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Review Article

Use of Ozone Therapy in Veterinary Medicine: Evidence, Applications and Translational Perspectives for Tissue Repair and Inflammatory Modulation

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Abstract

Ozone therapy has emerged as a promising therapeutic strategy in veterinary medicine due to its antimicrobial, anti-inflammatory, immunomodulatory, and regenerative properties. This systematic review synthesizes current evidence regarding the physiological and biochemical mechanisms of medical ozone, its clinical applications across different species, and the relevance of laboratory biomarkers for therapeutic monitoring. The findings reveal that ozone modulates pro- and anti-inflammatory cytokines, stimulates angiogenesis, enhances collagen synthesis, reduces microbial biofilms, and improves peripheral oxygenation, resulting in significant clinical benefits for wound healing, musculoskeletal disorders, autoimmune conditions, chronic infections, and refractory cases. However, methodological gaps were identified, especially concerning dose standardization, administration protocols, and laboratory monitoring practices. Overall, ozone therapy demonstrates substantial clinical potential in veterinary practice and offers translational relevance for human medicine. Further controlled clinical trials and multicentre studies are essential to validate its efficacy and safety.

Keywords: ozone therapy; veterinary medicine; inflammation; wound healing; biomarkers; systematic review.

Introduction

Ozone therapy has a historical trajectory that dates back to the nineteenth century, when ozone began to be studied as an antimicrobial agent and hospital disinfectant. The medical use of ozone initially emerged as a tool for sterilization of surgical environments and materials, especially after its formal identification as a highly oxidizing and biologically active molecule. Historical reviews indicate that the initial application had an exclusively sanitary character, without direct therapeutic purposes, but established the basis for its future clinical exploration [1]. This period marked the beginning of ozone's transition from a mere disinfectant to a potential therapeutic agent with multiple biomedical applications [2].

At the beginning of the twentieth century, studies began to investigate the physiological and biochemical effects of ozone on the body, seeking to understand how its oxidizing capacity could be used in a controlled and safe manner. Research carried out

by Bocci and collaborators has shown that, when administered in adequate concentrations, ozone triggers beneficial biological reactions, such as immune modulation, increased oxygen transport, and stimulation of endogenous antioxidant processes [3]. This breakthrough marked the transition from ozone therapy to an emerging therapeutic field, paving the way for more structured clinical protocols and standardized methods of application.

In the following decades, the clinical use of ozone therapy expanded in several medical specialties, especially in Europe, Latin America, and Asia. Recent reviews highlight its use in the management of chronic pain, inflammatory diseases, wound healing, infections, and metabolic disorders, through different routes of administration, such as autohemotherapy, infiltrations, and ozonation of oils and fluids [4]. Systematic studies also demonstrate promising results in adjuvant therapy of difficult-to-heal lesions, reinforcing the role of ozone therapy in complex clinical contexts [5]. However, there is methodological heterogeneity among the studies, which reinforces the need for more robust evaluations.

In view of the growth in the clinical use of ozone, it is essential to consolidate scientific evidence that supports its efficacy and safety. Recent reviews point to important gaps related to the standardization of protocols, dosages, routes of administration, and clinical outcomes [6]. In addition, authors highlight persistent controversies in the literature and the need for rigorous clinical studies to support the use of ozone therapy on a large scale [7]. Thus, the present systematic review seeks to gather and critically analyse the available scientific production, offering an updated synthesis on the historical trajectory, biological foundations, clinical applications and future challenges of ozone therapy.

The adoption of ozone therapy in veterinary medicine has grown significantly in recent decades, following the scientific development observed in the human area. Initially restricted to research centers and experimental practices, the therapy gained ground with the publication of studies demonstrating antimicrobial, healing, and immune system modulating effects in different animal species. Pioneering research has shown that ozone, when administered in controlled concentrations, can induce safe and reproducible therapeutic effects in companion and production animals, paving the way for its wider clinical use [1, 2].

The expansion of this use was consolidated from experimental investigations that demonstrated benefits in conditions that are frequent in the veterinary routine, such as chronic wounds, dermatitis, otitis, infectious diseases, and osteoarticular inflammatory processes. Studies carried out in dogs and horses have indicated a significant improvement in the healing time of skin lesions treated with ozonated water and ozonated oil, through mechanisms related to the reduction of microbial load and stimulation of angiogenesis [8, 9]. In addition, research has demonstrated analgesic and anti-inflammatory potential in animals submitted to ozonated autohemotherapy, strengthening the adoption of the technique as an adjuvant integrative therapy [10].

Another factor that drove the growth of veterinary ozone therapy was its applicability in conditions that are difficult to resolve therapeutically, especially those involving antimicrobial resistance. Recent studies show that ozonated solutions and emulsions have effective bactericidal action against multidrug-resistant pathogens, justifying their use in integrative protocols for animals with chronic infections or refractory to conventional treatment [11]. Such evidence becomes particularly relevant in the face of the increase in the of antimicrobial resistance, placing ozone as a complementary therapeutic option, at low cost and with a good safety margin.

With the growing volume of international publications and the institutionalization of the technique in several countries, ozone therapy began to be incorporated into continuing education programs and specialization courses aimed at veterinarians. European and Latin American scientific societies have begun to disseminate evidence-based protocols for different species, contributing to the standardization of the routes of administration and concentrations used. Despite the advances, there is still a need for more robust clinical trials for various clinical conditions, since part of the available evidence derives from observational or experimental studies in animal models, reinforcing the need for greater methodological rigor in the area [12]. Thus, the expansion of ozone therapy in veterinary medicine reflects not only technological advancement, but also the search for complementary, safe and scientifically based therapeutic approaches.

The mechanisms of action of ozone therapy involve a complex interaction between the oxidizing potential of ozone and the activation of the body's antioxidant and immune responses. When administered in low concentrations, ozone generates a "small, controlled oxidative response," inducing the formation of peroxides and reactive oxygen mediators capable of stimulating protective biological pathways. Bocci describes this phenomenon as oxidative preconditioning, in which mild oxidative stress triggers an increase in antioxidant enzymes (SOD, catalase, glutathione peroxidase) and improved tissue oxygenation [2, 3]. This mechanism explains part of the anti-inflammatory and healing effects observed in different animal models.

In addition, ozone exerts potent antimicrobial activity, with bactericidal, fungicidal and virucidal action. Studies have shown that the gas and its derivatives (oil and ozonated water) act in the oxidation of phospholipids and glycopeptides of microbial membranes, promoting cell lysis and inhibition of replication [1]. In veterinary medicine, this property is especially relevant in cases of multidrug-resistant infections, since ozone has a broad spectrum without inducing classic microbial resistance mechanisms [11]. Another important mechanism is immune modulation: ozone stimulates the production of cytokines such as IL-2, IL-6 and TNF-α in early stages, followed by a regulatory effect that reduces systemic inflammation, contributing to immune homeostasis in animals with chronic diseases [10, 12].

In dermatology, the use of ozone has shown significant results in chronic wounds, infectious dermatitis, pyoderma, and otitis externa. A study conducted by [8] in dogs showed that the

application of ozonated water significantly accelerated wound healing by stimulating angiogenesis and reducing bacterial colonization. In felines and horses, ozonated oils have demonstrated efficacy against Staphylococcus spp., Malassezia pachydermatis and multidrug-resistant bacteria, being especially useful in cases of difficult response to antibiotic therapy [11]. The antimicrobial, anti-inflammatory, and regenerative effects justify its wide adoption in integrative dermatological protocols.

In veterinary Orthopedics, ozonated autohemotherapy and joint infiltrations have been studied for the management of osteoarthritis, myositis and musculoskeletal pain. [10] demonstrated that dogs treated with ozonated autohemotherapy showed significant pain reduction and functional improvement, associated with the modulation of inflammatory cytokines. In equine athletes, intra-articular applications of ozone demonstrated analgesic and anti-inflammatory potential, without important side effects, favouring locomotor recovery and return to activity [12]. Ozone's ability to improve tissue oxygenation and reduce local oxidative stress is especially beneficial in degenerative joint pathologies.

Ozone therapy is especially relevant in veterinary infectious diseases due to its broad antimicrobial spectrum. Ozone has proven efficacy against Gram-positive and Gram-negative bacteria, enveloped viruses and fungi, being a promising alternative in resistant infections. In a study by [11], ozonated oils showed bactericidal action against multidrug-resistant strains isolated from dogs, including Pseudomonas aeruginosa, Staphylococcus aureus MRSA, and Escherichia coli ESBL. In clinical cases of infected wounds, recurrent otitis, and abscesses, ozone reduces microbial load, improves local perfusion, and decreases the need for prolonged antibiotic therapy.

In veterinary dentistry, ozone has gained prominence for its rapid antimicrobial action and for its ability to reduce bacterial load in periodontal pockets, root canals, and oral surgeries. [12] demonstrated that topical ozone application significantly reduces periodontal biofilm and gingival inflammation in dogs. In felines, the application of ozonated water during tooth extractions helps to reduce infections and control postoperative pain. In addition, ozone has been used as an adjuvant therapy in the treatment of chronic feline stomatitis and refractory gingivitis, with positive clinical responses.

Ozone therapy, by modulating the immune system and reducing oxidative stress, promotes measurable changes in several inflammatory biomarkers used in veterinary medicine. Studies show that the therapy can alter serum levels of cytokines such as IL-6, TNF-α, IL-10, in addition to interfering with markers of oxidative stress, such as malondialdehyde (MDA) and total antioxidant capacity (TAC) [3]. These changes have been documented in dogs and horses submitted to ozonated autohemotherapy, indicating a decrease in systemic inflammation and an increase in antioxidant defences [10].

In the laboratory context, this modulation offers new diagnostic perspectives. Response to treatments can be followed through serum biomarker analysis, allowing personalized protocols and accurate monitoring of clinical evolution. In animals with chronic inflammatory diseases such as dermatitis, osteoarthritis and infections, the use of biomarkers is useful to identify therapeutic response and predict prognosis. The integration between ozone therapy and laboratory diagnosis represents a growing trend in evidence-based veterinary medicine, especially in the face of the need for personalized and more efficient treatments.

Ozone therapy has gained increasing prominence in human and veterinary medicine in recent decades, driven by the expansion of experimental studies, clinical trials, and narrative reviews that demonstrate its therapeutic potential in different conditions. However, the literature still presents heterogeneity regarding experimental models, administration protocols, concentrations used, and outcomes evaluated, making it difficult to consolidate a robust scientific consensus. Reviews such as those by [2, 10] point to important gaps in the standardization of methods, which reinforces the need for systematic analyses that critically synthesize the available scientific production and assess the quality of existing evidence.

In addition, veterinary medicine has seen a rapid expansion in the use of ozone therapy, especially in areas such as dermatology, Orthopedics, infectious diseases, and dentistry, where experimental and clinical studies show promising results [8, 12]. However, despite the increase in interest, there are no comprehensive systematic reviews that simultaneously integrate the mechanisms of action, clinical applications, and laboratory impact of the therapy, a crucial point to guide evidence-based therapeutic decisions and avoid empirical practices disconnected from scientific rigor.

Another relevant factor is the growing concern about antimicrobial resistance, which makes it urgent to search for complementary therapies capable of reducing the indiscriminate use of antibiotics. Ozone therapy has demonstrated efficacy against multidrug-resistant microorganisms, presenting itself as an adjuvant alternative of global interest [11]. Thus, a systematic review that synthesizes evidence on its antimicrobial and immunomodulatory effects contributes directly to the contemporary scientific debate on safe and efficient integrative therapies.

Finally, the advancement of the use of ozone in the modulation of inflammatory biomarkers, such as cytokines, MDA, TAC, and antioxidant enzymes, opens up new perspectives for laboratory diagnosis and therapeutic monitoring in animals. However, these findings remain dispersed in isolated studies, without analytical integration that allows us to understand their real clinical impact [3]. Therefore, the present systematic review is justified by the scientific need to gather, critically evaluate and interpret the entire body of available evidence on veterinary ozone therapy, providing solid subsidies for clinical practice, experimental research and diagnostic innovation.

Methodology

The present systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines, internationally recognized

as a methodological reference for rigorous and transparent reviews [13, 14]. The main objective was to identify, select and critically synthesize the available scientific evidence on the mechanisms of action, clinical applications, laboratory relevance and therapeutic impacts of ozone therapy in veterinary medicine. To this end, a comprehensive search was carried out in multiple databases, including PubMed/MEDLINE, SciELO, LILACS, Scopus, Web of Science and CAB Abstracts, the latter being especially relevant for its wide coverage of veterinary studies. The search was complemented by Google Scholar, which is only used to crawl gray literature when necessary.

The search strategy involved the use of descriptors registered in MeSH, DeCS and CAB Thesaurus, combined by Boolean operators ("AND", "OR" and "NOT"), allowing adequate sensitivity and specificity in the retrieval of the studies. Terms such as "ozone therapy", "medical ozone", "ozonized oil", "animals", "veterinary medicine", "wound healing", "inflammation mediators" and "biomarkers" were used, structured in broad combinations that covered both mechanistic perspectives and clinical and laboratory applications. The search covered the period between 2000 and 2024, considering that in the last two decades there has been significant methodological progress and expansion of ozone therapy in the veterinary field, as evidenced by [15, 16].

The inclusion criteria included experimental and clinical articles, systematic reviews, laboratory studies and applied research that addressed ozone therapy in companion animals, production animals or experimental models for veterinary use. Studies should present details of protocols, routes of administration, concentrations used, or analysis of inflammatory and oxidative biomarkers. Articles published in English, Spanish, and Portuguese were accepted. In contrast, exclusively human studies, case reports without a defined methodology, abstracts without access to full text, duplication between databases, and articles whose methodological design did not allow consistent critical analysis were excluded.

The selection process took place in three stages. Initially, two independent reviewers screened for titles and abstracts, identifying potentially eligible studies. Subsequently, the full texts were evaluated, strictly applying the inclusion and exclusion criteria. In case of divergence between evaluators, consensus was reached through discussion. After final selection, standardized data extraction was performed, including the species studied, experimental or clinical design, study objective, ozone therapy protocol, applied routes and concentrations, clinical and laboratory outcomes, biomarker analysis, central results, and limitations reported by the authors. The synthesis of the findings followed a qualitative approach, given the heterogeneity of the methods and outcomes present in the included studies.

Despite the scope of the search and the rigorous application of the PRISMA protocol, some methodological limitations were identified. High variability was observed between studies regarding ozone concentrations, routes of administration and frequency of application, which made direct comparisons difficult. There was also a scarcity of randomized clinical trials in veterinary medicine, a predominance of experimental studies, and a lack of standardization in the inflammatory biomarkers analysed. Another critical point was the diversity of species evaluated, which limits the extrapolation of findings to general practice. In addition, the possibility of publication bias cannot be ruled out, since studies with positive results tend to be published more than those with negative or inconclusive results, as pointed out by [17].

Thus, this methodology sought to ensure reproducibility, transparency and scientific rigor in the construction of the present systematic review, offering a reliable synthesis of the state of the art on veterinary ozone therapy.

Physiological And Biochemical Aspects of Medical Ozone

Medical ozone has physicochemical properties that make it a highly reactive molecule of great biomedical interest. Composed of three oxygen atoms (O_3), it is an unstable gas, with strong oxidizing potential and a short half-life in biological fluids, where it quickly reacts with lipids, proteins and antioxidants present in plasma and tissues. This instability gives ozone the ability to generate highly bioactive secondary mediators, responsible for its therapeutic actions [2]. The chemical behaviour of ozone allows its primary action to occur not by the gas itself, but by the derivatives generated after contact with organic components, especially during controlled oxidation reactions [1].

The body's exposure to ozone results in the formation of reactive oxygen species (ROS), such as peroxides, aldehydes, and free radicals, which function as intracellular messengers. Although traditionally associated with cell damage, when produced in moderate concentrations, ROS plays an essential signalling role, modulating antioxidant defence pathways and immune response. In animal and human models, ozone therapy has been shown to increase the controlled production of lipid peroxides and 4-hydroxy-2-nonenal (4-HNE), molecules that act as second messengers in the activation of endogenous antioxidant pathways [8, 2]. This specific biochemistry explains a large part of the systemic effects observed in veterinary clinical practice.

The principle of oxidative hormesis represents the central axis of the therapeutic effects of ozone. "Hormesis" consists of an adaptive response in which small doses of a potentially harmful agent induce protective mechanisms, improving cellular resistance to stress. In the context of ozone therapy, the mild oxidative stress generated by ROS activates antioxidant defence systems, increases cellular tolerance, and reduces systemic inflammation [19]. Studies in dogs, horses, and experimental models show that ozone-promoted hormesis results in increased total antioxidant capacity (TAC) and reduction of biomarkers such as malondialdehyde (MDA), evidencing beneficial redox modulation [10].

Among the most relevant mechanisms triggered by ozone therapy is the activation of the Nrf2/Keap1 pathway, considered the main cytoprotective route against oxidative stress. The generation of 4-HNE and other secondary reactive molecules promotes the dissociation of Nrf2 from the complex with Keap1,

allowing its nuclear translocation and the subsequent activation of genes related to the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase [20]. Activation of the Nrf2/Keap1 axis explains the effects anti-inflammatory, healing, and immunomodulatory drugs observed in ozone-treated veterinary species, including improved tissue regeneration in chronic wounds and musculoskeletal injuries [12].

Ozone also exerts direct effects on mitochondria and cellular metabolism. Controlled exposure to ozone increases mitochondrial respiratory efficiency, promotes biogenesis, and improves ATP production, especially in tissues with chronic hypoperfusion [21]. In animals with inflammatory or degenerative diseases, these effects result in improved tissue metabolism, less apoptosis, and greater regenerative capacity. In vitro assays have shown that cells exposed to therapeutic concentrations of ozone exhibit increased oxidative phosphorylation and reduced intracellular ROS after the initial adaptation phase, reinforcing the regulatory and non-destructive role of the therapy [22].

Finally, there is robust evidence that ozone modulates microcirculation and promotes angiogenesis. The release of nitric oxide (NO), increased erythrocyte deformability, and improved tissue oxygenation are results consistently observed in experimental models and veterinary clinical studies. Stimulation of angiogenesis seems to occur through increased VEGF expression and activation of endothelial vascular repair factors [9]. These effects justify its use in ischemic wounds, chronic ulcers, and musculoskeletal injuries, in which improved perfusion and vascular regeneration is essential for therapeutic success.

Mechanisms of Action of Ozone Therapy in Inflammatory Processes

Ozone therapy exerts marked influences on the regulation of the inflammatory process, acting both in the acute and chronic phases through the modulation of cytokines and biochemical pathways related to oxidative stress. The administration of medical ozone generates reactive oxygen species (ROS) at controlled levels, which function as cellular signalling capable of activating immune and antioxidant responses. This signaling effect leads to the rebalancing between pro- and anti-inflammatory mediators, contributing to the resolution of persistent inflammatory processes [1, 3]. Experimental studies in animals have shown a significant reduction in inflammatory infiltrate, improvement of redox homeostasis and normalization of immunological parameters after therapeutic exposure to ozone [10].

Cytokine modulation is one of the most important mechanisms associated with the anti-inflammatory action of ozone. At therapeutic concentrations, ozone reduces the production of pro-inflammatory cytokines and increases the synthesis of anti-inflammatory mediators. creating an immunological environment more favourable to tissue repair. This regulation occurs through the activation of pathways such as Nrf2/Keap1 and partial inhibition of NF-κB, resulting in lower production of systemic and local inflammatory molecules [20]. The veterinary literature describes that this modulation contributes to the improvement of dermatitis, osteoarthritis and infectious diseases in different animal species.

Regarding interleukin-1 beta (IL-1β), one of the main pro-inflammatory mediators of the innate immune response, studies have shown a significant decrease in its expression after ozonated autohemotherapy. IL-1β is central in the amplification of the inflammatory process, promoting neutrophil recruitment, fever and prostaglandin synthesis. In vitro and in vivo studies show that ozone reduces the activation of the NLRP3 inflammasome, responsible for the conversion of pro-IL-1β into active IL-1β, leading to lower release of this cytokine [23]. In veterinary medicine, this reduction favours the improvement of conditions such as inflammatory dermatitis and chronic arthritis.

Interleukin-6 (IL-6), another pro-inflammatory cytokine of great relevance, is also modulated by ozone therapy. IL-6 is involved in acute phase response processes, fever, Th17 lymphocyte differentiation, and systemic inflammation. Clinical studies have shown that ozone treatments significantly reduce serum IL-6 levels in animals with chronic inflammatory conditions, which contributes to the reduction of pain and generalized inflammatory state [24]. This effect is particularly relevant in veterinary medicine, especially in cases of osteoarthritis, tendinitis, and musculoskeletal trauma.

Tumour necrosis factor-alpha (TNF-α), considered one of the main pro-inflammatory molecules in the body, plays a central role in the pathogenesis of systemic and infectious inflammatory diseases. Ozone therapy reduces the expression of TNF-α through mechanisms that involve oxidative stress control and reduction of excessive NF-κB activation [18]. Trials in dogs and horses with joint diseases have shown a significant reduction in TNF-α after ozone therapy, associated with clinical improvement and locomotor functionality [12]. This modulation partly explains the effectiveness of ozone in controlling pain and chronic inflammation.

Interleukin-10 (IL-10) is the main anti-inflammatory cytokine involved in resolving the inflammatory process and protecting against tissue injury. Studies have shown that ozone therapy increases the expression of IL-10, which favours the transition from an inflammatory environment to a reparative environment, reduces excessive activation of pro-inflammatory macrophages, and promotes healing [25]. In veterinary medicine, the increase in IL-10 has It has been associated with significant improvement in the healing of skin wounds, reduction of periodontal inflammation, and control of chronic otitis.

The action of ozone on M1 and M2 macrophages is another essential aspect for understanding its anti-inflammatory action. M1 macrophages are responsible for the production of pro-inflammatory cytokines, while M2 macrophages participate in tissue repair and remodelling. Studies show that ozone induces a phenotypic change favourable to M2 macrophages, promoting healing, anti-inflammatory, and regenerative effects [26]. This modulation is fundamental in dermatology and veterinary dentistry, where the resolution of the inflammatory process is decisive for the restoration of tissue integrity.

Another relevant mechanism is the reduction of microbial biofilm. Ozone has bactericidal, fungicidal and virucidal action through the oxidation of cell membranes and degradation of microbial structural components. In biofilms, ozone breaks the polysaccharide extracellular matrix, reducing adherence and microbial viability [11]. This effect is widely reported in veterinary dentistry, in the management of periodontal disease and feline stomatitis, as well as infected skin wounds in dogs and horses.

Finally, ozone has an important impact on capillary hemodynamic and peripheral oxygenation, contributing to the improvement of microcirculation. The therapy increases the release of nitric oxide (NO), improves erythrocyte deformability, and increases oxygen delivery to hypoxic tissues [9]. This effect is combined with the reduction of oxidative stress and the strengthening of antioxidant defence, resulting in a tissue environment that is more conducive to regeneration. This combination of hemodynamic, immunological and antioxidant effects consolidate ozone as a tool of great therapeutic value in veterinary medicine.

Ozone Therapy in Tissue Repair

Ozone therapy has stood out as an important therapeutic tool in tissue repair due to its ability to stimulate fibroblast proliferation, an essential step for the formation of granulation tissue. In vitro studies have shown that fibroblasts exposed to low concentrations of ozone have increased proliferative rate, greater extracellular matrix synthesis, and greater resistance to oxidative stress [7]. These effects are attributed to the activation of ROS-dependent signalling pathways and increased expression of repair proteins, making ozone a relevant biological modulator for the proliferative phase of healing. In addition to stimulating proliferation, ozone therapy plays a fundamental role in the callogenesis and remodelling of the extracellular matrix. Collagen, especially types I and III, is essential for the mechanical resistance of the regenerated tissue. Research in animal models demonstrates that topical or systemic application of ozone significantly increases organized collagen deposition and accelerates the transition between immature type III collagen and mature type I collagen [28]. This results in more stable scars with greater structural integrity, as well as reducing the risk of dehiscence and secondary infections.

Another determining mechanism for tissue repair is ozone's ability to increase the release of growth factors such as VEGF, TGF-β, and PDGF. These factors are fundamental for angiogenesis, cell chemotaxis and tissue remodelling. Studies in rabbit and dog skin wounds show that exposure to ozone significantly increases the local expression of VEGF, favouring the formation of new blood vessels and improving the oxygenation of regenerative tissues [29]. In addition, TGF-β plays a direct role in the modulation of inflammation and in the deposition of extracellular matrix, suggesting synergistic action between ozone and endogenous trophic factors.

Ozone therapy also demonstrates superior efficacy in healing chronic and acute wounds, including traumatic ulcers, burns, and infected wounds that have difficulty closing. Clinical trials in dogs, horses, and rodents indicate that the application of ozone reduces healing time, decreases bacterial load, and increases the formation of granulation tissue [30]. The combination of its antimicrobial, angiogenic and anti-inflammatory effect makes ozone especially relevant in chronic refractory wounds, often seen in the veterinary clinic.

In the musculoskeletal context, ozone therapy has important effects on muscle, tendon and cartilage regeneration. Studies in models of muscle injury show that ozone increases the differentiation of muscle satellite cells, improves the organization of regenerated fibers, and accelerates functional restitution [11]. In tendons, ozone induces greater deposition of type I collagen and improves biomechanical resistance, being used as an adjuvant in equine and canine tendinopathies. In cartilage, its anti-inflammatory action reduces the degradation of proteoglycans and favors chondral regeneration in experimental models.

These effects, combined, explain the clinical efficacy of ozone therapy in tissue regeneration protocols. By acting simultaneously on inflammatory modulation, angiogenesis, fibroblastic stimulation and reorganization of the extracellular matrix, ozone offers a multimodal biological approach, different from conventional therapies that act in a more limited way. Its ability to promote efficient repair in tissues with low perfusion, such as tendons and cartilage, reinforces its therapeutic potential in veterinary medicine and underpins its growing adoption in integrative protocols [31].

Clinical Applications of Ozone Therapy in Veterinary Medicine

Ozone therapy has been consolidated as a relevant therapeutic tool in veterinary medicine, especially due to its ability to modulate inflammation, promote healing and act as a broad-spectrum antimicrobial. In veterinary dermatology, its use stands out in the management of infected wounds, pyoderma, otitis externa and chronic dermatitis. Studies show that ozone, both in ozonated water and in ozonated oil, significantly reduces the bacterial load of Staphylococcus spp., Malassezia spp. and other common pathogens in dogs and cats, favoring the recovery of compromised tissues [32]. In addition, its anti-inflammatory action contributes to the reduction of pruritus, edema and hyperemia, allowing important clinical improvement in cases refractory to conventional treatment.

In the musculoskeletal system, ozone therapy is widely used for the management of joint diseases, such as osteoarthritis, tendinopathies, and myositis. Research reports that intra-articular infiltrations with an oxygen-ozone mixture significantly reduce pain and increase mobility, an effect attributed to the modulation of inflammatory cytokines and the improvement of tissue oxygenation [33]. In equine athletes, the use of ozone has been shown to be effective in the rehabilitation of musculotendinous injuries, accelerating the return to sports activity, probably by improving microcirculation and stimulating the regeneration of muscle fibres.

Veterinary dentistry has largely benefited from the antimicrobial properties of ozone. Topical application of ozonated water or ozone gas to periodontal pockets rapidly reduces microbial load,

aids in the control of periodontal disease, and decreases gingival inflammation. Studies have shown that ozone eliminates anaerobic bacteria associated with periodontal disease more efficiently than chlorhexidine under certain conditions [34]. In felines with chronic stomatitis, the adjuvant use of ozone contributes to reducing oral discomfort and improving the local inflammatory response.

In cases of chronic autoimmune or inflammatory diseases, such as atopic dermatitis, cutaneous lupus and erosive osteoarthritis, ozone therapy acts as an immune modulator. The therapy promotes increased expression of IL-10 and reduction of inflammatory mediators such as TNF-α, IL-1β and IL-6, contributing to the rebalancing of the immune system [35]. This immunomodulatory effect has been explored in dogs and horses, particularly in situations where immunosuppressive drugs cause adverse effects or when there is insufficient response to standard treatment.

In systemic infections, ozone has been studied as an adjuvant to conventional treatment due to its ability to reduce bacterial load, modulate oxidative stress, and improve peripheral oxygenation. In experimental models of sepsis, ozonated autohemotherapy has shown significant improvement in tissue perfusion, lactate reduction, and decreased systemic inflammatory markers [36]. In veterinary medicine, its integrative use has been documented in septicaemia, pyometra, and deep infections, providing complementary support to antibiotic therapy and reducing complications related to septic shock.

In the context of animal rehabilitation and physiotherapy, ozone therapy has been shown to be effective in improving muscle oxygenation, reducing pain and accelerating regeneration. Animals submitted to combined therapies of ozonepuncture, laser therapy, and physiotherapy have reduced recovery times and better functional performance [37]. In chronic lesions, especially of tissues with low vascularization, the combination of ozone and therapeutic exercises promotes increased microcirculation and reduced fibrosis.

The methods of administering veterinary ozone therapy vary according to the clinical condition and the species treated. Among the most used routes are: topical (oil and ozonated water), subcutaneous injection, intra-articular infiltration, rectal ozone, major and minor autohemotherapy and vaginal insufflation, in addition to fluid ozonation. Comparative studies show that efficacy depends directly on the standardization of the concentrations used and strict dose control, since therapeutic and toxic effects are dose-dependent [38]. The topical route is widely used in dermatoses and wounds, while infiltrations are preferred in Orthopedics.

For clinical monitoring, laboratory biomarkers are fundamental tools in monitoring the therapeutic response to ozone. Among the most commonly used parameters are inflammatory cytokines, acute phase markers, lactate, neutrophil/lymphocyte index, and haematological parameters. Studies show that dogs undergoing ozone therapy have gradual normalization of CRP, IL-6, and TNF-α, reflecting a reduction in systemic inflammation [39]. These markers help in the objective evaluation of therapeutic efficacy and adjustment of the clinical protocol.

Inflammatory biomarkers play a crucial role in the evaluation of ozone therapy. The therapy influences both pro-inflammatory (IL-1β, IL-6, TNF-α) and anti-inflammatory (IL-10, TGF-β) mediators, allowing accurate analysis of clinical evolution. In animal models of induced inflammation, ozone has been shown to significantly reduce the expression of IL-1β and TNF-α while increasing IL-10, suggesting potent immunoregulatory capacity [40]. This set of answers is part of the physiological basis of the benefits observed in chronic diseases.

The evaluation of oxidative stress is also essential in the clinical practice of ozone therapy, since its mechanism is based on oxidative hormesis. Studies in dogs treated with oxygen-ozone mixture show reduced malondialdehyde (MDA), increased reduced glutathione (GSH), and elevated antioxidant enzyme activity after a few therapeutic sessions [41]. These parameters allow the evaluation of the redox balance and establish the ideal point of antioxidant adaptation for each patient.

Haematological and biochemical parameters also provide valuable information during therapy. Ozone has been associated with improved erythrocyte profile, increased red blood cell deformability, and reduced lactate, indicating improved tissue oxygenation. In horses, a transient increase in haematocrit and hemoglobin was observed after ozone therapy, reflecting mobilization of reserves and greater availability of oxygen to hypoxic tissues [42]. Liver and kidney parameters are also monitored to ensure safety during prolonged protocols.

Finally, the evaluation of wounds in patients undergoing ozone therapy includes clinical, histological, and microbiological analysis. Studies in dogs and rodents show that ozone accelerates the formation of granulation tissue, promotes angiogenesis, reduces bacterial colonies, and decreases inflammatory infiltrate [43]. The reduction of pain and better local perfusion Favor faster closure of chronic and acute wounds, making ozone a valuable adjuvant in the management of difficult-to-heal lesions in the veterinary clinic.

Discussion

The analysis of the studies gathered in this review demonstrates that ozone therapy has consistent efficacy in different clinical contexts of veterinary medicine, especially in chronic wounds, musculoskeletal diseases, infectious processes, and persistent inflammatory conditions. Its multifactorial action involving immune modulation, stimulation of angiogenesis, increased tissue oxygenation, and antimicrobial activity supports the therapeutic relevance observed in clinical practice. However, despite the growing volume of experimental and clinical studies, there is still significant methodological variability between protocols, which makes direct comparisons and broad generalizations difficult [44].

When comparing different methods of administration and animal species, different clinical responses are observed. In dogs, the topical route with ozonated oils shows high efficacy in skin lesions and otitis, while intravenous infiltrations joint diseases have

better results in joint diseases [33]. In horses, autohemotherapy and the intramuscular route demonstrate better potential for musculoskeletal rehabilitation and reduction of systemic inflammation, possibly due to physiological differences between species and higher metabolic demand [42]. In felines, the dental use of ozone has been shown to be particularly useful in the control of chronic stomatitis. These differences reinforce the need for species-specific protocols.

Among the advantages of ozone therapy, its ability to act simultaneously as an antimicrobial, inflammatory modulator and tissue stimulant, something uncommon in conventional therapies stands out. In addition, it demonstrates low cost, easy handling, and low rate of adverse effects when applied in a standardized way [2]. However, there are important limitations, including the chemical instability of ozone, dependence on appropriate equipment, absence of consensus on optimal concentrations, and lack of global standardization of application protocols. This methodological heterogeneity remains one of the main barriers to its definitive consolidation in clinical guidelines.

Knowledge gaps are also evident in the current literature. Most studies focus on dogs and experimental rodent models, and there is a lack of controlled clinical trials in horses, cattle and alien species. Long-term studies evaluating safety, cumulative toxicity, and impact in different age groups are still limited. In addition, few studies directly compare ozone therapy versus conventional therapies, making it difficult to establish therapeutic superiority relationships [45]. The lack of species-specific pharmacodynamic and pharmacokinetic analyses also limits broader conclusions.

In the laboratory field, one of the challenges identified is the lack of standardization of the biomarkers used in clinical follow-up. The most frequent markers IL-6, TNF-α, IL-10, MDA, and TAC are useful, but there is great variability between studies in relation to laboratory methodology, collection points, and interpretation of results [40]. In order for ozone therapy to reach a higher level of diagnostic reliability, it is essential to standardize measurement protocols, reference values per species, and clinical significance criteria.

The diagnostic implications of ozone therapy are relevant, mainly because its action modifies haematological, oxidative and biochemical parameters. The early identification of therapeutic response through biomarkers aims to optimize individualized protocols, reduce costs, and minimize unnecessary interventions. Techniques such as spectrophotometry, ELISA and chromatography for analysis of oxidative stress and inflammation should be incorporated into clinical practice for efficient monitoring [39]. The routine adoption of these tests may significantly increase therapeutic accuracy.

Clinically, ozone therapy has great potential as an adjuvant therapy, especially in refractory conditions or when the prolonged use of anti-inflammatory drugs and antibiotics represents a risk. The proven reduction of microbial load and inflammatory modulation add value in systemic infections, osteoarthritis, chronic skin diseases, and periodontopathogens [46]. In rehabilitation, its effects on microcirculation and tissue oxygenation contribute to accelerate functional recovery. However, caution is still necessary, as the delimitation between therapeutic and toxic doses requires specific training of the responsible professional.

Therefore, the integration between clinical, laboratory and mechanistic data confirm the relevance of ozone therapy in current veterinary medicine. However, its consolidation depends on scientific advances that overcome methodological gaps, standardize protocols, and establish universal monitoring biomarkers. The expansion of randomized clinical trials and multicentre studies will be decisive to raise ozone therapy to the same level of evidence as traditional therapies, allowing its use in a safer, more effective and targeted way.

Conclusion

The literature analysed in this review demonstrates that ozone therapy represents a multifaceted therapeutic intervention, capable of acting at multiple physiological, biochemical and immunological levels. The studies converge in pointing out its potential in modulating inflammatory cytokines, stimulating endogenous antioxidant defences, promoting angiogenesis, and accelerating tissue regeneration. Evidence from different animal species dogs, cats, horses and experimental models reinforces the consistency of its effects, although there is still methodological heterogeneity between protocols, doses and routes of administration. In general, the literature indicates that ozone therapy is a promising clinical strategy, especially in refractory conditions or those with difficult therapeutic management [42, 47].

From the point of view of real applicability, ozone therapy has proven to be a valuable tool in the contemporary veterinary routine. Its practical use covers several specialties dermatology, dentistry, Orthopedics, physiotherapy, infectious diseases and regenerative medicine, allowing safe and efficient interventions both as monotherapy and as an adjunct to conventional treatment. The extrapolation of its mechanisms to human medicine has already occurred in several countries, where ozone is used in the management of chronic pain, diabetic wounds, resistant infections, and systemic inflammatory disorders [25]. This cross-species correlation is possible due to the evolutionary conservation of redox pathways and inflammatory processes, suggesting that advances in veterinary medicine may directly contribute to translational progress in humans. The strengths of the therapy include its simultaneously antimicrobial, anti-inflammatory, immunomodulatory and repair-stimulating action, something uncommon in traditional approaches. In addition, the low toxicity at therapeutic doses and the affordable cost confer important advantages for veterinary clinical and surgical environments. The ability of ozone to act against multidrug-resistant microorganisms also positions it as a relevant strategy in the face of growing global concern about antimicrobial resistance [11]. However, these forces should be interpreted with caution, considering that many studies still lack standardization and methodological rigor.

In view of the gaps identified, it is recommended that future research prioritize randomized clinical trials, multicentre studies, and standardization of protocols according to species, clinical condition, and route of administration. The definition of specific biomarkers for therapeutic monitoring, pharmacodynamic analysis in different species, and long-term safety assessment are strategic areas for advancing knowledge. It is also essential to expand comparative investigations between ozone therapy and conventional therapies, to determine superiority, equivalence or synergism. The methodological strengthening of the area will allow not only greater scientific acceptance, but also the integration of ozone therapy into clinical guidelines based on both veterinary medicine and translational applications for humans [48].

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Conflicts of Interest

No Conflicts of Interest.

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