



Evaluación de la eficacia del agua enriquecida con ozono en la cicatrización de heridas quirúrgicas en perros

Evaluation of the efficacy of ozone-enriched water in the healing of surgical wounds in dogs

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Keywords: Ozone therapy, Veterinary medicine, Ozonated water, recovery, histopathology

Summary

Introduction: Postoperative wound healing in canines, essential for recovery and prevention of complications, has led to the exploration of innovative therapies, such as ozone. Although human studies support its positive effects, its application in dogs requires further analysis due to physiological differences. This study in Guaranda evaluates ozone-enriched water in surgical wounds in dogs, seeking to improve postoperative protocols and strengthen veterinary practice with scientific evidence in an under-researched area to benefit animal health. **Objective:** To evaluate the efficacy of ozone-enriched water in the healing of surgical wounds in male and female dogs, in the Guaranda Canton, Bolívar Province, at the Pelos JE Veterinary Clinic and Pet Shop. **Methodology:** This study employs a descriptive experimental design carried out at the "de Pelos JE" Veterinary Clinic and Pet Shop in Guaranda during August and September 2023. 40 canine patients were randomly assigned to two groups: one treated with ozonated water and one untreated control. The population included 15 females and 15 males, undergoing ovariohysterectomy and orchiectomy, excluding pre-existing medical conditions or history of adverse reactions to ozone. Surgical procedures followed veterinary standards, with anesthetic protocol and pain assessment using the Glasgow Pain Scale. Tissue samples were collected on the third and seventh day for histopathological analysis. **Results:** The results indicate that the group treated with ozonated water shows a significant presence of fibrous connective tissue (FCT), with 33.3% (10/30) showing an abundant amount and 66.7% (20/30) with a moderate presence. In the control group, we observed a moderate presence of FCT in the majority (90%, 9/10). The statistical data reveal a marked difference ($P < 0.05$) on the seventh day, where the treated group exhibits 76.7% (23/30) with moderate neovascularization and 13.3% (4/30) with an abundant presence. In comparison, the control group shows 60% (6/10) with mild neovascularization and 40% (4/10) with moderate neovascularization. Regarding inflammation, 26.7% (8/30) of the treated group showed no inflammation and 73.3% (22/30) showed mild inflammation. In contrast, all patients in the control group showed mild inflammation. Regarding healing, 70% (21/30) of the treated group showed complete

healing at 6 days, 30% (9/30) at 5 days, while the control group achieved adequate healing at 8 days in 100% of cases. **Conclusion:** The application of ozone-enriched water significantly accelerated the healing process, improving the organization of collagen and other tissue components compared to the control group. In addition, an effective reduction of inflammation in surgical wounds was observed, indicating a decrease in the inflammatory reaction of the surrounding tissue. These results suggest a positive and potentially beneficial impact of ozonized water on the speed of recovery.

Keywords:

Ozonotherapy,
Veterinary,
Ozonized water,
Recovery,
Histopathology

Abstract

Introduction: Postoperative wound healing in dogs, crucial for recovery and complication prevention, has prompted exploration of innovative therapies like ozone. While human studies support its positive effects, applying ozone in dogs demands a deeper analysis due to physiological differences. This study in Guaranda assesses ozone-enriched water's impact on surgical wounds in dogs, aiming to enhance postoperative protocols and strengthen veterinary practice with scientific evidence in an underexplored area for the benefit of animal health. **Objective:** Evaluate the efficacy of ozone-enriched water in the healing of surgical wounds in male and female dogs in the Canton of Guaranda, Bolivar Province, at the Veterinary Clinic "de Pelos JE" and Pet Shop. **Methodology:** This study employed a descriptive experimental design conducted at the Veterinary Clinic "de Pelos JE" and Pet Shop in Guaranda during August and September 2023. Forty canine patients were randomly assigned to two groups: one treated with ozonated water and another control group without treatment. The population included 15 females and 15 males undergoing ovariohysterectomy and orchiectomy, excluding pre-existing medical conditions or a history of adverse reactions to ozone. Surgical procedures followed veterinary standards, with anesthetic protocol and pain evaluation using the Glasgow Pain Scale. Tissue samples were collected on the third and seventh days for histopathological analysis. **Results:** The results indicate that the group treated with ozonated water shows a significant presence of fibrous connective tissue (FCT), with 33.3% (10/30) exhibiting an abundant amount and 66.7% (20/10) with a

moderate presence. In the control group, we observed a moderate presence of FCT in the majority (90%, 9/10). Statistical data reveal a marked difference ($P < 0.05$) on the seventh day, where the treated group exhibits 76.7% (23/30) with moderate neovascularization and 13.3% (4/30) with an abundant presence. In comparison, the control group shows 60% (6/10) with mild neovascularization and 40% (4/10) with moderate neovascularization. Regarding inflammation, 26.7% (8/30) of the treated group shows absence, and 73.3% (22/30) shows mild inflammation. In contrast, all patients in the control group exhibit mild inflammation. As for healing, 70% (21/30) of the treated group shows comprehensive healing at 6 days, 30% (9/30) at 5 days, while the control group achieves adequate healing by the 8th day in 100% of cases. Conclusion: The use of ozone-enriched water significantly accelerated the healing process, enhancing the organization of collagen and other tissue components compared to the control group. Additionally, there was an effective reduction in inflammation in surgical wounds, indicating a decrease in the inflammatory response in the surrounding tissue. These results suggest a positive and potentially beneficial impact of ozonized water on the speed of recovery.

Introduction

According to Odani et al. (2022), Surgical wound healing in canines is a critical element in postoperative recovery and in the prevention of post-surgical complications; various approaches and therapies have been explored in the constant search for methods to improve this fundamental process. As stated by Kosachenco et al. (2018), and in this context, the use of ozone has emerged as a potentially beneficial agent, thanks to its antimicrobial, anti-inflammatory and healing-promoting properties. Although significant advances have been documented in the application of ozone in human medicine, its effectiveness in the veterinary field, particularly in dogs, requires further examination.

According to Carreno (2018) Previous studies have highlighted the positive effects of ozone on wound healing in humans, supported by studies that underline its ability to improve local oxygenation, reduce bacterial load and modulate the inflammatory response; however, the direct extrapolation of these results to veterinary practice should

be approached with caution, given that the biological characteristics and physiological responses of dogs differ significantly from those of humans.

This work is located in the Guaranda Canton, Bolívar Province, specifically in the Veterinary Clinic "de Pelos JE" and Pet Shop, with the purpose of evaluating the effectiveness of ozone-enriched water in the healing of surgical wounds in male and female dogs undergoing ovariohysterectomy and orchiectomy, respectively. The relevance of this research lies in the need to optimize postoperative management protocols in veterinary practice, providing scientific evidence on the applicability of ozone in this specific context.

The scientific problem addressed is focused on the lack of comprehensive studies supporting the use of ozone in the healing of surgical wounds in dogs, as well as the limited information available on its possible effects in this group of patients. The justification for carrying out this study is based on the clinical relevance of improving postoperative healing, which would not only contribute to the well-being of animals, but would also strengthen veterinary practice with innovative approaches.

In this context, the main objective of the present research is to systematically evaluate the efficacy of ozone-enriched water in healing surgical wounds in male and female dogs, thus providing a valuable contribution to the field of veterinary medicine.

Wound healing in canines

It is a sequential process that seeks to restore the integrity of the affected tissue, it begins with hemostasis, stopping blood loss by forming a clot; in the inflammation phase, inflammatory cells remove debris and prepare the site for repair, proliferation involves collagen synthesis and the formation of new blood vessels, while remodeling strengthens scar tissue and any alteration in these phases can negatively affect healing; this understanding serves as a basis for investigating the impact of ozone-enriched water on this process, seeking to improve efficacy and reduce complications in surgical wounds in dogs.(Perdomo et al., 2018).

According to Benavides et al. (2018), in the case of ovariohysterectomy and orchiectomy, the wound resulting from these surgeries must undergo adequate healing to prevent complications such as infection and dehiscence. Assessment of healing involves observing the integrity of the incision, the absence of signs of infection, minimal inflammation, and the formation of healthy scar tissue. Implementation of measures to accelerate and improve the healing process becomes an area of interest.

Factors that influence healing

Taking into account Lima et al. (2016) Wound healing in dogs is influenced by a number of intrinsic and extrinsic factors; internally, the dog's age, genetics and general health play a critical role, whilst external factors such as nutrition and exposure to infections are also essential; medical conditions, such as diabetes, can compromise wound healing, and a balanced diet is crucial for cell development and collagen synthesis.

As stated Santos et al. (2009), in the case of surgical wounds, specific considerations arise, the surgical intervention alters the anatomy of the tissue, affecting the biological response. From the point of view of Martinez (2019), factors such as the choice of surgical technique, the quality of the suture, such as polyglycolic acid which synthetic coating, composed of polycaprolactone and calcium stearate, gives it a unique combination of strength and flexibility, facilitating its passage through the tissues and guaranteeing ease, precision and safety in the knotting process, as well as asepsis in the operating room are crucial elements that can influence the healing process, understanding these factors is essential to evaluate the effectiveness of interventions such as ozone-enriched water in improving healing in dogs, especially in the context of surgical wounds.

Properties of Ozone

Quoting Alvarez et al. (2021) Ozone, a gas composed of three oxygen atoms (O₃), exhibits remarkable physical and chemical properties that have sparked interest in the medical field; from a physical perspective, ozone is a powerful oxidant and disinfectant, capable of interacting with organic and inorganic molecules; chemically, its unique molecular structure confers antimicrobial, anti-inflammatory and antioxidant properties.

According to Carreno (2018), scientific literature has extensively documented the use of ozone in human and veterinary medicine; in humans, its application has been explored in a variety of conditions, from chronic wounds to systemic diseases; in the veterinary field, studies have examined its efficacy in improving wound healing in different species.

The proposed mechanisms of action of ozone in wound healing encompass several biological dimensions. It is suggested that ozone modulates the inflammatory response, reducing the release of proinflammatory mediators. (Carreno, 2018). Also according to Castro (2022), is postulated to improve local oxygenation, stimulating angiogenesis and facilitating the delivery of essential nutrients to cells at the wound site, these combined effects could contribute to accelerate the healing process and improve the quality of the repaired tissue, understanding these properties and underlying mechanisms supports the evaluation of the efficacy of ozone-enriched water in healing surgical wounds in dogs.

Previous studies on ozone in wound healing

Previous research has explored the impact of ozone on wound healing, both in human and animal contexts. Studies in human medicine have pointed to the potential benefits of

ozone in improving wound healing, highlighting its ability to reduce bacterial load, modulate inflammatory response and stimulate regenerative processes.(Castro, 2022).

From the position ofHernandez et al. (2016)In the veterinary field, research has been conducted examining the effects of ozone on various species. These studies have addressed both traumatic and surgical wounds, seeking to evaluate the influence of ozone on the speed and quality of healing.

It is relevant to highlight important findings from previous studies, such as the acceleration in wound resolution and the improvement in scar tissue quality. However, limitations have also been pointed out, such as the variability in results between studies and the need to address species-specific aspects.(Mateus, 2020).

As Mateus (2020) points out, the compilation of this previous research provides essential context for evaluating the efficacy of ozone-enriched water in healing surgical wounds in dogs, allowing for a more complete understanding of the potential benefits and challenges associated with this intervention.

Healing of surgical wounds in canines and current protocols

According toPacheco et al. (2019), sConventional protocols covering key aspects of postoperative management have been established. These protocols include the choice of appropriate surgical techniques, suture quality, and postoperative care measures such as the administration of analgesics and antibiotics.

From the position ofPeña et al. (2017),The comparison of these results contributes to the understanding of the relative efficacy of conventional protocols, identifying areas where they can be improved. This analysis contextualizes the evaluation of the impact of ozone-enriched water on surgical wound healing in dogs, providing a basis for understanding how the application of ozone can complement or improve existing protocols.

Mechanisms of action of ozone in healing

Ozone intervenes in specific cellular and molecular processes to improve the regenerative process; its participation focuses on key modulations that positively impact the effectiveness of the healing process.(Cofré et al., 2021).

As it is proposedSparrowhawk (2022), sIt is postulated that ozone can modulate the inflammatory response, reducing the release of proinflammatory mediators and limiting associated tissue damage. In addition, it is believed to improve local oxygenation at the wound site, stimulating angiogenesis and facilitating the delivery of essential nutrients to growing cells. This combined action could accelerate tissue repair and improve the quality of the healing process.

Ozone can affect the composition of the extracellular matrix, which plays a key role in the structure and function of scar tissue. Modifying the extracellular matrix could positively influence the quality of healing, as well as being able to stimulate the release of growth factors, which are essential proteins for cell regeneration and proliferation.(Diaz & Castellanos, 2001).

Ethical and safety considerations in the use of ozone

When applying ozone to animals, it is crucial to address ethical considerations to ensure the well-being of canine patients. The ethical discussion focuses on ensuring that the ozone intervention is beneficial, respectful and does not cause any unnecessary harm to the animals. This involves considering the real need for ozone treatment, ensuring informed consent from owners and addressing any concerns related to animal welfare.(Vidal et al., 2009).

In terms of safety, it is essential to carefully review the possible side effects associated with the use of ozone in dogs. According toPerez et al. (2015),Although ozone, when administered appropriately, can be safe, the potential risks need to be fully understood, with possible side effects including localized irritation, allergic reactions, and changes in the normal microbiota. From the position ofCofré et al. (2021), the safety review is carried out with the aim of minimising any risks and ensuring that the benefits of ozone treatment significantly outweigh any potential risks to animal health.

Ethical consideration and thorough safety assessment are essential elements when exploring the application of ozone in surgical wound healing in dogs, ensuring the integrity and ethical care of animal patients involved in the research.(Hidalgo & Torres, 2013).

Methodology

The research adopted an experimental design with a descriptive approach, carried out at the Veterinary Clinic "de Pelos JE" and Pet Shop in Guaranda, during the period between August and September 2023. 40 canine patients were randomly assigned to two groups: one treated with ozonated water and another control group without ozone treatment. The study population included 15 females and 15 males, undergoing ovariohysterectomy and orchietomy, respectively. In addition, 5 males and 5 females were designated as controls without ozone application. Patients classified as ASA I were included and those with pre-existing medical conditions or history of adverse reactions to ozone were excluded. Surgical procedures followed veterinary standards, applying the anesthetic protocol and evaluating pain using the modified Glasgow pain scale. Tissue samples were taken on the third and seventh day for histopathological analysis.

Results

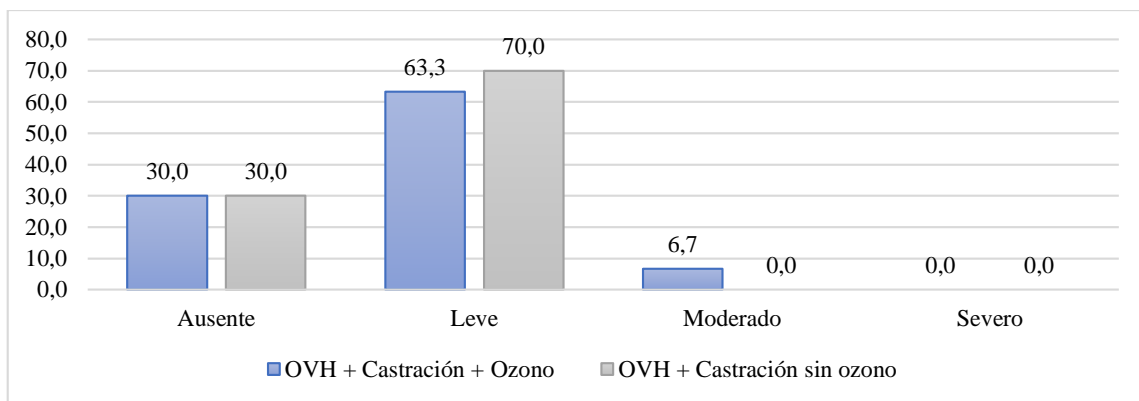
During the study period, 40 patients undergoing surgical procedures were evaluated according to the methodology used, yielding the results shown below:

Fibrous connective tissue

Regarding the fibrous connective tissue observed in the histopathological study, we observed that for the sampling on the third day post-surgery, for the group of surgical patients treated with ozonated water, 63.3% (19/30) of patients had a slight presence of fibrous connective tissue, while the control group presented 70% (7/10) of patients with a slight presence of fibrous tissue, giving us to notice that no marked statistical difference was found ($P > 0.05$) in the sampling on the third day.

Figure 1

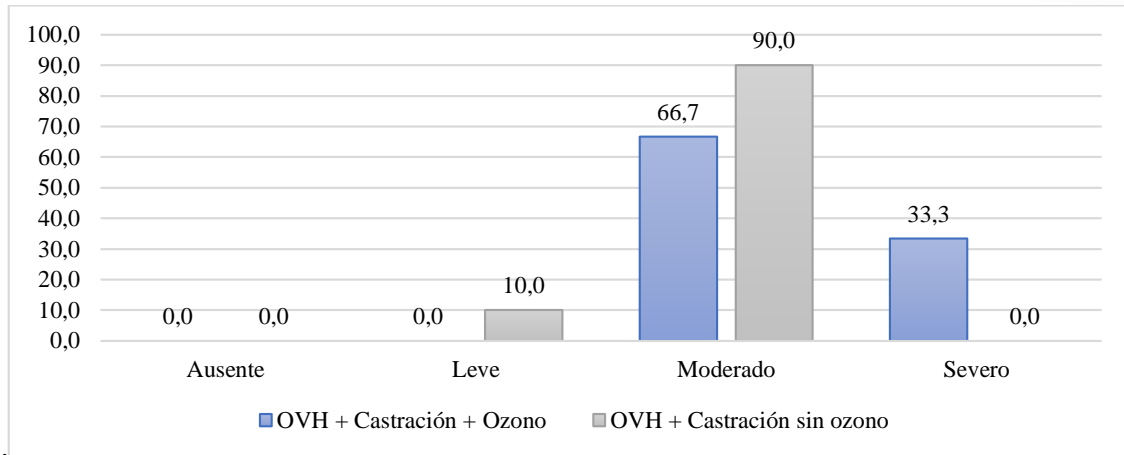
Fibrous connective tissue (3rd day)



For the seventh day sample we obtained results that indicate the presence of a large amount of fibrous connective tissue for 33.3% (10/30) and 66.7% (20/10) with moderate presence of TCF for the study group subjected to treatment with ozonated water; while for the control group we did not observe a large presence of TCF and the majority (9/10) that is, 90% presented a moderate presence of fibrous connective tissue in the vicinity of the wound, allowing us to notice the marked statistical difference ($P < 0.05$) in the intake on the seventh day.

Figure 2

Fibrous connective tissue (7th day)

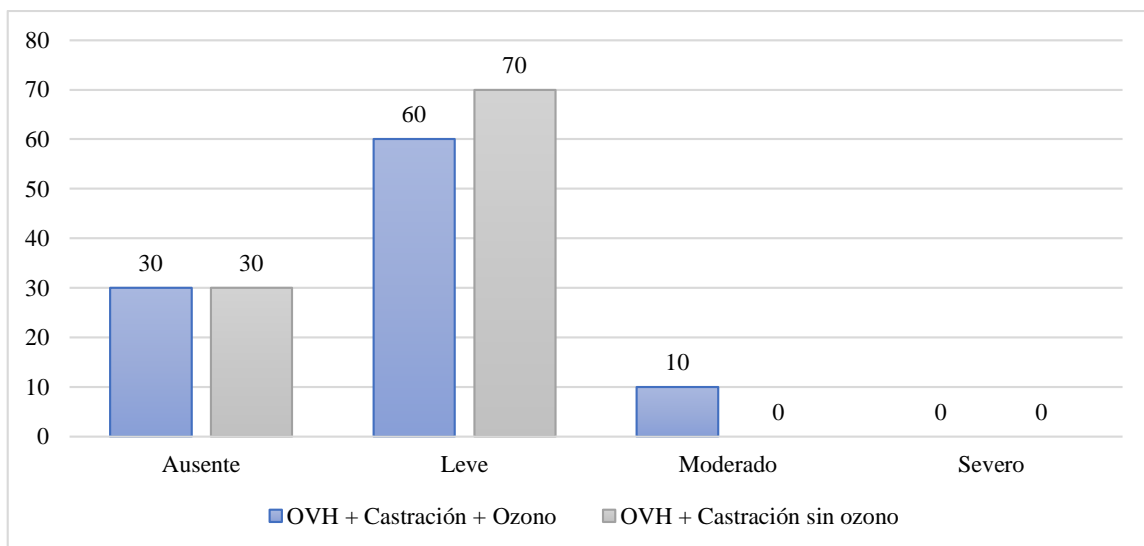


Neovascularization

Regarding neovascularization, to measure the effectiveness of healing and the progress of healing, we observed that, from the third intake, the group treated with ozonated water began to show greater formation of blood vessels with 10% (3/30) with a moderate presence of blood vessels, and the majority 60% (18/30) with a slight presence of blood vessels; for the control group, 70% (7/10) presented slight neovascularization and 30% (3/10) had no neovascularization.

Figure 3

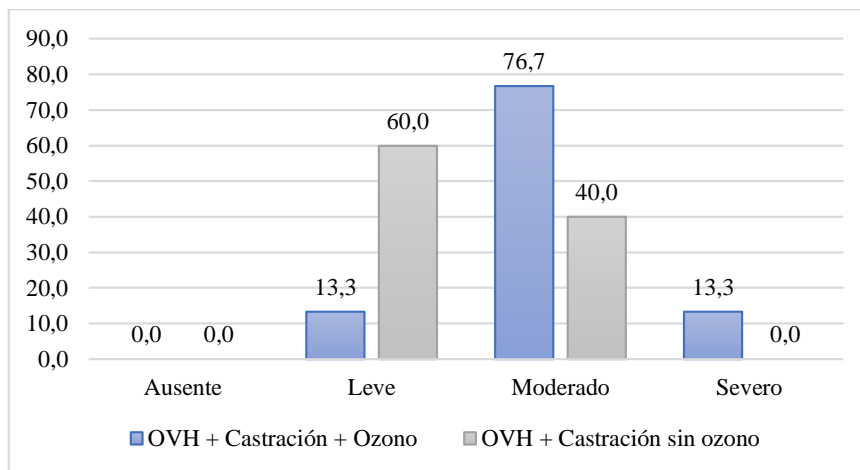
Neovascularization (3rd day)



The statistical value data, where it was found a marked statistical difference ($P < 0.05$) was observed on the seventh day of the study, finding a contrast between the two groups under study; observing the difference between the presence and formation of new blood vessels at the site of the surgical procedure; in the group treated with ozonated water, 76.7% (23/30) exhibited a moderate presence of neovascularization and 13.3% (4/30) an abundant presence of this; on the other hand, the control group, mostly 60% (6/10) showed a mild neovascularization, while the remaining 40% (4/10) exhibited a moderate presence of localized blood vessels.

Figure 4

Neovascularization (7th day)



Re-epithelialization

Revitalization in a wound refers to the process in which epithelial cells migrate and proliferate to cover the wound surface and restore the skin barrier, being important to evaluate the speed and effectiveness of the healing process; as for this tangible value, as for the third and seventh day intake, no data were found. a marked statistical difference ($P > 0.05$) since the values obtained for the group treated with ozonized water and the control group behaved statistically similar to each other.

Figure 5

Re-epithelialization (3rd day)

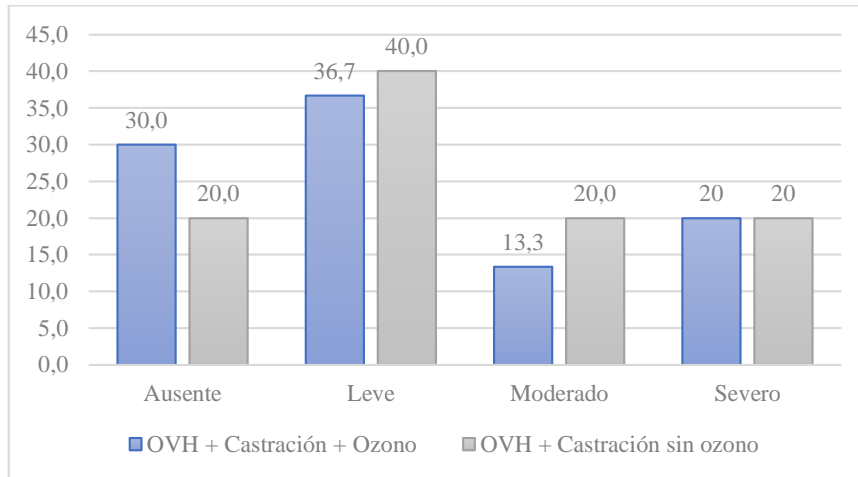
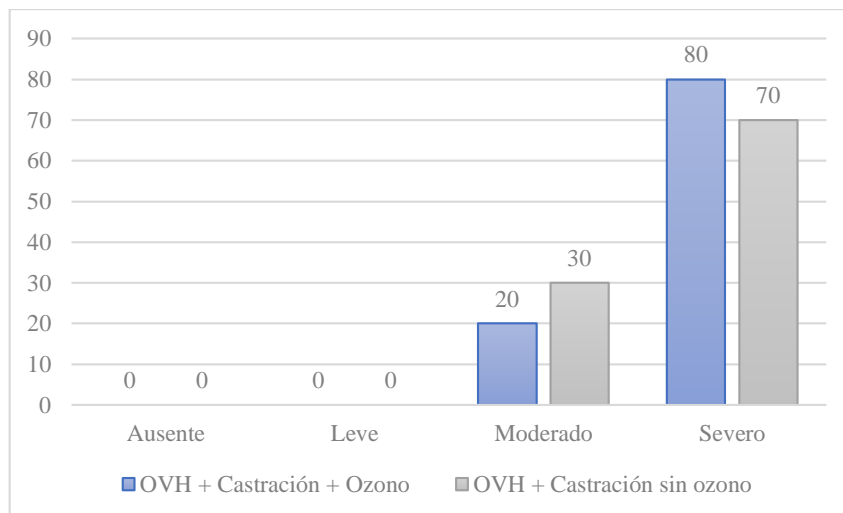


Figure 6

Reepithelialization (7th day)

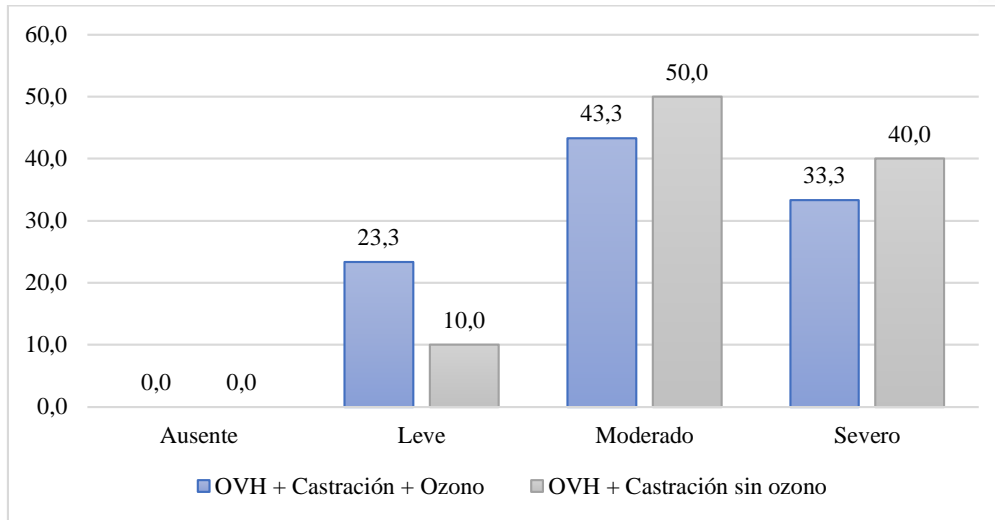


Inflammation

Inflammation in a wound is a crucial component of the body's response to tissue damage. Measuring inflammation is essential to assess the progression and effectiveness of the healing process; in the histopathological analysis, on the third day for both the group treated with ozonated water and the control group, we observed that no inflammation was found. marked statistical difference ($P > 0.05$), observing a similar behavior.

Figure 7

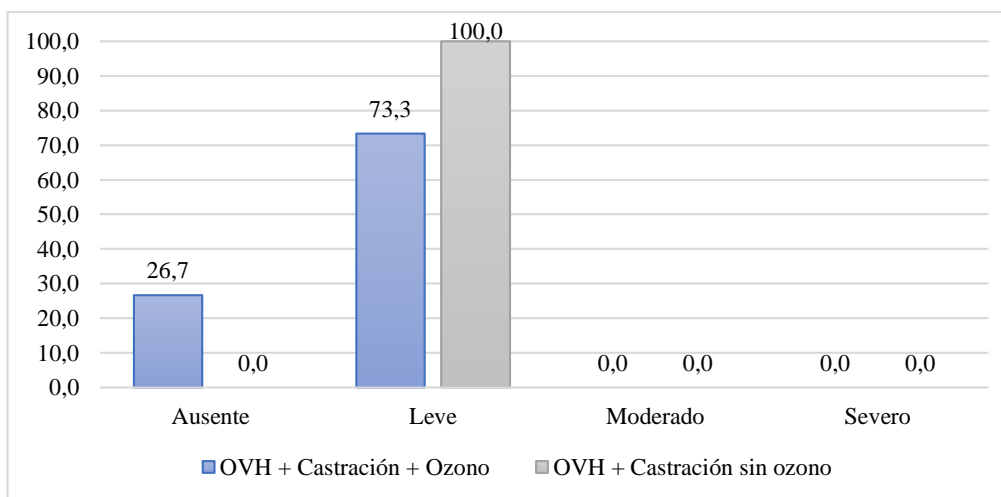
Inflammation (3rd day)



While for the seventh day sample, interesting data were observed; the group treated with ozonated water, presented 26.7% (8/30) of surgical patients, with no inflammation and 73.3% (22/30) with mild inflammation located at the wound site; while for the control group, all patients (100%) presented mild inflammation at the time of the histopathological sample. Let us notice the marked statistical difference ($P < 0.05$) in the sample on the seventh day.

Figure 8

Inflammation (7th day)

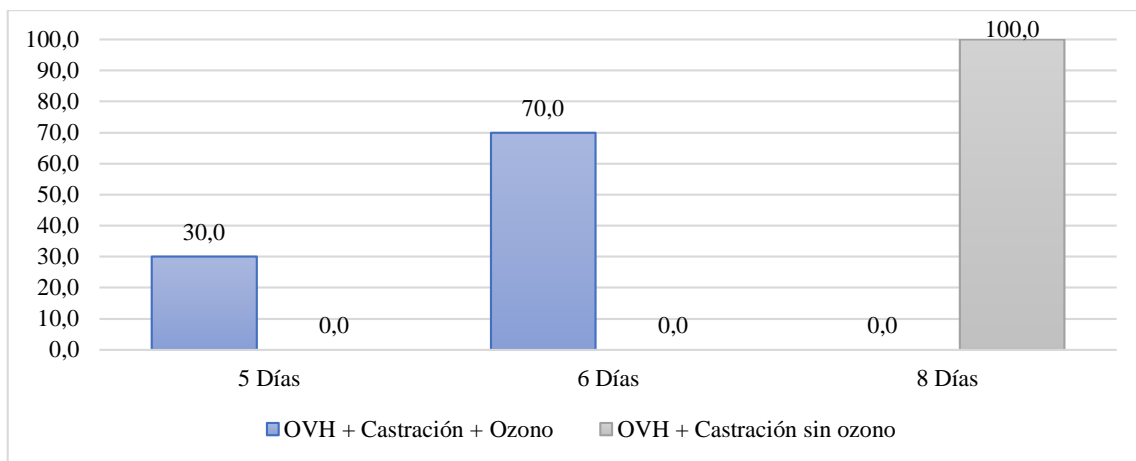


Healing days

For the days of healing we took into account the period of time determined from the surgical procedure to the actual healing, taking into account the parameters mentioned above in the histopathological study; as for the study group treated with ozonated water, 70% of surgical patients (21/30) presented complete healing 6 days after the procedure; 30% (9/30) at 5 days and finally; for the control group, without the use of ozonated water as an adjuvant in the healing of surgical wounds, 100% presented appropriate healing 8 days after the operation.

Figure 9

Healing days



Discussion

Taken together, the results obtained suggest that ozone-enriched water may be a promising tool in the management of surgical wound healing in dogs, offering benefits in terms of accelerating the process, reducing inflammation, improving scar tissue quality, and preventing infections. These findings support the need for further research and may have important implications in veterinary clinical practice (Peteoaca et al., 2020).

Regarding the data obtained in the histopathological study, we observed that, for the formation of fibrous connective tissue, neovascularization and inflammation; the post-surgical treatment of the wound with ozonated water presented a positive effect compared to the control group; regarding tissue reepithelialization, there was no marked effect in comparison; it is important to clarify whether the method of application of ozonated water influenced the results obtained.

For the days of healing, we observed a decrease in the wound healing time compared to the control group; although other variables such as wound size, pain, presence of infection in addition to wound care, nutritional factors, and age of the patient must also be taken into account.(Anguieta, 2023).

Conclusions

Based on the significant data obtained in the research we can reach the following conclusions:

- The study group treated with ozone-enriched water showed a significant acceleration in the healing process compared to the control group, as demonstrated by the histopathological results obtained for the formation of fibrous connective tissue, as well as collagen and connective fibers themselves; the organization of collagen and other tissue components suggests a positive influence on the formation of a more functional scar.
- The application of ozonized water proved to be effective in reducing inflammation in surgical wounds, this may indicate anti-inflammatory properties of ozone, which contributes to a more favorable environment for healing, the comparison made between the groups in study suggests a decrease in the inflammatory reaction of the surrounding tissue of the surgical lesion.
- The reduction in time required for complete healing suggests a positive and potentially beneficial impact of ozone-enriched water on recovery speed; the antimicrobial properties, reduction of inflammation and stimulation of angiogenesis, could improve the supply of nutrients and oxygen to the wound site, thus promoting healing and reducing wound healing time to a minimum.
- Thus demonstrating the beneficial properties of ozone and its interaction with biological healing processes.

Conflict of interest

The authors declare that there is no conflict of interest.

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