REVIEW

https://doi.org/10.21708/avb.2022.16.4.10330

Ozone therapy in veterinary medicine: clinical indications and techniques

Ozonioterapia na medicina veterinária: técnicas e indicações clínicas

Juliana Midori Sumida[1]*, Ayne Murata Hayashi[2]

ABSTRACT: Ozone therapy application and research have increased recently. The mixture of oxygen-ozone (O_2-O_3) has been used as a therapeutic agent for the treatment of several diseases with beneficial effects. This brief literature review has the objective of disclosing the mechanisms of action and main clinical indications and possibilities of ozone therapy for different conditions. The local and systemic approaches and techniques described for human treatment can be easily transposed for use in animals, such as rectal insufflation; bag therapy; ozonated oil; intradiscal and paravertebral applications; in acupuncture points; minor autohemotherapy, and major autohemotherapy. The possibilities of clinical indications and dosages were also described, including immunological and infectious diseases. Although it is a minimally invasive and relatively safe approach, more clinical studies are necessary to standardize techniques, doses, and clinical indications.

KEYWORDS: pain; antioxidant; disk disease; chemical acupuncture; autohemotherapy; COVID-19.

RESUMO: A aplicação e a pesquisa da terapia com ozônio aumentaram recentemente. A mistura de oxigênio-ozônio (O_2-O_3) tem sido utilizada como agente terapêutico para o tratamento de diversas doenças com efeitos benéficos. Esta breve revisão de literatura tem o objetivo de divulgar os mecanismos de ação e as principais indicações clínicas e possibilidades da ozonioterapia para diferentes condições. As abordagens e técnicas locais e sistêmicas descritas para o tratamento em humanos podem ser facilmente transpostas para uso em animais, como insuflação retal; terapia tópica de "bagging"; óleo ozonizado; aplicações intradiscal e paravertebral; em pontos de acupuntura; auto-hemoterapia menor e auto-hemoterapia maior. Também foram descritas as possibilidades de indicações clínicas e dosagens, incluindo doenças imunológicas e infecciosas. Embora seja uma abordagem minimamente invasiva e relativamente segura, mais estudos clínicos são necessários para padronizar técnicas, doses e indicações clínicas.

PALAVRAS-CHAVE: dor; antioxidante; discopatia; acupuntura química; autohemoterapia.

INTRODUCTION

Therapeutic ozone is generated when pure medicinal oxygen is converted through ozone generators, producing a mixture of the two gases, oxygen-ozone (O_2-O_3), with its ozone concentration ranging from 0.05 to 5% (1 to 107 µg/mL) and consequently oxygen concentration ranging from 99,95% to 95% (OLIVEIRA JUNIOR; LAGES, 2012; SCHWARTZ *et al.*, 2020).

Ozone therapy is a modality increasingly used for various diseases in medicine and veterinary. Although in veterinary medicine most publications are on large animals, their use in dogs and cats has grown exponentially in recent years, requiring further research and studies (TEIXEIRA *et al.*,2013, HAYASHI; FRIOLANI, 2018, KAWAHARA *et al.*, 2019, SCIORSCI *et al.*, 2020).

The oxidative effect of ozone promotes bactericidal, fungicidal, and virucidal properties. It is used in the treatment of circulatory arterial diseases, external ulcers, skin lesions, immunodeficiency, hepatitis, supportive therapy in cancer patients, inflammations, and dental treatments. Because of the ozone virucidal effect and indirect immunity stimulation, several studies for the treatment of herpes and human immunodeficiency are being done (PENIDO; LIMA; FERREIRA, 2010).

Since 2011, a consensus is being published: "Madrid Declaration on ozone therapy". In 2020 an update included

[1]Médica Veterinária Autônoma
[2]Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo
*Corresponding author: jmsumida@alumni.usp.br
Received: 05/25/2021. Accepted: 04/19/2022

a veterinary and a dentistry addendum (SCHWARTZ; SÁNCHEZ, 2012; SCHWARTZ; SÁNCHEZ; SABAH, 2015; SCHWARTZ *et al.*, 2020).

The purpose of this brief literature review is to disclose the mechanisms of action, main indications, and possibilities for the use of ozone therapy under different conditions in veterinary medicine. Also, to describe the several types of applications in small animals.

HISTORY

Martinus van Marum discovered ozone in 1781 and Christian Friedrich Schonbein characterized ozone in 1840, when, working with a voltaic pile in the presence of oxygen, he noticed the appearance of gas with an "electric and pungent smell", which they called *ozone*, from the Greek *ozein (*odor). And Nikola Tesla patented a generator for producing ozone in 1896. The use of ozone in medicine developed during the last century and it was stimulated by the lack of antibiotics and the positive disinfectant ozone properties (BOCCI, 2005; MASAN; SRAMKA; RABAROVA, 2020; SCHWARTZ; SÁNCHEZ, 2012).

In the 1970s, Alexander Balkanyi in Zurich was perhaps the first to inject small volumes of ozone into patients affected by tendonitis and myofascial pain. Others used ozone to treat polyarthritis, osteoarthritis, epicondylitis, carpal tunnel syndrome, and Morton's disease or neuroma with intra-articular or periarticular insufflation of a gas mixture of O_2-O_3 (BOCCI *et a*l., 2015).

MECHANISM OF ACTION

The ozone (O_3) is a potent oxidizer and it has a more selective activity over organic compounds (PENIDO; LIMA; FERREIRA, 2010). The likely mechanisms of action of this mixture of oxygen-ozone (O_2-O_3) are due to the biochemical properties of O_3 producing analgesic, anti-inflammatory, antioxidant and immunomodulatory effects. The events that promote these effects can be resumed by: activation of cellular metabolism, reduction of proinflammatory prostaglandins synthesis or release of algogenic compounds, increase of release of immunosuppressive cytokines, reduction of oxidative stress through the induction of the synthesis of antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase), increase of O_2 supply at tissues and stimulation of angiogenesis (BOCCI, 2006; LATINI *et al.*, 2019; PENIDO; LIMA; FERREIRA, 2010).

The therapeutic efficacy of ozone therapy is due to the controlled and moderate oxidative stress produced by O_3 reactions with various biological components (Figure 1). The calculated and transient oxidative stress induced by O_3 generates several second messengers in several intracellular signals (BOCCI, 2006; LATINI *et al.*, 2019). This property of ozone is known as paradoxical action because it plays a role as an oxidizing molecule and can increase the antioxidant properties of regions affected by the disease (SCIORSCI *et al.,* 2020).

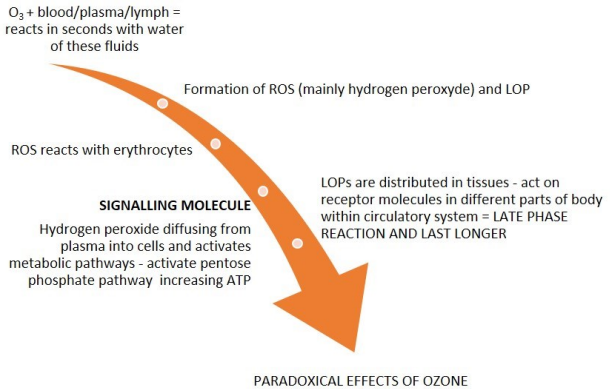
INSTRUMENTAL AND OZONE APPLICATION TECHNIQUES

Several instruments and techniques of ozone application will be described below. As it is a highly unstable molecule, the proximity of the ozone generator device during the application is necessary, so that its collection is performed moments before the treatment, allowing the reliability of the correct dose and concentrations. The equipment necessary to produce the ozone is described as well as the several applications (items 4.1 and 2.2)

Ozone generator devices

There are several medical ozone generator devices with easy purchase and certified by Agência Nacional de Vigilância Sanitária (ANVISA). The costs can range approximately from R$ 7000 to R$ 11000,00 (https://www.ozonioline.com.br/produto/gerador-de-ozonio-c-maleta-com-rodas-para-ozonioterapia-certificado-anvisa-com/362507; https://loja.philozon.com.br/gerador-de-ozonio-philozon-medplus-v) according to the manufacturers. Usually, a complete device includes the medical ozone generator and an oxygen rechargeable cylinder when the treatment is not performed at a veterinary clinic or hospital with oxygen support.

Commercial ozone production is carried out by the electric discharge process, also called the corona process. This consists of two electrodes subjected to a high potential difference (approximately 1000 V). Ozone is generated by the passage of air or pure oxygen between the two electrodes (SCHWARTZ; SÁNCHEZ, 2012; SILVA *et al.*, 2011). When the electrons have enough energy to dissociate the oxygen molecule, collisions start to occur that cause the dissociation of oxygen and the consequent formation of ozone (SCHWARTZ; SÁNCHEZ; SABAH, 2015).



O_3 = ozone; ROS = Reactive Oxygen Species; LOP= Lipid Oxidation Product; ATP= Adenosine Triphosphate. Font: LATINI et al., 2019

Figure 1. Oxidative effects of ozone

Ozone (O_3) is generated when a diatomic oxygen molecule (O_2) is separated. The free oxygen resulting from the breakdown of the molecule reacts with other diatomic oxygen (O_2) molecules forming the triatomic ozone molecule (O_3). For the oxygen molecule to break down, a large amount of energy is required which may be due to electrical discharges, chemical electrolysis, and ultraviolet light radiation. It can still be produced by thermal, radiochemical, and electrochemical methods (SCHWARTZ; SÁNCHEZ, 2012; SILVA *et al.*, 2011).

The triatomic form is unstable and its stability is temperature-dependent. Thus, at 20 ° C the average life of the O_3 gas is 40 minutes and at 30 ° C, the average life is 25 minutes (BOCCI, 2005). Thus, it cannot be stored and its use must be carried out right after its collection.

Ozone Therapy techniques

Several techniques of applying medical ozone are described. Both local and systemic via are described. The different ways of ozone therapy can be used alone or together to obtain a synergistic effect. Usually, the frequency of applications is variable according to the general conditions of the patient, age, and primary disease, it can be done two times a week, but adaptations must be done in each case. The general rule is each five ozone sessions, an increase of ozone dose as a cycle, and this cycle can vary between 15 or 20 sessions. Clinical improvement is observed between five to ten applications, due to activation of the self-defense antioxidant mechanism. All materials must be ozone resistant, like glass, silicon probes/tubes/syringes (SCHWARTZ; SÁNCHEZ; SABAH, 2015). It is described as a therapeutic window of secure ozone concentrations between 10 to 60 µg/mL, without toxicity or side effects (KAWAHARA *et al.*, 2019).

Local techniques

A- Rectal Insufflation

The rectal application of drugs is an initially local modality, but it can be used for the administration of systemic drugs. Rectal ozone insufflation has low toxicity and corresponds to a popular via in veterinary medicine due to its easy application and because it is an alternative to the use of major autohemotherapy (SANCHEZ; RE, 2012; TEIXEIRA *et al.*,2013).

It consists of the ozone rectal application assisted with an urethral probe. The gas quickly dissolves within the content of the intestinal lumen, reacts with mucoproteins and other released substances with antioxidant activity, and produces reactive oxygen species (ROS) and lipid peroxidation products (LOPs) that penetrate the mucosa. They are absorbed via blood and lymphatic circulation (SCHWARTZ; SÁNCHEZ; SABAH, 2015).

The rectal insufflation dosage of ozone can below (10-15 µg/mL), medium (20-25 µg/ m) and high (30-35 µg/ mL) doses. The volume to apply is 3mL/kg of weight (SCHWARTZ *et al.*, 2020).

B - Ozone Bagging

The ozone bagging is widely used in veterinary medicine for wounds and purulent skin infections, ulcers, and skin diseases. The O_2-O_3 mixture is pumped into an ozone-resistant bag, such as silicone or polypropylene, that is then placed around the area to be treated but previously moistened with distilled water to facilitate the penetration through the lesion site. Ozone reacts with minerals, so the bi-distilled/distilled water or water purified by the reverse osmosis process has lesser reagents to produce not desirable products (BHATT *et al.*, 2016; SCHWARTZ; SÁNCHEZ; SABAH, 2015). The O_2-O_3 mixture is inflated by a vacuum system until the bag is full. The wound to be treated will be surrounded by the gas, and it must be acting for 20 to 30 minutes (Figure 2) (SCHWARTZ; SÁNCHEZ; SABAH, 2015). The concentration usually used is 15-30 µg/mL in a low dose, 30-40 µg/mL in a medium dose, and 40- 70 µg/mL in a high dose (SCHWARTZ *et al.*, 2020).

C - Ozonated oil

The oil is the result of the interaction between vegetable oil and ozone and produces a mixture of chemical compounds such as ozonides and peroxides with a germicidal effect. (LINCHETA *et al.*, 1998). Ozonated oil can be used topically and orally. This kind of ozone application was very safe and has demonstrated activity for use in the prevention and treatment of chronic local infections, topical antimicrobial agents, in treatment of wounds and foot ulcers in patients with diabetes, anaerobic infections, herpetic infections (HSV I and II), trophic ulcers and burns, cellulitis, abscesses, anal fissures, decubitus ulcers (bed sores), fistulae, fungal diseases, furunculosis, gingivitis, and vulvovaginitis (BOCCI, 2005; UGAZIO et al., 2020). Ozonated oil is produced by incorporating the O_2-O_3 mixture into triglycerides where gaseous ozone chemically reacts with unsaturated substrates leading to therapeutically active

Figure 2. Ozone generator device and a dog receiving ozone bagging for skin wound with an ulcer at the distal extremity of the right thoracic limb. (SUMIDA, 2020)

ozonated derivatives (ozonide). The ozonide compositions deliver active O_2 and/or other useful species deep within the lesion without causing primary skin irritation. They can eliminate the pathogens and then, by releasing oxygen (O_2), activate the proliferation of fibroblasts, hence the building of an intercellular matrix with the consequent proliferation of keratin blasts and successive healing. The most commonly used oils are olive oil, sesame oil, and sunflower oil (TRAVAGLI *et al.*, 2010; UGAZIO *et al.*, 2020). Successful treatment with the topic of ozonated oil was described for skin lesions in a dog (SILVA JÚNIOR *et al.*,2019). Oral treatment can be also administered for H.pylori infection in human patients (SOTO; ROMO-VASQUEZ; WEBER-CHULIÁ, 2018).

D - Intradiscal

The intradiscal route, consists of the $O_2 – O_3$ mixture application in the altered intervertebral disk space, assisted by radiographic control using the radiological arc mobile or fluoroscopy or computed tomography. Ozone dissolves in the interstitial fluid and reacts with the biomolecules of the local tissue. A cascade of ROS is generated by reacting with the proteoglycans, of the nucleus pulposus, leading to their rupture and degeneration of the matrix with progressive decrease and disappearance of the herniated material. Because of the reduction of mechanical irritation, the sensitivity of axons decreases, although there are other mechanisms of pain in disk disease and spinal cord lesions (BOCCI *et al.*, 2015). The intradiscal route can be performed by using a 2.5 inch 22 G spinal needle inserted through the skin and epaxial muscles and positioned from the lateral side of the articular facet to the center of the herniated disk. Immediately after production by an ozone generator device, a volume of 1.5 to 2 ml O_2-O_3 moisture with an O_3 concentration of 32 µg/µl (HAN *et al.*, 2007).

E - Paravertebral

The ozone is immediately produced by a medical device and collected by a syringe. With the help of a 27G scalp or 30 G hypodermic needle (13 x 30mm) is applied to the paravertebral region. The paravertebral dosage of ozone can be low (5 µg/ mL), medium (10 µg/ m) and high (20 µg/ mL) doses. The volume to apply is about 0.5 - 10 mL/application (SCHWARTZ *et al.*, 2020).

It consists of the application of $O_2 – O_3$ in the paravertebral region, being minimally invasive, safe, and effective to relieve pain. The advantage observed was that this technique is easy to perform especially in small animals and horses, not requiring computed tomography or anesthesia (BIAZZO; CORRIERO; CONFALONIERI, 2018).

The paravertebral muscles are used as a route for O_2–O_3 infiltration, (Figure 3). A systematic review and meta-analysis of ozone therapy for low back pain secondary to herniated disk

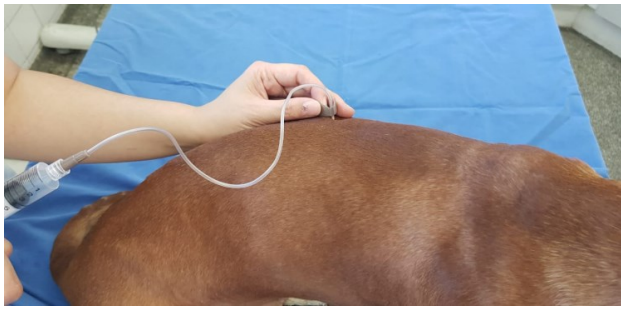


Figure 3. Paravertebral ozone application with a syringe and 27G scalp in a dog suffering thoracolumbar disk disease, also called chemical acupuncture. (SUMIDA, 2020)

indicated the level of evidence is II-3 (evidence obtained from diagnostic studies of uncertainty) for ozone therapy applied intradiscally and II-1 (evidence obtained from at least one properly conducted diagnostic accuracy study of adequate size) for ozone therapy applied at the paravertebral muscle and perifo-raminally for long-term pain relief based on USPSTF - levels of evidence-based on the quality data available in the literature (USPSTF- U.S. Preventive Services Task Force criteria). The available evidence produced a 1C strength of recommendation (strong recommendation, low quality or very low-quality evidence - may change when higher quality evidence becomes available) for ozone therapy applied to the disk and 1B (strong recommendation, moderate-quality evidence - can apply to most patients in most circumstances without reservation) for ozone applied at the paravertebral muscles or periforaminally (HARRIS *et al.*, 2001; MAGALHÃES *et al.*, 2012).

F- Vesicourethral insufflation

Direct ozone into vesicourethral is described as inflammatory, neoplasia, interstitial, bacterial, or radiation cystitis (TEKE *et al.*, 2017). It can be done with an urethral probe. The medium dosage is 15 µg/mL. It can be previously combined with ozonated water insufflation (CLAVO *et al.*, 2005; SCHWARTZ; SÁNCHEZ; SABAH, 2015; SCIORSCI *et al.*, 2020).

The low dosage is 10 µg/mL, the medium dosage is 15 µg/m Land the high dosage is 25 µg/mL The volume can vary from 5 to 50 mL/application (SCHWARTZ *et al.*, 2020).

4.2.1 Systemic application techniques

Ozone can be used for systemic action and the most common will be described. The local ozone rectal insufflation is also considered systemic, but it has been previously described.

A- Intraperitoneal

This kind of ozone application is considered in the initial study phase and is not yet recommended for application in humans and animals. However, it is described in experimental studies for the treatment of neoplasms (SCHULZ

et al., 2008; ROSSMANN *et al.*, 2014) and demonstrated improvement in neurodegenerative changes in the cerebral cortex of elderly rats (EL-MEHI; FARIED, 2020). This kind of insufflation is experimental and recommended an ozone generator device with an integrated intra-abdominal pressure control which avoids high abdominal pressures during the process. Also a sterile plastic tube and stopcock to the "backflush" of the ozone generator device and to connect to a catheter to insert through the abdominal wall (SCHULZ *et al.*, 2008). Intraoperative abdominal lavage is described in humans with peritonitis using 5 to 10 liters of ozonized saline solution in a concentration of 4 a 6 µg/mL for 20 minutes (SCHWARTZ *et al.*, 2020).

B - Major ozonated autohemotherapy

The Major ozonated autohemotherapy (MAH) consists of collecting a sample blood volume in a bag containing anticoagulant, then mixing it with O_2–O_3 and administering intravenously. Variations in microcirculation and metabolic changes that have been stable over time have been observed, such as an increase in cerebral oxygenation after about 1.5 hours of ozonated blood infusion and an increase in cytochrome c oxidase activity and concentration for up to 40 min after the end of treatment (BALLARDINI, 2006; RIMINI *et al.*, 2016; TSUZUKI *et al.*, 2015).

One can collect 1mL/kg of blood sample and ozone dosage can be low (10-20 µg/ mL), médium (20-30 µg/ mL) and high (30-35 µg/ mL). The total volume of application is calculated: 1-1,5 mL/kg of weight (SCHWARTZ *et al.*, 2020).

It is indicated for arterial circulation diseases, infections, rheumatic arthritis, immunostimulation, and carcinoma in geriatric patients (PENIDO; LIMA; FERREIRA, 2010). The AMD (dry age-related macular degeneration) has been treated with major ozonated autohemotherapy with a positive influence on visual acuity (BORRELLI *et al.*, 2012).

C - Minor ozonated autohemotherapy

It works as an autovaccine, widely used for immunomodulation. It consists of the application of autologous blood collected through a syringe with previously ozone content. After homogenization of blood with ozone, it is applied intramuscularly or at an acupuncture point (Figure 4) with the purpose of immunomodulation. Indications are in general for dermatological diseases like dermatitis, psoriasis, allergies, and adjuvant for oncological disease and chronic debilitating diseases. A blood sample can be collected at 0,1-0,5mL/kg (SCHWARTZ; SÁNCHEZ; SABAH, 2015; SCHWARTZ *et al.*, 2020). The ozone dosage can below (10-15 µg/ m), medium (15-30 µg/ mL) and high (30-40 µg/ mL) and the volume of application is 0.1-0.2 mL/kg (SCHWARTZ *et al.*, 2020).

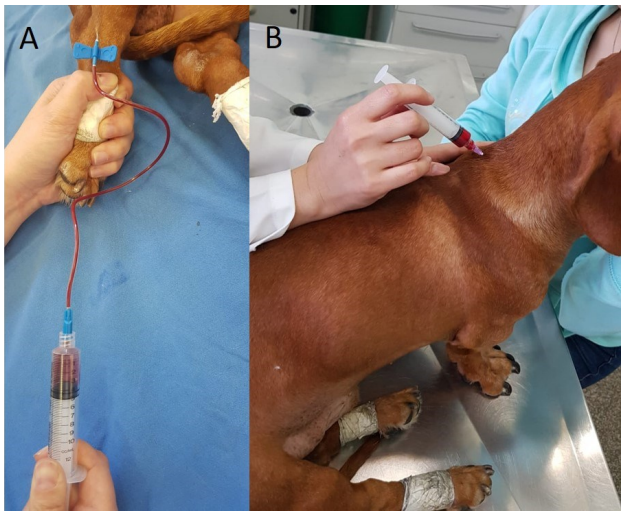


Figure 4. A- Autologous blood collection with ozone already in a syringe for minor autohemotherapy. B - Minor autohemotherapy applied at an acupuncture point - GV14, localized between C7 -T1 vertebrae for immunological modulation. The dog had seizures (suspected imune-mediated etiology) and thoracolumbar disk disease. (SUMIDA, 2020)

The therapeutic ozone dosages are divided according to their mechanism of action and route of application. The low doses are used for immunomodulatory effect and when the immune system is very much compromised, like in neoplasia, elderly and debilitated patients. The medium doses are used for immunomodulatory function and can stimulate the antioxidant enzyme defense system. It is indicated for chronic degenerative diseases such as diabetes, atherosclerosis, chronic obstructive pulmonary disease (COPD), Parkinson's syndrome, Alzheimer's, and senile dementia. The high doses are used for the inhibitory effect on the mechanisms, which occur in autoimmune diseases like rheumatoid arthritis and lupus, or to treat ulcers or infected injuries (SCHWARTZ *et al.*, 2020).

Other routes of ozone administration can be used such as intra-articular, subcutaneous, paratendon, and trigger points. For intraarticular ozone route, the dosage can vary from low (8 µg/ mL), medium (10 µg/ mL) and high (25 µg/ mL). The volume varies from 0.5 to 10 mL per application (SCHWARTZ *et al.*, 2020).

CONTRAINDICATIONS, SIDE EFFECTS, AND CARE

Contraindications for the use of ozone in humans are described, such as Glucose-6-Phosphate-Dehydrogenase deficiency (Favism, acute hemolytic anemia), hemochromatosis, toxic hyperthyroidism (Basedow-Graves' disease). In severe thrombocytopenia in humans, it is recommended not to use when below 50,000 platelets. Also, if there is severe cardiovascular instability, acute myocardial infarction, alcohol intoxication, treatment with iron or copper, and seizure status it is not recommended the ozone application (SCHWARTZ; SANCHEZ; SABAH, 2015). There are no studies in veterinary medicine to establish the recommended limits.

The incidence of side effects of ozone therapy-related in literature is very low, estimated at 0.0007%. One can manifest euforia, nausea, headaches, and fatigue (HERNÁNDEZ *et al*, 2021).

One must take care during the use of ozone and not direct inhale it because it is toxic to the upper airway and lungs. Signs of epiphora, rhinitis, cough, headache, and less common, nausea and vomiting are side effects (BHATT *et al.*, 2016; BOCCI, 2005; SCIORSCI *et al.*, 2020). Important interactions with ozone are the antioxidants, like vitamin C and E, so they must be administered before or after ozone therapy, not during the therapy, because of the interference with ozone mechanisms of action and results. Ozone increases the effects of angiotensin-converting enzyme inhibitors. Treatment with copper and iron is contraindicated together with ozone therapy. However, synergic effects are observed with laser, magnetic therapy, acupuncture, and diathermy (SCHWARTZ; SANCHEZ; SABAH, 2015).

Overall, it is a safe therapy if applied correctly with the recommended dose, avoiding complications with air embolism using the correct practice and certified equipment (HERNÁNDEZ *et al*, 2021).

POSSIBILITIES OF CLINICAL INDICATIONS

In small animals, the great application of ozone therapy has been in pain control (BIAZZO *et al.*, 2018; SUMIDA; MATERA; HAYASHI, 2019; TEIXEIRA *et al.*, 2013), intervertebral disk disease (HAN *et al.*, 2007), arthropathies (AVILÉS, 2013; TARTARI *et al.*, 2020), dermatopathies (BORGES *et al.*, 2019; JORDAN *et al.*, 2019) and in the treatment of infectious diseases such as ehrlichiosis (GARCIA *et al.*, 2010), leishmaniasis (MODA *et al.*, 2014), and feline viral immunodeficiency (KAWAHARA *et al.*, 2019). In large animals, in addition to diseases common to small animals such as arthropathies, disk disease, and treatment of pain and wounds in horses (BHATT *et al.*, 2016), mastitis and reproductive disorders in domestic ruminants (ĐURIČIĆ *et al.*, 2016; IMHOF *et al.*, 2019; SAMARDŽIJA *et al.*, 2017).

A chronic increase in oxidative stress is seen in serious conditions such as myocardial infarction, stroke, chronic limb ischemia, COPD, type II diabetes, and AMD. Initial inflammation occurs followed by excessive release of ROS, causing a diffuse cell injury that must be corrected by an inducible expression of the innate detoxifying and antioxidant system. ROS could activate the transcription factor (nuclear factor erythroid 2-related factor 2) Nrf2, which increases the expression of antioxidant enzymes. Nrf2 could be activated by ozone therapy, and when properly activated, can restore redox homeostasis, and possibly improve health (BOCCI; VALACCHI, 2015). Reports of studies with various diseases in animals have demonstrated the effect of ozone therapy in neutralizing oxidative stress such as an improvement in the antioxidant capacity after MAH application in thoroughbred horses (TSUZUKI *et al.*, 2015), cisplatin-induced nephrotoxicity (BORREGO *et al.*, 2004), reduced lipid and protein oxidation markers and decreased lipofuscin pigment deposition in rat liver and kidneys in a pre-aging ozone administration (SAFWAT *et al.*, 2014), reperfusion injury of hepatic and renal ischemia (AJAMIEH *et al.*, 2004; YU *et al.*, 2017) and diabetic nephropathy (MORSY; HASSAN; ZALAT, 2010) and improvement in neurodegenerative changes in the cerebral cortex in rats (EL-MEHI; FARIED, 2020).

The most frequent animal diseases treated with ozone therapy and their most common application routes can be seen elsewhere. For example, gastrointestinal diseases, pancreatitis, chronic gastroenteritis, anemias, and immune-mediated thrombocytopenias with Ht >20% and acute and chronic kidney disease could have the ozone application route like major autohemotherapy, minor autohemotherapy, and rectal insufflation (SCIORSCI *et al.*, 2020; SCHWARTZ *et al.*, 2020).

Neurological diseases

Ozone therapy is described in the treatment of neurodegenerative diseases such as Multiple Sclerosis, Parkinson's disease, Alzheimer's and Wilson's disease, amyotrophic lateral sclerosis, Huntington's disease, and cognitive disorders. Its effect can be explained by its neuroprotective action, minimizing the effects of oxidative stress in these pathologies (MASAN; SRAMKA; RABAROVA, 2020). For example, studies in rats have shown that intraperitoneal application therapy with controlled ozone induces oxidative preconditioning, thus reversing oxidative stress, with an improvement in neurodegenerative changes in the cerebral cortex of elderly rats (EL-MEHI; FARIED, 2020).

Inflammatory and infectious diseases

Recently, cohort studies and case reports using ozone therapy as a complementary treatment have been published in patients diagnosed with the pandemic vírus COVID-19. The articles describe the ozone techniques used: major autohemotherapy (HERNÁNDEZ *et al.*, 2020; HERNÁNDEZ *et al.*, 2021; ZHENG; DONG; HU, 2020; WU *et al.*, 2020), intramuscular application (BROWNSTEIN *et al.,* 2020), rectal insufflation (FERNÁNDEZ-CUADROS *et al.*, 2020; FERNÁNDEZ-CUADROS *et al.*, 2020) and intravenous ozonized saline solution (SCHWARTZ *et al.*, 2021). In these studies, improvement in symptoms such as dyspnea, weakness, and decrease in body temperature was observed and corresponded to an improvement in chest radiographic findings, oxygen saturation, and laboratory findings due to the decrease in inflammation markers (D-dimer, urea, ferritin, fibrinogen, LDH, IL-6 and C-reactive protein). A reduction in mortality was seen and side effects were not noticed (FERNÁNDEZ-CUADROS, M.E. *et al*, 2020; SCHWARTZ, *et al.*, 2020).

A twice-daily ozonated autohemotherapy for 5 consecutive days in 18 human patients with COVID-19 severe pneumonia was associated with a significant reduction in the time of clinical improvement (HERNÁNDEZ *et al.*, 2021).

Some reports related good results on ozone therapy for inflammatory and infectious diseases in veterinary medicine. An application of rectal ozone insufflation in animals with positive PCR for canine parvovirus study, demonstrated 20 times higher mortality in the control group when compared to the dogs of the treated group (TRALDI, 2019). Garcia *et al.* (2010) reported the use of ozonated MAH in canine ehrlichiosis treatment. This therapy was effective in reversing different hematological parameters and improving renal damage. The use of ozone therapy as an adjuvant treatment for visceral leishmaniasis is described by Moda *et al.* (2014). Infected dogs undergoing rectal insufflation treatment showed a decrease in serum urea and creatinine values at the end of treatment, showing better performance than the intra-abdominal treatment.

Kawahara *et al.* (2019) described the successful treatment of a cat with feline viral immunodeficiency with ozone therapy using minor and major autohemotherapy and rectal insufflation. No side effects were observed.

Musculoskeletal diseases

Avilés (2013), studied the anti-inflammatory and analgesic effects of ozone and growth factors derived from ozone-activated platelets in dogs with osteoarthritis in the hips and with spinal pain or pain in the extremities. O_2–O_3 was applied in the periarticular region and painful paravertebral regions, resulting in a significant improvement.

An experimental study in horses evaluated the transient inflammatory reactions induced by intra-articular administration of medicinal ozone affecting joint components, by in vivo inflammatory evaluation, anti-inflammatory, and oxidative biomarkers, and extracellular matrix degradation products in synovial fluid in healthy horse's joints. Analyzes of synovial fluid did not reveal significant changes in the concentrations of the main biomarkers of cartilage inflammation and catabolism, indicating that intra-articular application of medicinal ozone in horses seems to be safe (VENDRUSCOLO *et al.*, 2018). Another study verified the action of ozone therapy application on an experimental model of rheumatoid arthritis in Wistar rats. It was observed effectively reduced inflammation through the reduction of pro-inflammatory cytokines and activation of IL-10 anti-inflammatory cytokine (TARTARI *et al.*, 2020).

Coelho *et al.* (2015), reported a mare case diagnosed with chronic laminitis (grade IV according to Obel) with signs of lameness and reluctance to walk, radiographic measurements revealed displacement of the distal phalanx. Corrective cutting and administration of O_2–O_3 (intramuscularly, peritendinous and rectal insufflation) was performed. The animal showed improved body condition, and better ambulation and the radiological evaluation after treatment showed a normal relationship between the dorsal hull wall.

Intervertebral disk diseases

For the treatment of disk disease, two methods are described: direct and indirect. The direct method consists of an intradiscal application and the indirect method, in a paravertebral application. This last form is also considered chemical acupuncture, due to the beneficial results being obtained with the application of ozone as a chemical reagent through the insertion of the needle. The role of this technique is related to obtaining a complex series of neurological and chemical reactions that lead to decreased pain in the majority of patients with pain in the spine, with positive responses in 70-80% of cases (BOCCI *et al.*, 2015). In an observational and prospective study realized in humans in Guantanamo, ozone therapy was applied paravertebrally in diseases of the spine. Most patients moved to a lower pain category and 80.7% were evaluated in the mild-moderate category after ozone therapy (CUBA RODRIGUEZ *et al.*, 2019).

The effects of the application of intradiscal and paravertebral ozone are: inhibition of prostaglandin E2 and phospholipase A2, similar to steroids; inhibition of other pro-inflammatory cytokines (IL 1, 2, 8. 12, 15, interferon- α); (IL 1, 2, 8. 12, 15, interferon- α); increased release of immunosuppressive cytokines (IL10, factor B1) producing analgesic and anti-inflammatory effects; increase of local microcirculation; reduce venous stasis and hypoxia in the nerve root producing an analgesic effect; effect on the mucopolysaccharides and proteoglycans of the nucleus pulposus, which is called ozonolysis, producing a chemical discolysis with the loss of water and dehydration; matrix degeneration, which is replaced by collagen fibers reducing the volume of the disk (SCHWARTZ; SANCHEZ, 2012).

Han *et al.* (2007), reported the percutaneous application of intradiscal O_2–O_3 guided by fluoroscopy in dogs with thoracolumbar disk disease. A significant reduction of the intervertebral disk volume was observed in computed tomography and all dogs recovered their gait function and did not present recurrence. Jang *et al*, (2009) described the application of ozone on a Shih-Tzu dog with spinal cord compression in the cervical region and presenting progressive tetraplegia. O_2–O_3 was applied intraoperatively in the affected intervertebral spaces together with ventral decompression surgery in the intervertebral space. After the procedure, there was no more cervical pain and complete resolution of neurological deficits.

Painful conditions

Biazzo *et al.* (2016), reported the application of O_2–O_3 in intramuscular paravertebral lumbar regions, every seven days

for a total of six sessions, for the treatment of low back pain in humans. It was observed that 79% of the patients showed improvement in pain conditions.

Teixeira *et al.* (2013), investigated the postoperative analgesic effects of ozone administered intrarectally or at acupuncture points compared with meloxicam, in bitches submitted to ovariohysterectomy. All groups obtained satisfactory analgesia for 24 hours, but the group that received ozone at the acupuncture points needed analgesic rescue (n=2), however without significant differences in pain scales. The authors used a modified Glasgow pain scale and the visual analog scale. Ozone had no measurable adverse effects and was a good option to promote pain relief, being the intrarectal route with analgesic control in 24 hours.

Ballardine (2005) and Vigliani *et al.* (2005) reported the use of O_2–O_3 in sports horses with musculoskeletal disorders and pain. Ozone´s subcutaneous and paravertebral application was performed, remission of symptoms was good, and pain relief was noticed.

Two dogs with neck pain refractory to analgesic medication have been treated by ozone. The O_2–O_3. was applied by rectal insufflation and at acupuncture points bilaterally in the two paravertebral cervical points, GV14, GB21, BL23, lumbar Bai Hui, obtaining pain control, with DIVAS=0 after four applications (SUMIDA; MATERA; HAYASHI, 2019).

Oncologic conditions

Rossmann *et al.* (2014) reported a considerable efficacy of the application of O_2–O_3 intraperitoneally in rabbits implanted with papilomavírus associated auricular carcinoma VX2, which serves as a model for the study of head and neck cancer in humans. Host acquired tumor resistance and tumor regression were observed. Another study by Schulz *et al.* (2008) using rabbits inoculated with squamous cell carcinomas of the head and neck, observed a complete remission of 50% of the animals treated also with intraperitoneal administration.

Other studies in an animal model and humans, refer to the use of ozone as an adjuvant in the treatment of cancer, which may accelerate the healing after tumor resection surgery. Other effects described were the ozone`s capacity to induce direct damage to tumor cells, enhance the radiotherapy and chemotherapy effects, immune modulation, alter locoregional blood flow and cause tumor hypoxia that provides additional support for potential beneficial effects during cancer treatment (CLAVO *et al.*, 2018).

Dermatologic conditions

In veterinary medicine, skin diseases are very common conditions and can be one of the main indications for the application of ozone therapy. For modulation of immunity both methods, systemic and local applications can be used. The local application also has antiseptic, antibacterial, antifungal, and healing stimulation effects. Borges *et al.* (2019) and Jordan *et al.* (2019) described the use of ozone bagging and rectal insufflation in dermatologic cases and obtained remission of circumscribed, alopecic skin lesions, distributed diffusely along the body, with crusty and scaly, mild itching, and various bacterial infections.

Lincheta *et al.*, 1998 reported the effective result of using ozonized oil in the treatment of herpes simplex, fungal dermatitis, and pyodermas.

Hepatic diseases

The combination of rectal ozone insufflation and the chinese formula (Yigan Fuzheng Paidu) was used in the induced acute liver injury. This treatment combination was superior when compared with the other groups (ozone or chinese formula alone, and control). It managed to control the dog's general conditions and also, and the survival rates were higher with normal levels of liver enzymes and less liver damage on histopathological examination (LI *et al.*, 2007).

Ophthalmologic diseases

There are reports of ozone application for ophthalmic diseases in the treatment of dry eye syndrome, diabetic retinopathy, endophthalmitis, choroidal dystrophy, AMD, retinitis pigmentosa, and chronic glaucoma in human medicine (BORRELLI *et al.*, 2012; SCHWARTZ; SÁNCHEZ; SABAH, 2015). In veterinary medicine, Marchegiani *et al.* (2019) and Spadea *et al.* (2018), described the use of the Ozodrop® liposomal ozone lubricating ophthalmic solution (Fb Vision, Ascoli Piceno, Italy) based on hypromellose, liposomes, and ozonized oil It was observed that ozone-based eye drops have anti-inflammatory and bactericidal activity, promote tissue repair, and can be successfully used in various ophthalmic pathologies like recurrent conjunctivitis and chronic keratitis.

CONCLUSION

Recently, the use of ozone therapy has been intensified in clinical practice due to its multiple applications, minimally invasive, and relatively safe approach. However, the understanding of all biochemical and pharmacodynamic mechanisms is not yet fully elucidated. More scientific clinical studies are essential to expand its applicability and standardize effective doses and techniques for several diseases in veterinary medicine.

FUNDING

The authors would like to thank São Paulo Research Foundation (FAPESP), Grant#2018/06769-8. This study

was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

DECLARATION

This article will be part of the Master's Degree dissertation of the corresponding author.

ABBREVIATIONS

AMD: dry age-related macular degeneration; AHM: Major Autohemotherapy; COPD: chronic obstructive pulmonary disease; CRP: C-Reactive protein; LDH: Lactate Dehydrogenase; LOP: Lipid Oxidation Product; MiAH: Minor Autohemotherapy; O_3: Ozone; O_2: Oxygen; ROS: Reactive Oxygen Species; RIO_3: Rectal Insufflation

REFERENCES

AJAMIEH, H.H. *et al*. Effects of ozone oxidative preconditioning on nitric oxide generation and cellular redox balance in a rat model of hepatic ischemia - Reperfusion. **Liver International**. v. 24, n. 1, p. 55–62, 2004.

AVILÉS, M.H. Use of ozone and ozonated growth factors in musculoskeletal disorders of the canine species. **Revista Española de Ozonoterapia**, v. 3, n. 1, pp. 95-98, 2013.

BALLARDINI E. Oxygen-Ozone therapy for spinal muscle disorders in the horse. **Rivista Italiana di Ossigeno-Ozonoterapia,** v.4, p.70-73, 2005.

BALLARDINI, E. Changes in Haemochromocytometric Values in Horses after Ozone Auto-haemotransfusion. **Rivista Italiana di Ossigeno-Ozonoterapia**, v.5, p.47-52, 2006.

BHATT, J. *et al*. An overview of ozone therapy in equine- an emerging healthcare solution. **Journal of Experimental Biology and Agricultural Sciences**, v. 4, p. S203–S210, 2016.

BIAZZO, A.; CORRIERO, A.S.; CONFALONIERI, N. Intramuscular oxygen-ozone therapy in the treatment of low back pain**. Acta Biomedica**, v. 89, n. 1, p. 41–46, 2018.

BOCCI, V. **Ozone**: A New Medical Drug. Netherlands: Springer, 2005, 259 p.

BOCCI, V. Scientific and medical aspects of ozone therapy. State of the art. **Archives of Medical Research**, v. 37, p. 425-435, 2006.

BOCCI, V.; BORRELLI E.; ZANARDI I.; TRAVAGLI V. The usefulness of ozone treatment in spinal pain. **Drug Design, Development and Therapy,** v. 9, p. 2677–2685, 2015.

BOCCI, V.; VALACCHI, G. Nrf2 activation as target to implement therapeutic treatments. **Frontiers in Chemistry**, v. 3, p.1-6, 2015.

BORGES, T.L. *et al*. Ozonioterapia no tratamento de cães com dermatite bacteriana: relato de dois casos. **Revista Científica Eletrônica de Medicina Veterinária**, n.32, 11 p, 2019.

BORREGO, A. *et al*. Protection by ozone preconditioning is mediated by the antioxidant system in cisplatin-induced nephrotoxicity in rats. **Mediators of Inflammation,** v.13, p. 13-19, 2004.

BORRELLI, E. *et al*. Effects of major ozonated autohemotherapy in the treatment of dry age related macular degeneration: a randomized controlled clinical study. **International Journal of Ophthalmology,** v.5, p.708-713, 2012.

BROWNSTEIN, D. *et al*. 2020. A Novel Approach to Treating COVID-19 Using Nutritional and Oxidative Therapies**. Science, Public Health Policy, and The Law**, v.2, p. 4-22, 2020.

CLAVO, B. *et al*. Intravesical Ozone Therapy for Progressive Radiation-Induced Hematuria. **The Journal of Alternative and Complementary Medicine,** v.11, n.3, p. 539–541, 2005.

CLAVO, B. *et al*. Ozone Therapy as Adjuvant for Cancer Treatment: Is Further Research Warranted? **Evidence-Based Complementary and Alternative Medicine**, v.9. p. 1-11, 2018.

COELHO, C.S. *et al*. Use of ozone therapy in chronic laminitis in a horse. **Journal of Ozone Therapy,** v.1, n.1, p. 1-4, 2015.

CUBA RODRIGUEZ, L.C. *et al*. Ozonoterapia paravertebral en la patología de la columna vertebral lumbar. Revista Información Científica**,** *v.*98, n.3, p. 64-73, 2019.

ĐURIČIĆ, D. *et al*. Comparison of intrauterine antibiotics versus ozone medical use in sheep with retained placenta and following obstetric assistance. **Reproduction in Domestic Animals,** v. 51, p. 538–540, 2016.

EL-MEHI, A.E.; FARIED, M.A. Controlled ozone therapy modulates the neurodegenerative changes in the frontal cortex of the aged albino rat. **Annals of Anatomy**, v. 227, p. 1-8, 2020.

FERNÁNDEZ-CUADROS, M.E. *et al*. Effect of Rectal Ozone (O_3) in Severe COVID-19 Pneumonia: Preliminary Results**. SN Comprehensive Clinical Medicine**, v. 2, p. 1328–1336, 2020.

FERNÁNDEZ-CUADROS, M.E. *et al*. Ozone (03) and SARS-CoV-2: Physiological Bases and Their Therapeutic Possibilities According to COVID-19 Evolutionary Stage. **SN Comprehensive Clinical Medicine,** v. 2, p. 1094–1102, 2020.

GARCIA, C.A. *et al*. The use of ozonated major autohemotherapy in canine ehrlichiosis' treatment: case report. **Revista CENIC. Ciencias Biológicas**, v. 41, p. 1–6, 2010.

HAN, H.J. *et al*. Fluoroscopic-guided intradiscal oxygen-ozone injection therapy for thoracolumbar intervertebral disc herniations in dogs. **In Vivo,** v. 21. p.609-614, 2007.

HARRIS, R.P.*et al*. Current Methods of the U.S. Preventive Services Task Force: A Review of the Process. **American Journal of Preventive Medicine,** v. 20, n. 3, p. 21–35, 2001.

HAYASHI, M.P., FRIOLANI M. Aplicabilidade clínica cirúrgica da ozonioterapia em pequenos animais: Revisão de Literatura. **Revista Unimar Ciências**, v.27, n.1-2, 2018.

HERNÁNDEZ, A. *et al*. Potential Role of Oxygen–Ozone Therapy in Treatment of COVID-19 Pneumonia. **American Journal of Case Reports,** v.21, p.1-6, 2020.

HERNÁNDEZ, A. *et al*. Ozone therapy for patients with COVID-19 pneumonia: preliminary report of a prospective case-control study. **International Immunopharmacology,** v. 90, 2021.

IMHOF, S. *et al*. Therapy of retained fetal membranes in cattle: comparison of two treatment protocols. **Animal Reproduction Science**, v. 206, p. 11–16, 2019.

JANG, H.Y. *et al*. A case of intradiscal oxygen-ozone injection therapy for cervical herniated intervertebral disc in a dog. **Journal of Veterinary Clinics,** v.26, n.3. p. 273-275, 2009.

JORDAN, M.V.R. *et al*. Evaluación de Ozonoterapia en Dermatopatía Generalizada en Perros. **European Scientific Journal ESJ,** v. 15, n. 6, p. 364–379, 2019.

KAWAHARA, R. *et al*. Ozonioterapia no tratamento da Imunodeficiência Viral Felina (FIV): relato de caso. **Revista Nosso Clínico**, n.132, 2019.

LATINI, E. *et al*. Ultrasonography for oxygen-ozone therapy in musculoskeletal diseases. **Medical Gas Research**, v.9, n.1, p. 18-23, 2019.

LI, L-J. *et al*. Protective effects of medical ozone combined with traditional chinese medicine against chemically-induced hepatic injury in dogs. **World Journal of Gastroenterology,** v. 13, n. 45, p. 5989-5994, 2007.

LINCHETA, F. L. *et al*. Aceite ozonizado en Dermatologia. Experiencia de 9 anos. **Revista CENIC Ciencias Biologicas**, v. 29, p. 192-5, 1998.

MAGALHAES, N.D.O. *et al*. Review Ozone Therapy as a Treatment for Low Back Pain Secondary to Herniated Disc: A Systematic Review and Meta-analysis of Randomized Controlled Trials. **Pain Physician**, v.15, p. E115-129, 2012.

MARCHEGIANI, A. *et al*. Preoperative topical liposomal ozone. dispersion to reduce bacterial colonization in conjunctival sac and periocular skin: Preliminary study in dogs. **Experimental Eye Research**. v.179, p.1-6, 2019.

MASAN J.; SRAMKA M.; RABAROVA D. The possibilities of using the effects of ozone therapy in neurology. **Neuro Endocrinol Lett,** v. 42, n. 1, p.13-21, 2021.

MODA, T.F.*et al*. Efeitos da ozonização intra-abdominal e intra-retal sobre a avaliação renal de cães acometidos por leishmaniose visceral. In: XXIV CONGRESSO BRASILEIRO DE ENGENHARIA BIOMÉDICA, Uberlândia, 2014, p.1900-03.

MORSY, M.D.; HASSAN, W.N.; ZALAT, S.I. Improvement of renal oxidative stress markers after ozone administration in diabetic nephropathy in rats. **Diabetology and Metabolic Syndrome,** p. 1–7, 2010.

OLIVEIRA JUNIOR, J.O.; LAGES, G.V. Ozonioterapia em lombociatalgia. **Rev Dor,** v. 13, n. 3, p. 261-270, 2012.

PENIDO, B.R.; LIMA C.A.; FERREIRA L.F.L. Aplicações da ozonioterapia na clínica veterinária. **Pubvet**, v.4, n.40, 2010.

RIMINI, D. *et al*. The speed of reinfusion affects the vascular system during ozone major autohemotherapy. **Ozone Therapy**, v. 1, p. 56-60, 2016.

ROSSMANN, A. *et al*. Intraperitoneal oxidative stress in rabbits with papillomavirus-associated head and neck cancer induces tumoricidal immune response that is adoptively transferable. **Clinical Cancer Research**, v. 20, n. 16, p. 4289–4301, 2014.

SAFWAT, M.H. *et al*. Ozone ameliorates age-related oxidative stress changes in rat liver and kidney: Effects of pre- and post-ageing administration. **Biochemistry Moscow**, v.79, p. 450–458, 2014.

SAMARDŽIJA, M. *et al*. Intrauterine ozone treatment of puerperal disorders in domestic ruminants: a review. **Veterinarski Arhiv**, v. 87, n. 3, p. 363–375, 2017.

SANCHEZ, G.M.; RE L. Rectal administration and its application in ozonetherapy. **International Journal of Ozone Therapy**, v.11, n.1, p.41-49, 2012.

SCHWARTZ, A.; SÁNCHEZ, G.M. Ozone Therapy and Its Scientific Foundations. **Revista Española de Ozonoterapia,** v. 2, n. 1, p. 199-232, 2012.

SCHWARTZ, A.; SÁNCHEZ, G.M.; SABAH, F**. Declaración de Madrid sobre la Ozonoterapia** 2rd ed. 2015.

SCHWARTZ, A. *et al*. **Declaración de Madrid sobre la Ozonoterapia**, 3rd ed. 2020.

SCHWARTZ, A. *et al*. Complementary Application of the Ozonized Saline Solution in Mild and Severe Patients with Pneumonia Covid-19: A Non-randomized Pilot Study. **Journal of Pharmacy & Pharmacognosy Research**, v.9, n. 2, p. 126-142, 2021.

SCHULZ, S. *et al*. Treatment with ozone/oxygen-pneumoperitoneum results in complete remission of rabbit squamous cell carcinomas. **International Journal of Cancer**, v. 122, n. 10, p. 2360–2367, 2008.

SCIORSCI, R.L. *et al*. Ozone Therapy in Veterinary Medicine: A Review. **Research in Veterinary Science**, v. 130, p. 240–246, 2020.

SILVA, S.B. *et al*. Potencialidades do uso do ozônio no processamento de alimentos. **Semina: Ciências Agrárias**, v.32, n.2, p.659-682, 2011.

SILVA JÚNIOR, J.I.S. *et al*. Topical Ozone Therapy in the Treatment of Pharmacodermia in a Dog (Canis lupus familiaris). **Acta Scientiae Veterinariae**, v.47, p. 1-5, 2019.

SOTO, M.A.; ROMO-VÁZQUEZ, C.A.; WEBER-CHULIÁ, N. Endoscopy evidence; H. Pylori infection Ozonetherapy treated, Mexican cases report. **Revista Española de Ozonoterapia,** v.8, n.1, p. 171-179, 2018.

SPADEA, L. *et al*. Use of ozone-based eye drops: a series of cases in veterinary and human spontaneous ocular pathologies. **Case Reports in Ophthalmology**, v.9, n.2, p. 287-298, 2018.

SUMIDA, J.M.; MATERA, J.M.; HAYASHI; A.M. Ozone therapy as pharmacoacupuncture for cervical pain in dogs. In: Proceedings of the 45th IVAS Annual Congress on Veterinary Acupuncture, Wroclaw, 2019, p 91.

TARTARI, A.P.S. *et al*. Anti-inflammatory Effect of Ozone Therapy in an Experimental Model of Rheumatoid Arthritis. **Inflammation,** v.43, p.985-993, 2020.

TEIXEIRA, L.R. *et al*. Comparison of intrarectal ozone, ozone administered in acupoints and meloxicam for postoperative analgesia in bitches undergoing ovariohysterectomy. **Veterinary Journal**, v. 197, n. 3, p. 794–799, 2013.

TEKE, K. *et al*. Preventive effect of intravesical ozone supplementation on n-methyl-nitrosourea-induced non-muscle

invasive bladder cancer in male rats. **Experimental Animals,** v. 66, n. 3, p. 191-198, 2017.

TRALDI, R.F. **Uso da ozonioterapia como terapia complementar em cães diagnosticados com parvovirose**. 2019. 40 f. Dissertação (mestrando em anestesiologia, dor e acupuntura: Área de concentração em Cirurgia Animal) – Universidade Estadual Paulista Júlio de Mesquita Filho - Botucatu, 2019.

TRAVAGLI, V. *et al*. Ozone and Ozonated Oils in Skin Diseases: A Review. **Mediators of Inflammation,** 2010.

TSUZUKI, N. *et al*. Effects of ozonated autohemotherapy on the antioxidant capacity of Thoroughbred horses**. Journal of Veterinary Medical Science**, v.77, n. 12, p.1647-1650, 2015.

UGAZIO, E. *et al*. Ozonated Oils as Antimicrobial Systems in Topical Applications. Their Characterization, Current Applications, and Advances in Improved Delivery Techniques**. Molecules**, v. 25, n. 2, p. 1–24, 2020.

VENDRUSCOLO, C.D.P. *et al*. Effects of medical ozone upon healthy equine joints: clinical and laboratory aspects. PLoS One**, v.** 13, p.1-18, 2018.

VIGLIANI, A.; BONIPERTI, E.; SCUDO, E. Paravertebral O_2–O_3 treatment in mechanical lumbar pain in riding horses. **Rivista Italiana di Ossigeno-Ozonoterapia**, v. 4, p. 64-69, 2005

ZHENG, Z.; DONG, M.; HU, K. A preliminary evaluation on the efficacy of ozone therapy in the treatment of COVID-19. **Journal of Medical Virology**, v. 92, n.11, p. 2348-2350, 2020.

YU, G. et al. The NLRP3 inflammasome is a potential target of ozone therapy aiming to ease chronic renal inflammation in chronic kidney disease. **International Immunopharmacology**, v. 43, p. 203–209, 2017.

WU, J. et al. Case Report: Recovery of One ICU-Acquired COVID-19 Patient Via Ozonated Autohemotherapy. **The Innovation**. 8 Apr 2020.