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THE USE OF OZONE THERAPY IN VETERINARY MEDICINE: A SYSTEMATIC REVIEW

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Abstract

In the past decades, veterinary medicine has endorsed more alternative medicine practices, among which ozone therapy. Ozone therapy is a therapeutic method associated with integrative medicine by which ozone is administered to an organism in order to treat or ameliorate certain pathologies, however, controversy exists due to the lack of sufficient scientific proof of efficacy. This review aims to gather and summarize the current knowledge about the potential uses of ozone in veterinary medicine. A search in several biomedical databases such as PubMed, Science Direct, Research Gate, Google Scholar, Scopus but also in the archives of dedicated ozone journals, was performed using specific keywords. The results of the literature retrieval (117 papers) were grouped by pathology, affected system, or species in which ozone therapy can be applied or has been studied. Although the number of potential uses for ozone therapy quoted is large, there aren't enough studies to demonstrate the efficacy of each use. Most articles have positive yet uncertain conclusions, confirming the therapeutic effect of ozone but recommending further studies. The literature mentions clinical improvement after the use of ozone and suggests ozone therapy as an alternative approach to specific pathologies, or as an adjuvant to therapeutic protocols. Almost half of the work published is experimental, on animal models, and among the clinical studies, just a small percentage is related to small animal medicine. The therapeutic array of ozone therapy is yet to be defined.

***Key words**: animal, autohemotherapy, ozone insufflation, oxygen-ozone, ozone therapy, veterinary.*

INTRODUCTION

Ozone therapy has received more attention from veterinary professionals in the past two decades, and even more in the past couple of years. However, the use of ozone in medical practices is documented for more than 100 years (Rubin, 2001). This molecule was discovered in 1840 by a German chemist who named it after the Greek word "ozein" which translates as smell, odour, due to it's unpleasant, evident smell (Rubin, 2001; 2002). Ozone is a molecule consisting of three atoms of oxygen, that share the same electrons, this particularity making it unstable. It has a half-life of 40 minutes at 20ºC, of 140 minutes at 0ºC, and 3 months at -50ºC. The fact that at room temperature it has a very short life is the most considerable disadvantage, as it can't be stored. Ozone, compared to oxygen, is ten times more soluble in water. In nature, ozone is produced after a substantial electric discharge such as lightening through the atmospheric oxygen or by a UV solar emission that catalysis ozone production. In the stratosphere, in the ozone layer, placed at approximately 22 km from the earth's surface, at a concentration of 10 ppm (parts per million volume), the quantity of ozone seems to remain somewhat constant due to an equilibrium between formation and dissociation of this molecule. This layer absorbs the UV rays with mitogenic properties. Human activity and the subsequent pollution of air by the emission of certain substances led to an increased ozone concentration. Ozone, combined with other elements, forms the photochemical smog that has toxic effects on human and animal health. Studies made to determine the influence of these substances on different tissues such as skin, respiratory mucosa, eyes, lungs, led to the general opinion that ozone is a toxic gas (Valacchi et al., 2005). Recently the focus has shifted to research on the therapeutic effects of this gas, the concentration, doses, and route of administration to make it useful rather than toxic. Given the fact that for an extended period, ozone was considered to be toxic, its "migration" into the medical field is not

entirely accepted by the medical community (Bocci, 2002; 2004; 2011).
The ozone concentration has been reported in µg/ml, mg/L, and ppm, which produces confusion. The conversion rate is the following: *467ppm=1.0 µg/ml or 1 mg/L or 1* g/m^3*; 1000 ppm=2.1 µg/ml or 2.1 mg/L or 2.1* g/m^3.
The first use of ozone was as a disinfectant, for water as well as surfaces; leading to its current use in sterilizing operating theatres. The FDA approved the use of ozone as an antimicrobial agent related to the food industry. Water plants all over the world confirm the efficacy of ozone as a disinfectant as well as its effect on improving the organoleptic features of water (Langlais et al., 1991; Rodriguez et al., 2008; Martinelli et al., 2019).
Medical ozone is produced by an ozone generator that uses pure oxygen (100%) that is exposed to an electric arc. Similar to what happens in nature with the electrical discharge, the two molecules of oxygen are split, and part of them bound in pairs of three as ozone molecules. The ozone generator has to be made out of ozone resistant materials and to be able to measure the concentration of ozone produced.
The resulting gas is an oxygen-ozone mixture, in which the concentration of ozone is 3-5% of the mix defined as medical ozone. Seeing as ozone is an unstable molecule with a short therapeutic window, it is necessary to prepare it before each use. The ozone generator can produce ozone with a concentration that ranges between 1 and 80 µg/ml. The total dose of ozone delivered is obtained by multiplying the volume in ml with the preselected concentration.
This systematic review aims to gather and update relevant knowledge related to the use of ozone therapy in veterinary medicine. The paper intends to underline the worldwide interest for this therapy and the major need for further research to completely understand itsextent.
The data is presented in a systematic matter, the lack of similar data making it impossible to use a statistical approach as needed to perform a meta-analysis.

MATERIALS AND METHODS

The PRISMA Checklist 2009 was used to perform this systematic review. A search in several medical databases such as PubMed, Science Direct, Research Gate, Scopus, Google Scholar, as well as the archives of the ozone journals, was performed using the following key words: ozone therapy, veterinary, autohemotherapy, animal, ozone insufflation, oxygen-ozone.
The exclusion criteria selected were the publishing year and the field of application. The articles taken into consideration were published between January 2000 and March 2020.
Although the number of papers on ozone therapy is considerably larger in human medicine, to accomplish the aim of this review we only included those referring to studies, case reports, or reviews associated with veterinary medicine, performed on animals or in vitro. All articles found on this topic were included regardless of the impact of the journals, the type of the paper, or the methods used for the studies.
After the initial search performed by two different operators, 3554 results were obtained to which we added 20 other papers from other sources such as archives of Italian and Spanish ozone journals.
After a thorough screening, the duplicates and all articles not related to veterinary medicine were removed. At the end of the eligibility assessment, a total of 117 papers were taken into consideration for this systematic review and subsequently grouped into several major categories.

RESULTS

To facilitate understanding, the major routes of medical ozone administration are presented (Table 1), without details about the action mechanism, focusing on the clinical aspect of this therapy (Kozat and Okman, 2019; Schwartz-Tapia et al., 2015; Baeza et al., 2015; Khan et al., 2017; Re, 2018, Sciorci et al., 2020; Repciuc, 2016).

Table 1. Ozone administration routes

Parenteral	Topical/locoregional
Intravenous (major autohemotherapy)	Oral
Intramuscular (minor autohemotherapy/ infiltration)	Auricular
Subcutaneous	Vaginal
Intraperitoneal/ intrapleural	Urethral/intra bladder
Intra/periarticular	Rectal
Intradiscal	Cutaneous
Intralesional	Dental

Major autohemotherapy (MAHT)
Autohemotherapy is the most common form of ozone administration both in human and veterinary medicine and is considered to have a systemic effect, through immunomodulation and its anti-inflammatory and analgesic effects. It enhances oxygen delivery and consumption. The principle of this technique is to ozonize the patient's blood extracorporeally and reintroduce it into the vascular bed. A certain amount of blood is collected from one of the peripheral veins, exposed to ozone at a preset concentration, and transfused back to the patient. All equipment used has to be ozone resistant, sterile, and respect all criteria required for intravenous administration.
This method has been perfected over time and multiple ways of drawing and transfusing blood have been proposed. Nowadays, ozone generators are equipped with vacuum systems that help draw the blood through extension lines into a recipient with anticoagulant, where it is ozonated before transfusion, thus forming a closed system which is controlled by the machine, with little human interaction with the blood.

Minor autohemotherapy (mHAT)
mHAT is a technique used for unspecific immunomodulation by intramuscular administration of ozonized blood. More than 50 years ago, blood or sterile milk was injected into the body based on the same principle, with a similar desired response. Peripheral blood is drawn into a syringe, to which is added an equal volume of ozone of the same concentration used in autohemotherapy. The syringe is vigorously agitated to break the erythrocytes and obtain a homogenous mixture of blood-ozone. This product is then administered as an intramuscular injection in the hind leg.

Rectal/vaginal insufflation
The oxygen-ozone mix is administered through a rectal catheter at a lower concentration, but a greater volume is delivered. Some ozone generators have an insufflation function while others can only produce the ozone, that can be collected into a syringe and then administered locally.

Ozone infiltration
This method refers to the administration of gaseous ozone directly into different tissues, for its anti-inflammatory and analgesic effect. The ozone can be administered subcutaneously, intramuscularly, intraarticularly, peri-articularly, or even in or around a lesion or tumour.

Ozone bagging
This method is used for the topical administration of ozone. It was conceived for use in extremities that can easily be introduced into an ozone resistant bag and then exposed to ozone at a high concentration. The main indications are chronic unhealing wounds and dermatology.
The ozone can be used to produce ozonated saline or oil that can be applied topically, used to lavage different cavities or wounds (urinary bladder, ear, uterus, peritoneal cavity, fistulas).
The medical uses of ozone, which have considerably expanded, are increasingly being recommended as a possible approach to different pathologies.
The search results were grouped into several categories, as follows: wound management, buiatrics, musculoskeletal disorders, oncology, dentistry, ozone sterilization and antibacterial effect, equine pathology, urinary pathologies, ozone toxicosis, and miscellaneous studies.

Wound management
This is an immense and continuously evolving topic. Many commercial and "homemade recipes" were studied and documented over time (Mocanu et al., 2015; Bodea et al., 2017). In the past couple of decades, ozone was introduced as a potential therapy for wound healing. A large number of articles approach the use of ozone for chronic wound healing in human subjects. In veterinary medicine, just a

few studies have been published on this topic, although it seems that it is used on a much larger scale but without being documented (Roman, 2013).

In wound management, ozone is mainly applied topically as a gas during the bagging technique or through perilesional infiltration, as ozonated saline lavage (ozone hydrotherapy) or as ozonated oils and creams that act as healing enhancement agents (Al-Saadi et al., 2015; Travagli et al., 2010; Yildirim, 2018). Ozone plays a role in different phases of wound healing. It first helps eliminate contamination, then releases oxygen, which activates fibroblastic proliferation that leads to the formation of healthy granulation tissue and subsequent epithelisation (Krkl et al., 2016; Orsini, 2007; Zeng and Lu, 2018).

Another study was performed on guinea pigs to determine the effect of topical application of ozonated oil on wound healing. were used to determine the difference between the groups. Superior values were obtained (immunohistochemical analysis and collagen fibres and fibroblasts count) for the ozonated oil group showing that this product can accelerate cutaneous wound healing (Kim et al., 2009).

A similar study performed on SKH1 mice showed the efficacy of ozonated sesame oil as a healing enhancer when applied at three different concentrations. The level of peroxide in the ozonated oil was analysed in correlation with the healing rate (Valacchi et al., 2010; 2013).

A paper referring to induced gingival wounds in pigs concluded that ozone may be effective in the early stages of wound healing, although there was no statistical difference between the tested and the control group (Eroglu et al., 2018).

Ozone was used both systemically as major autohemotherapy and locally in dogs, but the proof of its efficiency was evaluated only based on clinical improvement (a qualitative granulation tissue with fast epithelization and normalization of haematological values) (Gayon-Amaro & Flores-Colin, 2019b; Kosachenco et al., 2018).

A case report revealed the protocol used in a horse with lesions from cutaneous habronemiasis treated locally with ozonized water and ozonized oil, fully healed in two months (Berbert et al., 2010).

Although there are recommendations for ozone use in dermatological diseases, our search revealed a single experimental study on 30 dogs with generalized dermatopathies such as traumatic dermatitis or intertrigo, in which ozone was applied locally as gas. They obtained remission, on average, after day 21 (Jordan et al., 2019). *In vitro*, Ouf (2016) demonstrated the anti-fungal potential of ozone against dermatophytes.

Another interesting study analyzed the toxic (not therapeutic) effect of ozone on wound healing in correlation with age. Lim et al. (2006), confirmed their hypothesis that oxidant pollutants delay wound healing in adult subjects, that were exposed for more extended periods.

Three studies published in consecutive years, review the oxidative, antioxidant, or toxic effect of ozone after controlled exposure of rat skin to this gas (Valacchi et al., 2002; 2003; 2004).

Buiatrics

Veterinarians working with cattle showed a great interest in ozone therapy by studying its potential uses to overcome the deficits of conventional protocols. In this area of veterinary medicine, ozone is used mainly as an alternative to antibiotics in the treatment of metritis, fetal membranes retention, mastitis, or as an adjuvant in improving reproductive efficacy in dairy cows.

A group of researchers from Croatia published several articles related to this subject and a review of the use of ozone in buiatrics (Duricic et al., 2015). One of the studies was performed on 404 Holstein cows, divided into three groups to assess reproductive performance after administering ozone pearls and ozone foam. The results showed an improvement when the products were administered in the early puerperal period (Djuricic et al., 2011). A similar study on cows with retained fetal membranes demonstrated that using intrauterine ozone improves reproductive performance (Djuricic et al., 2012). Sheep also demonstrated better outcomes when treated with ozone as compared to antibiotics when fetal membranes were retained after normal delivery (Djuricic et al., 2016).

In another study, rectal temperatures above 39.7ºC and the number of escape therapies were used as an indicator of the efficacy of ozone therapy (Imhof et al., 2019). Ozone has been used by another group as uterine ozone-flushes to improve fertility rate (Zobel et al., 2014), as a treatment for retained placenta in dairy cattle (Zobel and Tkalcic, 2012; Samardzija et al., 2017) and to overcome the reproductive consequences of urovagina (Zobel et al., 2012).

Another sector in which ozone has been intensively used is in the treatment of the mammary gland in ruminants. A small study on 18 quarters concluded that the administration of 1 ppm ozone intramammary for three days may be useful for treating mastitis (Kwon et al., 2005). A similar one performed in Turkey, on 22 cows considers ozone as a significant alternative to antibiotics in treating acute mastitis, with some limitations (no response in mastitis caused by *Streptococcus* spp. and *Candida* spp.) (Sertkol et al., 2018). Another study demonstrated a 50% improvement in subclinical mastitis after four days of ozone therapy (Aguirre et al., 2019). The ozone was administered directly as a gas into the mammary gland as in a study performed in Japan (Ogata and Nagahata, 2000), as ozone water to determine the efficacy of mammary irrigation in cows with acute coliform mastitis (Shinozuka et al., 2008) or even as ozonated oil infused into the affected quarters (Jo et al., 2005). Most of the studies on this subject encourage the use of intramammary ozone, having a lower treatment cost, and zero waiting period compared with a classic approach (Koseman et al., 2019; Enginler et al., 2015; Aguirre et al., 2019). The intramammary use of ozone-oxygen mixture combined with a platelet concentrate in an oil vehicle was researched as part of doctoral studies in Italy (Bignotti, 2014).

The versatility of the ozone molecule is shown in one paper that describes the use of ozone to fumigate food storage facilities, with a good outcome in preventing recurrent mastitis in dairy buffaloes (Atef et al., 2016).

The changes that occur in blood and milk cellularity where observed both in *in vitro* and *in vivo* studies in cattle after ozone was administered as gas *in vitro* or as autohemotherapy. Immunological changes have been noticed. (Ducusin et al., 2003; Ohtsuka et al., 2006; Terasaki et al., 2001).

Musculoskeletal disorders

Ozonetherapy in musculoskeletal disorders has two main directions: orthopaedic and spinal injuries both in large and small animals. It is used for chronic pain relief in osteoarthritis, muscular disorders, disc herniation, nerve damage, and osteomyelitis (Khan et al., 2018).

Ozone has gained much terrain in clinical orthopaedic practice, leading to a considerable number of publications. Balardini (2005) obtained a positive response after using ozone infiltration in four horses with spinal muscular pain. More data about the use of ozone in spinal muscle disorders was presented in a review that mentions ozone in horses (Seyam et al., 2010). Remission of clinical signs was obtained in a case of chronic laminitis in a horse after the administration of ozone intramuscularly, peritendinous and intrarectally as shown in a recent case report (Coelho et al., 2015; Flores-Colin and Gayon-Amaro, 2019a). In dogs with intervertebral disc herniation, ozone can be administered directly into the herniated disk, leading to its shrinkage and subsequent decompression and gait improvement (Han et al., 2007; Jang et al., 2009). Another experimental study on miniature pigs describes the mechanisms of action of ozone in intradiscal administration (Murphy et al., 2016). Clinical improvement was described in a study made on two unrelated canine groups: first group diagnosed with osteoarthritis and the second one presenting pain or lameness due to spinal injuries (Hernandez Aviles, 2013). Castrini (2002) published a treatment protocol for herniated discs in dogs, using ozone infiltrations. An abstract mentions the use of ozone in dogs with orthopaedic conditions (Scrollavezza, 2019).

To determine if ozone is safe for intraarticular administration in horses, the effect of ozone on joint components was analyzed through physical examination, lameness evaluation, ultrasonography, and synovial fluid analysis. Ozone was given to two groups in different concentrations, both being considered safe after no cartilage damage or significant inflammation was identified (Vendruscolo et al., 2018). The same hypothesis was confirmed

in a histological study on rats (Iliakis et al., 2008). A second study on rats measured the level of several tumour necrosis factors after ozone intraarticular administration in subjects with rheumatoid arthritis and found that at a concentration of 40 µg/mL, ozone suppresses joint inflammation (Chen et al., 2013).
Another recent study describes the combined effect of ozone and methylprednisolone in sciatic nerve regeneration after a crush injury, with potential beneficial effects (Ozturk et al., 2016). The effect of ozone on the crushed sciatic nerve was of interest in another experimental study (Somay et al., 2017). The therapeutic and preventive effect of ozone has been supported by a study on rats with experimentally-induced osteomyelitis (Gonenci et al., 2017). Experimental studies have also evaluated bone regeneration and tendon healing in association with ozone therapy (Alpan et al., 2016; Duman et al., 2017; Ozdemir et al.,2013; Kizilkaya et at., 2018)
Ozone therapy has been tested as a potential therapy for pododermatitis in the form of autohemotherapy in cows (Scrollavezza et al., 2002) or as a combined technique of ozone therapy and platelet-rich plasma in sheep (Szponder et at., 2017).

Oncology

In this field, ozone is considered an adjuvant to conventional oncological therapy. To better understand the full extent of the effect that ozone has on a subject that develops a cancerous process, several experimental studies on animal models have been performed (Gonzalez et al., 2004; Kesik et al., 2009; Kuroda et al., 2018; Teke et al., 2017; Rossmann et al., 2014; Clavo et al., 2018; Caquetti et al., 2017).
Two preclinical studies were published on the effect of ozone on mice inoculated with Ehrlich ascitic tumour cells. Ozone was administered via rectal insufflation in one study, and directly into the peritoneal cavity in the other. The results were concentration-dependent, at higher ozone concentrations a lower number of tumour cell per mice, and a lower number of pulmonary metastases were observed (Menéndez et al., 2008; Kızıltan et al., 2015). Another group of researchers used a rabbit model to administer ozone intraperitoneally after they induced squamous cell carcinoma metastases. They considered that ozone had an indirect effect, as it had no direct contact with the cancer cells, and suggested that stimulation of the immune system might be responsible for the significant regression of the tumour (Schulz, et al., 2008). An experimental rat model was used to determine the impact of ozone and radiotherapy on tongue cancer as monotherapy or in combination. Survival rates were higher in the group in which ozone and radiation therapy were used combined compared with the use of radiotherapy alone or ozone therapy alone (Dogan et al., 2018).
A case report series describes the protocols used in the treatment of four dogs with different types of cancer. No specific parameters were objectively analyzed to determine the effect of ozone, but the improved outcome was associated with longer survival rates, a better quality of life, and a lower therapeutic cost (Hernandez et al., 2016).

Dentistry

Ozone has recommendations in human medicine such as oral cavity lavage, periodontal disease, infected wound cavities, ulcers, wound healing, and osseointegration (Ahmed et al., 2013). All of these uses can be extrapolated to veterinary medicine, and are gaining terrain in daily practice, but a small number of studies have been published.
Experimental studies in rats evaluated the effect of ozone in dental pathologies such as treating periodontitis or as a tooth whitening agent in tetracycline-stained rat incisors. In the ozone groups, a positive response was observed, the intraperitoneal route being more effective in the first study (Saglam et al., 2019; Tessier et al., 2010). A positive response was also observed after the use of ozone as a healing enhancer in peri-implant lesions in rabbits (Yildirim, 2018).

Antibacterial and sterilization role

Several studies demonstrate the disinfectant and sterilizing effect of ozone, for spaces, objects, and organisms.
The antibacterial activity of ozonized oil was tested on several bacterial species. This product has an important antibacterial activity when tested *in vitro* (Sechi et al., 2001).
Another *in vitro* study shows that *Escherichia coli* releases a smaller amount of endotoxin

when exposed to ozone compared to antibiotics, making ozone sterilization a viable method (Shinozuka et al., 2007).
Another paper showed that ozonized water can be efficient against *Escherichia coli*, with best results when is applied at a concentration of 3 ppm and for more than 1 second (Leusink and Kraft, 2012)
A preliminary study in dogs, showed a reduction of the bacterial load at the level of the conjunctival sac and periocular skin, after using topical liposomal ozone (Marchegiani et al., 2019).
The antibacterial activity was analyzed in a couple of studies on animal models, experimental creating ozone pneumoperitoneum (Schulz et al., 2003; Silva et al., 2009; Souza et al., 2010).
A negative response, that supported the need for further research in this field, was obtained at the end of a study on rats, where ozone was administered as pre-treatment in subjects with experimental induced sepsis. The survival rate was reduced in the group where ozone was administered before sepsis treatment (Torossian et al., 2004).
In 2003, a Canadian company obtained clearance to market its method to use ozone as a sterilizing agent for heat-sensitive materials in hospitals (Dufresne et al., 2004). A thorough review gathered literature data concerning ozone sterilization in the health care field, describing its limitations and need for further studies, but also its great potential (Sousa et al., 2010).

Equine medicine

The use of ozone has been studied for various equine pathologies.
A work developed collaboratively between the surgical and pathology departments of a veterinary research institute lists the potential benefits of ozone therapy and describes its mechanism of action (reviewed in Bhatt et al., 2016).
Besides the articles already discussed on topics such as laminitis, lumbar pain, or cutaneous wound healing already mentioned in other categories, they describe the use of ozone to inactivate the Venezuelan equine encephalitis virus. To verify the toxicity of ozone in horses, several studies analyzed the oxidative stress produced by this gas by various ways of administration. In a comparative study between healthy horses and horses affected by recurrent airway obstruction, no difference was found in antioxidant capacity, as ozone induced a non-significant level of inflammation when inhaled (Deaton et al., 2005).
One study focused on the haematological changes in horses after intravenous ozone administration. There was a variation in platelet count and increased hematocrit. The fluctuation depended on the sex of the animal, but not on the volume of ozone. (Haddad et al., 2009; Ballardini, 2006). Autohemotherapy in horses helps to improve the biological antioxidant potential of these subjects (Tsuzuki et al., 2015).

Urinary system

Although several practitioners recommend and use ozone for urinary tract disease, especially cystitis, few papers exist on this topic. An experimental study on rabbits used ozone to treat interstitial cystitis by direct administration into the urinary bladder (Bayrak et al., 2014). In rats, the effect of ozone has been tested on different kidney injuries, with beneficial effects (Caliskan et al., 2011; Calunga et al., 2005).

Toxicity

Most of the quoted sources support the idea that ozone can have therapeutic effects, with little to no side effects, by all routes of administration described, except inhalation. Inhaled ozone, especially at high concentrations or for large periods, transforms from an irritant of the respiratory tract to a toxic gas for the organism. Many studies were written about air pollution and ozone implication in different injuries. Manufacturers of emission protective gear mention the fact that small animals can die from exposure to ozone over the concentration of 15 ppm for more than 2 hours.
In more recent articles on ozone toxicity, lung inflammation, lung injuries, and the effect of air pollution on humans, animals, and plants are described in detail (Iriti & Faoro, 2017; Last et al., 2018). Most refer to exposure to atmospheric ozone, but ozone from air purifiers can be harmful to small animals as well. A recent case report describes noncardiogenic pulmonary oedema in three kittens after inhaling ozonized air produced by an ozone air-

purifier device. Respiratory signs disappeared after three days of intensive care treatment, considered to be rapidly reversed by the oxygen supplementation and medical treatment (Caudal et al., 2018). Experimental studies on animals tried to distinguish between the therapeutic and toxic effects of ozone, describing respiratory system injuries and analyzing tumorigenesis after ozone exposure (Kim & Cho, 2009; Dorado-Martínez et al., 2001; Alfaro et al., 2004; Rodriguez, 2009; Schelegle et al., 2003).

Miscellaneous studies

Besides the work on the subjects already mentioned, scientists studied the therapeutic potential of ozone in other diseases, such as endometrial injuries in rats (Wei et al., 2018) and atrophic rhinitis in an albino rat model (Altas et al., 2018). Good results were obtained using ozone to prevent testicular damage in an experimental cryptorchid rat model (Bicer, 2018), similar to the results obtained in a study on a testicular torsion model rat in which ozone therapy prevented ischemia and reperfusion injury (Aydos et al., 2014). A clinical study on dogs compared the analgesic effect of ozone and meloxicam after ovariohysterectomy. To measure, the efficacy of the three analgesic protocols used (two with ozone and one with meloxicam), the modified Glasgow scale and the visual analogue scale were used. Satisfactory analgesia was provided by the two ozone protocols, considering it as an alternative pain reliever.

Ozone therapy was tested as a therapeutic option for diabetes mellitus in dogs and rat models to diminish oxidative and endothelial damage, but with uncertain conclusions due to a small number of cases and parameters evaluated in these studies (Castrini et al., 2002; Al-Dalain et al., 2001).

The versatility of ozone has been documented as a combination therapy with Chinese medicine to treat hepatic injuries in dogs and as ozone-based eye drops in both humans and animals (Li et al., 2007; Spadea et al., 2018). Another experimental study on mice uses ozone administered subcutaneously to prevent allodynia, with favourable outcomes (Fuccio et al., 2009).

DISCUSSIONS

Almost two-thirds of the articles selected were published in the past ten years, while the rest were published between 2000 and 2010, demonstrating the increased interest for ozone therapy in the past years.

Fifty out of the 117 papers documented experiments performed on laboratory animals or in vitro, supporting the idea that a major part of ozone therapy is still experimental, compared to the low number of clinical studies quoted for each pathology.

Positive, homogenous results were obtained in studies on the use of ozone in cattle and small ruminants. The therapeutic properties of ozone are confirmed by a considerable number of articles written on this topic. For this field, the ozone was tested on a large number of animals, in clinical studies that use standardized, reproducible protocols.

Although research shows that ozone associated with air pollution has negative effects on the skin, a large number of papers found clinical improvement when using ozone therapy in wound management. A small number of papers included clinical cases, the few that did were case reports, the majority of articles concerning the use of ozone as a healing agent referred to experimental studies.

Equine medicine embraced ozone therapy with good results for several pathologies.

Most studies also encourage the use of ozone for musculoskeletal disorders, urinary system disease, or as adjuvant therapy in oncological patients.

However, not all papers used standardized methods or analyzed objective, quantifiable parameters to measure the efficacy of ozone therapy. Some of them have a small number of subjects, which is insufficient for the studies to have statistical significance.

The papers published on this topic favour the insertion of ozone into clinical practice, however, there is a need for more clinical studies to evolve from empirical ozone administration to standardized methods, which will lead to protocols that can be reproduced in similar circumstances.

Ozone has no side effects when administered through the recommended routes, but it has a restriction for the respiratory system, as it is toxic when inhaled. Once longer, more extensive studies performed using validated methods will be carried out, more accurate restrictions and dosage limitations may be set.

CONCLUSIONS

Ozone therapy is still a new field, with uncharted areas. The scientific proof that exists related to veterinary medicine is vastly scattered through the medical field and difficult to group. Both clinical and experimental data support the use of ozone for various pathologies and encourage further research.
Ozone therapy deserves more attention, as its therapeutic range has not yet been fully defined.

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