. Dev Cell. 6(2):193–203. doi: 10.1016/s1534-5807(04)00022-x.

Medica P, Cravana C, Bruschetta G, Ferlazzo A, Fazio E. 2018a. Breeding season and transport interactions on the pituitary-adrenocortical and biochemical responses of horses. J Vet Behav. 23:91–96. doi: 10.1016/j.jveb.2017.11.003.

Medica P, Cravana C, Bruschetta G, Ferlazzo A, Fazio E. 2018b. Physiological and behavioral patterns of normal-term thoroughbred foals. J Vet Behav. 26:38–42. doi: 10.1016/j.jveb.2018.04.002.

Medica P, Giunta RP, Bruschetta G, Ferlazzo AM. 2020. The influence of training and simulated race on horse plasma serotonin levels. J Equine Vet Sci. 84:102818. doi: 10.1016/j.jevs.2019.102818.

Mohammad-Zadeh LF, Moses L, Gwaltney-Brant SM. 2008. Serotonin: a review. J Vet Pharmacol Ther. 31(3):187–199. doi: 10.1111/j.1365-2885.2008.00944.x.

Mwangi WE, Mogoa EM, Mwangi JN, Mbuthia PG, Mbugua SW. 2018. A systematic review of analgesia practices in dogs undergoing ovariohysterectomy. Vet World. 11(12):1725–1735. doi: 10.14202/vetworld.2018.1725-1735.

Nichols DE, Nichols CD. 2008. Serotonin receptors. Chem Rev. 108(5):1614–1641. doi: 10.1021/cr078224o.

Pal D, Lipinski WJ, Walker AJ, Turner AM, Mashour GA. 2011. State-specific effects of sevoflurane anesthesia on sleep homeostasis: selective recovery of slow wave but not rapid eye movement sleep. Anesthesiology. 114(2):302–310. doi: 10.1097/ALN.0b013e318204e064.

Pick J, Chen Y, Moore JT, Sun Y, Wyner AJ, Friedman EB, Kelz MB. 2011. Rapid eye movement sleep debt accrues in mice exposed to volatile anesthetics. Anesthesiology. 115(4):702–712. doi: 10.1097/ALN.0b013e31822ddd72.

Prajwalita K, Mistry J, Chaudhary S. 2013. Oxidative stress due to ovariohysterectomy in bitches. *Indian Vet J*. 34(1):59–60.

Salavati S, Mogheiseh A, Nazifi S, Shojaee Tabrizi A, Taheri P, FJJoVBCA K, et al. 2018. Changes in sexual hormones, serotonin, and cortisol concentrations following oral administration of melatonin in castrated and intact dogs. J Vet Behav. 27:27–34.

Sarrouilhe D, Mesnil M. 2019. Serotonin and human cancer: a critical view. Biochimie. 161:46–50. doi: 10.1016/j.biochi.2018.06.016.

Senoner T, Schindler S, Stättner S, Öfner D, Troppmair J, Primavesi F. 2019. Associations of oxidative stress and postoperative outcome in liver surgery with an outlook to future potential therapeutic options. Oxid Med Cell Longev. 2019:3950818–3950818. doi: 10.1155/2019/3950818.

Soeters PB, Wolfe RR, Shenkin A, Nutrition E. 2019. Hypoalbuminemia: pathogenesis and clinical significance. JPEN J Parenter Enteral Nutr. 43(2):181–193. doi: 10.1002/jpen.1451.

Srithunyarat T, Höglund OV, Hagman R, Olsson U, Stridsberg M, Lagerstedt A-S, Pettersson A. 2016. Catestatin, vasostatin, cortisol, temperature, heart rate, respiratory rate, scores of the short form of the Glasgow composite measure pain scale and visual analog scale for stress and pain behavior in dogs before and after ovariohysterectomy. BMC Res Notes. 9(1):381. doi: 10.1186/s13104-016-2193-1.

Szczubial M, Kankofer M, Bochniarz M, Dąbrowski R. 2015. Effects of ovariohysterectomy on oxidative stress markers in female dogs. Reprod Domest Anim. 50(3):393–399. doi: 10.1111/rda.12501.

Tsuchiya H, Ueno T, Tanaka T, Matsuura N, Mizogami M. 2010. Comparative study on determination of antioxidant and membrane activities of propofol and its related compounds. Eur J Pharm Sci. 39(1-3):97–102. doi: 10.1016/j.ejps.2009.11.001.

Tung A, Bergmann BM, Herrera S, Cao D, Mendelson WB. 2004. Recovery from sleep deprivation occurs during propofol anesthesia. Anesthesiology. 100(6):1419–1426. doi: 10.1097/00000542-200406000-00014.

Tung A, Szafran MJ, Bluhm B, Mendelson WB. 2002. Sleep deprivation potentiates the onset and duration of loss of righting reflex induced by propofol and isoflurane. Anesthesiology. 97(4):906–911. doi: 10.1097/00000542-200210000-00024.

Viñuela-Fernández I, Jones E, Welsh EM, Fleetwood-Walker SM. 2007. Pain mechanisms and their implication for the management of pain in farm and companion animals. Vet J. 174(2):227–239. doi: 10.1016/j.tvjl.2007.02.002.

Walther DJ, Peter J-U, Bashammakh S, Hörtnagl H, Voits M, Fink H, Bader M. 2003. Synthesis of serotonin by a second tryptophan hydroxylase isoform. Science. 299(5603):76–76. doi: 10.1126/science.1078197.

Wheeler CR, Salzman JA, Elsayed NM, Omaye ST, Korte DW. 1990. Automated assays for superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase activity. Anal Biochem. 184(2):193–199. doi: 10.1016/0003-2697(90)90668-y.

Xu S, Rouzer CA, Marnett LJ. 2014. Oxicams, a class of nonsteroidal anti-inflammatory drugs and beyond. IUBMB Life. 66(12):803–811. doi: 10.1002/iub.1334.

Yang J, Chai Y-F, Gong C-Y, Li G-h, Luo N, Luo N-F, Liu J. 2009. Further proof that the spinal cord, and not the brain, mediates the immobility produced by inhaled anesthetics. Anesthesiology. 110(3):591–595. doi: 10.1097/ALN.0b013e3181974bfd.