

**Is ozone effective in reducing pain, edema, and trismus after third molar surgery? A  
systematic review**

**O ozônio é eficaz em reduzir dor, edema e trismo após cirurgia de terceiro molar? Uma  
revisão sistemática e meta-análise**

**¿El ozono es efectivo para reducir dolor, edema y trismo tras una cirugía de tercer  
molar? Una revisión sistémica**

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### **Abstract**

This study aimed to evaluate the efficacy of ozone as a supporting therapy in reducing pain, edema, and trismus after lower third molar extraction. The protocol was registered in PROSPERO. Six electronic databases (PubMed, Scopus, LILACS, SciELO, Embase, and Web of Science) were used. Only randomized clinical trials were included, without restriction of year, language, and publication status. The JBI tool was used to assess the risk of bias. The GRADE approach assessed the certainty of evidence. The search yielded 3386 results, from which only three articles were eligible. The studies were published between the years 2013 and 2017, resulting in a sample of 133 patients. Ozone was used in the form of gas or gel. All studies found significant results for pain reduction after one, three, and seven days. Success in reducing trismus and edema varied between studies. The risk of bias varied between moderate and low. All outcomes were classified as a very low level of certainty. Although presenting favorable results for pain reduction, there is insufficient evidence to indicate the use of ozone as a complementary therapy during the extraction of third molars.

**Keywords:** Edema; Ozone; Trismus; Pain.

### **Resumo**

Este estudo teve como objetivo avaliar a eficácia do ozônio como terapia complementar na redução da dor, edema e trismo após a extração do terceiro molar inferior. O protocolo foi registrado no PROSPERO. Foram utilizadas seis bases de dados eletrônicas (PubMed, Scopus, LILACS, SciELO, Embase e Web of Science). Apenas ensaios clínicos randomizados foram incluídos, sem restrição de ano, idioma e status de publicação. A ferramenta JBI foi usada para avaliar o risco de viés. A abordagem GRADE avaliou a certeza das evidências. A busca resultou em 3.386 resultados, dos quais apenas três artigos foram elegíveis. Os estudos foram publicados entre os anos de 2013 e 2017, resultando em uma

amostra de 133 pacientes. O ozônio foi usado na forma de gás ou gel. Todos os estudos encontraram resultados significativos para redução da dor após um, três e sete dias. O sucesso na redução do trismo e edema variaram entre os estudos. O risco de viés variou entre moderado e baixo. Todos os resultados foram classificados como um nível de certeza muito baixo. Apesar de apresentar resultados favoráveis para redução da dor, não há evidências suficientes para indicar o uso do ozônio como terapia complementar durante a exodontia de terceiros molares.

**Palavras-chave:** Dor; Edema; Ozonio; Trismo.

### **Resumen**

Este estudio tuvo como objetivo evaluar la eficacia del ozono como terapia complementaria en la reducción del dolor, edema y trismo tras la extracción del tercer molar inferior. El protocolo se registró en el PROSPERO. Se utilizaron seis bases de datos electrónicas (PubMed, Scopus, LILACS, SciELO, Embase y Web of Science). Solo ensayos clínicos aleatorizados fueron incluidos, sin restricción de año, idioma y estatus de publicación. La herramienta JBI se usó para evaluar el riesgo de sesgo. El abordaje GRADE analizó la certeza de las evidencias. La búsqueda generó 3.386 resultados, de los cuales solo tres artículos eran elegibles. Los estudios fueron publicados entre los años 2013 y 2017, originando una muestra de 133 pacientes. El ozono se usó en forma de gas o gel. Todos los estudios encontraron resultados significativos para la reducción del dolor tras uno, tres y siete días. El éxito en la reducción del trismo y edema varió entre los estudios. El riesgo de sesgo varió de moderado a bajo. Todos los resultados fueron clasificados como un nivel de certeza muy bajo. Apesar de presentar resultados favorables la para reducción del dolor, no hay evidencias suficientes para indicar el uso do ozono como terapia complementaria durante la exodoncia de terceros molares.

**Palabras clave:** Dolor; Edema; Ozono; Trismo.

### **1. Introduction**

Lower third molar extraction is often associated with postoperative complications from the inflammatory process (Lago-Méndez et al., 2007). Symptoms such as pain, edema, and trismus relate to the complexity of the surgical procedure and individual characteristics of the patients (Pell and Gregory, 1993). In general, painful symptoms after third molar removal are acute and may vary from moderate to severe (Barden et al., 2004). Pain reaches maximum

intensity 5–6 h after the surgical procedure and it remains for approximately two days, gradually decreasing until the seventh day and presenting a negative impact on the quality of life of patients during this period (Chuang et al., 2008).

Alternative therapies for controlling complications after impacted lower third molar extraction, such as cryotherapy (Libonati et al., 2019), low-level laser therapy (Bittencourt et al., 2017), and ozone therapy (Osunde et al., 2014; Ahmedi et al., 2016) are acknowledged. Ozone can be administered parenterally or topically (Bocci, 2006) in the form of gas, gel, or liquid (Sivalingam et al., 2017). The therapeutic efficacy of ozone therapy may be partly due to the controlled oxidative stress produced by the reactions of ozone with several biological components. In optimal doses, ozone can react with blood components and affect positively oxygen metabolism and cell energy, activating antioxidant defense systems (Bocci, 2004). In dentistry, ozone therapy has been used to treat caries (Lim and Ngeow, 2017), endodontic (Ajeti et al., 2018) and periodontal diseases (Walker et al., 1995), and temporomandibular joint dysfunction (Dray, 1995; Domb, 2014). Moreover, this therapy has been used during maxillofacial surgery to promote hemostasis, enhance local oxygen supply (Bianco et al., 2019), and minimize postoperative discomfort (Wang et al., 2018).

Despite the versatility of ozone therapy, the clinical results obtained in the literature are controversial regarding the reduction of postoperative complications after third molar extraction. Thus, this systematic review aims to answer the following question: Is local ozone useful for controlling pain, edema, and trismus after impacted third molar surgery?

## **2. Material and Methods**

This is a systematic review performed according to the Cochrane Collaboration (Higgins et al., 2019) guidelines for systematic reviews and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Statement (Moher et al., 2009). The research protocol was registered in the Prospective International Register of Systematic Reviews (PROSPERO) (CRD # 42019134207).

### **2.1 Eligibility criteria**

The systematic review was designed to answer the guiding question, based on the PICO strategy: Population (individuals submitted to lower third molar extraction);

Intervention (ozone therapy); Control (individuals not undergoing ozone therapy); Outcome (pain, edema, and trismus).

Only randomized controlled trials evaluating the influence of postoperative ozone therapy on pain, edema, and trismus after lower third molar surgery were included, even if assessing only one of the outcomes. The quantitative data available were obtained by evaluating impacted third molars of similar difficulty according to the classification proposed by Pell and Gregory. The search was unrestricted for year and language.

The following were excluded: 1) Studies outside the objective; 2) Review studies, case reports, brief communications, observational studies, editorials or letters to the editor, monographs, conference summaries, and book/book chapters; 3) Studies including teeth other than third molars; 4) Studies including patients under 18 years old.

## **2.2 Search strategy**

The primary sources were Embase, Latin American and Caribbean Health Sciences Literature (LILACS), PubMed (including MEDLINE), SciELO, Scopus, and Web of Science databases. Open Gray and OATD were used to partially capture the “gray literature”. Additionally, a manual search was performed in the references of the eligible articles after the electronic search. All steps were performed to minimize selection and publication biases.

The MeSH (Medical Subject Headings), DeCS (Health Sciences Descriptors), and Emtree (Embase Subject Headings) resources were used to select search descriptors according to the specificity of each database (Table 1). The bibliographic search was performed in February 2019. The results obtained were exported to the EndNote Web™ software (Thomson Reuters™, Toronto, Canada), in which duplicates were automatically removed. The remaining results were exported to Microsoft Word™ 2010 (Microsoft™ Ltd, Washington, USA) and the remaining duplicates were removed manually.

**Table 1.** Strategies for database search.

Database	Search Strategy (February, 2019)
<b>PubMed</b> <a href="http://www.ncbi.nlm.nih.gov/pubmed">http://www.ncbi.nlm.nih.gov/pubmed</a>	((("Somatosensory Disorders" OR "Neurosensory Disorders" OR "Pain" OR "Edema" OR "Swelling" OR "Trismus" OR "Mouth Opening") AND ("Ozone" OR "Ozonotherapy" OR "O3" OR "Ozone Therapy")))
<b>Scopus</b> <a href="http://www.scopus.com/">http://www.scopus.com/</a>	((("Somatosensory Disorders" OR "Neurosensory Disorders" OR "Pain" OR "Edema" OR "Swelling" OR "Trismus" OR "Mouth Opening") AND ("Ozone" OR "Ozonotherapy" OR "O3" OR "Ozone Therapy")))
<b>LILACS</b> <a href="http://lilacs.bvsalud.org/">http://lilacs.bvsalud.org/</a>	tw:(pain AND ozone) AND (instance:"regional") AND ( db:("LILACS")) Trismus AND Ozone tw:(swelling AND ozone) AND (instance:"regional") AND ( db:("LILACS")) edema AND ozone AND (instance:"regional") AND ( db:("LILACS")) tw:(dolor AND ozono) AND (instance:"regional") AND ( db:("LILACS")) [Spain] Trismo AND Ozono [Spain] tw:(edema AND ozono) AND (instance:"regional") AND ( db:("LILACS")) [Spain]
<b>SciELO</b> <a href="http://www.scielo.org/">http://www.scielo.org/</a>	Pain AND Ozone Trismus AND Ozone Swelling AND Ozone Edema AND Ozone Dolor AND Ozono [Spain] Trismo AND Ozono [Spain] Edema AND Ozono [Spain]
<b>Embase</b> <a href="http://www.embase.com">http://www.embase.com</a>	('somatosensory disorders'/exp OR 'somatosensory disorders' OR 'neurosensory disorders' OR 'pain'/exp OR 'pain' OR 'edema'/exp OR 'edema' OR 'swelling'/exp OR 'swelling' OR 'trismus'/exp OR 'trismus' OR 'mouth opening'/exp OR 'mouth opening') AND ('ozone'/exp OR 'ozone' OR 'ozonotherapy' OR 'o3' OR 'ozone therapy'/exp OR 'ozone therapy')
<b>Web Of Science</b> <a href="http://apps.webofknowledge.com/">http://apps.webofknowledge.com/</a>	((("Somatosensory Disorders" OR "Neurosensory Disorders" OR "Pain" OR "Edema" OR "Swelling" OR "Trismus" OR "Mouth Opening") AND ("Ozone" OR "Ozonotherapy" OR "O3" OR "Ozone Therapy")))
<b>OpenGrey</b> <a href="http://www.opengrey.eu/">http://www.opengrey.eu/</a>	Pain AND Ozone Trismus AND Ozone Swelling AND Ozone Edema AND Ozone
<b>Open Access Theses and Dissertations (OATD)</b> <a href="https://oatd.org/">https://oatd.org/</a>	Pain AND Ozone Trismus AND Ozone Swelling AND Ozone Edema AND Ozone

Source: Elaborated by the authors (2020).

In Table 1 is shown the descriptors used to perform the search in the electronic database. It is important to notice that each strategy was adequate for the respective database.

### 2.3 Study selection

First, as a calibration exercise, three reviewers discussed the eligibility criteria and applied them to 20% of the sample to determine inter-examiner agreement. After obtaining an adequate level of agreement ( $Kappa \geq 0.81$ ), the studies were selected in two moments and two eligibility reviewers (RPS and VLA) methodically reviewed the titles and abstracts, independently. These reviewers were not blinded to the names of authors and journals. Studies that did not answer the research question were deleted at this time. Studies whose

titles corresponded to the study objectives but did not have abstracts available were fully analyzed.

In the second stage, the preliminary eligible studies had their full texts obtained and evaluated to verify whether they met the eligibility criteria. When both reviewers could not agree, a third one (LRP) was consulted to make a final decision. The studies rejected were recorded separately, explaining the reasons for exclusion.

## **2.4 Data extraction and assessment of the risk of bias**

Two reviewers extracted the data from the articles, independently. The following information was collected: authors, country and year of publication, sample number, average age, dental position classification, anesthetic solution used, surgery time, postoperative drug protocol, ozone administration method, postoperative pain evaluation method, mouth opening evaluation method, and edema assessment method.

The risk of bias was assessed using the JBI Critical Appraisal Checklist for Randomized Controlled Trials tool (Tufanaru et al., 2017). Two authors assessed independently each study according to the PRISMA recommendations (Moher et al., 2009). Each study was categorized according to the percentage of positive answers obtained with the assessment tool. The risk of bias was considered high when the study obtained up to 49% of "yes" answers; moderate when the study obtained 50% to 69% of "yes" answers, and low when the study had more than 70% of "yes" answers.

In all phases, any disagreement between the reviewers was solved by discussing the items evaluated and, when they could not reach an agreement, a third reviewer was consulted to make a final decision.

## **2.5 Data analyses**

The data collection process was performed with an analysis of the studies selected and the result was presented in a descriptive/narrative manner, analyzing the methodological heterogeneity of the eligible studies. A meta-analysis was planned in case the data from the eligible studies were homogeneous.

## **2.6 Certainty of evidence**

The certainty of evidence and strength of recommendation were assessed with the Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) approach (Murad et al., 2017). The GRADE pro-GDT software (<http://gdt.guidelinedevelopment.org>) was used for summarizing the results. According to this system, randomized clinical trials start at a high level of evidence and can be downgraded based on study design, methodological limitations, inconsistency, indirect evidence, imprecision, and publication bias (Balshem et al., 2011; Murad et al., 2017). The level of certainty among the evidence identified was characterized as high, moderate, low, or very low (Balshem et al., 2011).

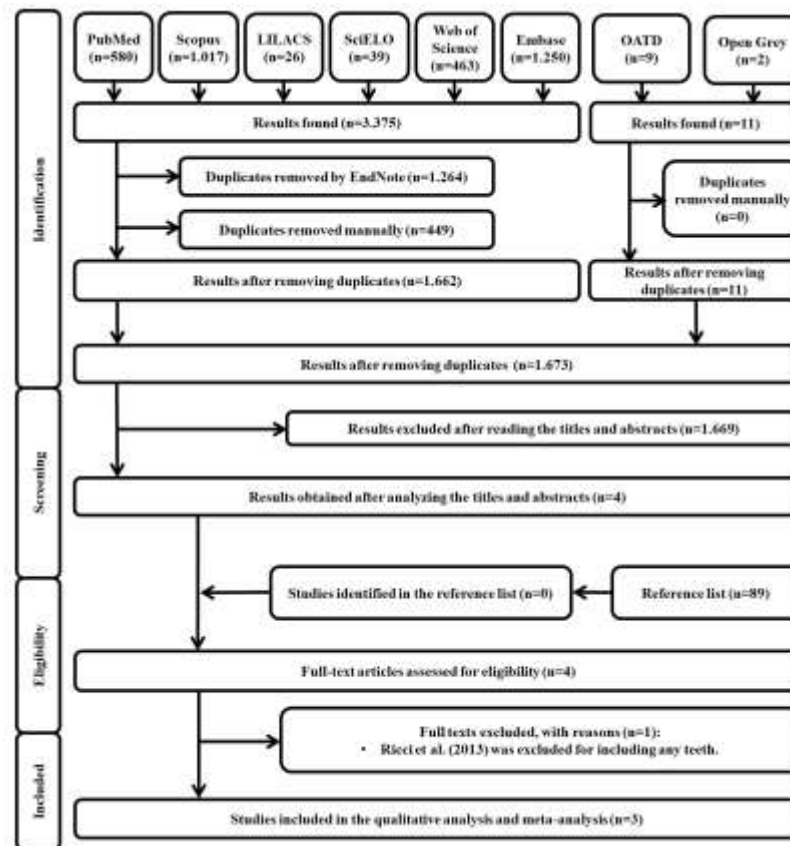
## **3. Results**

### **3.1 Study selection**

The search found 3386 results. After screening the titles and abstracts, four studies were eligible for full-text analysis. Their references were carefully evaluated, and no additional studies were selected. After reading the full text, one text was eliminated for including the extraction of teeth other than third molars. Thus, three studies were selected for qualitative and quantitative analyses (Figure 1).



**Figure 1.** Flowchart of the search and selection process for eligible studies.



Source: Elaborated by the authors (2020).

In Figure 1 is shown the selection process for eligible studies. It is important to notice that from 3375 registers, only three studies were included in the qualitative analysis.

### 3.2 Study characteristics

The studies were published between 2013 and 2017 and they were conducted in India (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b) and Turkey (Sivalingam et al. 2017). All three studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b; Sivalingam et al. 2017) respected the ethical parameters established and collected a consent form from all volunteers who participated in the study. Only one study (Sivalingam et al. 2017) mentioned using CONSORT guidelines and none reported the registration in clinical trial databases (Table 2).

**Table 2.** Summary of the main features of the eligible studies.

Author, country, and year of publication	Sample (n)	Average age (Years)	Dental arrangement classification	Anesthetic solution used	Surgery time (minutes)	Postoperative medication protocol	Method of ozone therapy administration	Postoperative pain assessment method	Mouth opening assessment method	Edema assessment method
<b>Kazancıoğlu et al., 2014a</b> Turkey	GC: 20 EG: 20	22.6 ± 2.3	Class III B (Pell and Gregory)	2.5% articaine hydrochloride + 1: 100.000 epinephrine	CG: 25 ± 11 EG: 22 ± 9	1 g amoxicillin and 550 mg oral naproxen sodium when needed.	The ozone generator was applied extraorally at the insertion point of the masseter muscle immediately after surgery and on the first, third, and seventh postoperative days, with intensity of 80% for 10 seconds.	Visual analog scale (VAS)	Maximum interincisal opening	Measurements of the distances from the tragus to the corner of the mouth (T-C) and from the tragus to the pogonion (T-P)
<b>Kazancıoğlu et al., 2014b</b> Turkey	Control and Experimental groups (Split mouth method): 60 32 ♂ 28 ♀	22.6 ± 2.3	Class III B (Pell and Gregory)	2.5% articaine hydrochloride + 1: 100.000 epinephrine	CG: 25 ± 11 EG: 22 ± 9	1 g amoxicillin and 550 mg oral naproxen sodium when needed.	The ozone generator was applied extraorally at the insertion point of the masseter muscle immediately after surgery and on the first, third, and seventh postoperative days, with intensity of 80% for 10 seconds.	Visual analog scale (VAS)	Maximum interincisal opening	Measurements of the distances from the tragus to the corner of the mouth (T-C) and from the tragus to the pogonion (T-P)
<b>Sivalingam et al., 2017</b> India	Control and Experimental groups (Split mouth method): 33 16 ♂ 17 ♀	25.6 ± 4.4	+	2% lidocaine hydrochloride + 1: 80.000 adrenaline	CG: 20 ± 12 min EG: 22 ± 14 min	Ibuprofen 400 mg paracetamol (333 mg) three times a day for two days. 500 mg of amoxicillin and 400 mg of Flagyl every 8 hours for 5 days. <sup>1</sup>	Ozone gel was administered topically to the extraction site twice a day for three days.	Visual analog scale (VAS)	Maximum interincisal opening	Mean measurements of the distance from the tragus to the corner of the mouth (T-C), from the tragus to the pogonion (T-P), and from the lateral corner of the eye to the lowest point of the mandible angle.

<sup>1</sup> Only the control group received antibiotic therapy. CG: Control group. EG: Experimental group.  
Source: Elaborated by the authors (2020).

Table 2 shows the main characteristics of the eligible studies. It is important to notice that the total sample included 133 patients subjected to third molar extraction surgery. In all studies, the patients were prescribed anti-inflammatory postoperatively. Only one study (Sivalingam et al. 2017) did not prescribe antibiotics postoperatively. The mean operation time ranged from 20 to 25 minutes. One study had a negative control group (Kazancioglu et al., 2014b) and one study had a positive control group (Sivalingam et al. 2017) (Table 2).

In two studies, ozone was applied in the masseter region on the 1<sup>st</sup>, 3<sup>rd</sup>, and 7<sup>th</sup> postoperative days by injecting ozone gas produced by a generator (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b). In the third study, ozone therapy was performed with the application of an ozone gel in the socket region where the surgery was performed, twice a day for three days after the surgical procedure (Sivalingam et al. 2017). In all studies, postoperative pain was assessed using a visual analog scale (VAS) (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b; Sivalingam et al. 2017).

All studies assessed trismus by measuring the maximum interincisal opening. In two studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b), edema was measured with the distances from the tragus to the corner of the mouth (T-C) and from the tragus to the pogonion (T-P) and, in one study (Sivalingam et al. 2017), it was measured with the mean of the distances from the tragus to the corner of the mouth (T-C), from the tragus to the pogonion (T-P), and from the lateral corner of the eye to the lowest point of the mandible angle.

### **3.3 Risk of individual bias of the studies**

Two studies (Kazancioglu et al., 2014b; Sivalingam et al. 2017) showed a low risk of bias and one study (Kazancioglu et al., 2014a) showed a moderate risk of bias. Detailed information on the risk of bias of the studies included can be found in Table 3. Item 1 was marked as “Unclear” in two studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b) as the randomization method was not explicit.

Only one study (Sivalingam et al. 2017) explained that patients were blinded to the group in which they were allocated, so two studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b) were marked as “No” in item 4. Item 5 was marked as “No” in one study (Sivalingam et al. 2017) because the same author who performed the surgery applied the ozone gel topically.

**Table 3.** Risk of bias assessed by the Joanna Briggs Institute Critical Appraisal Tools for use in JBI Systematic Reviews for Randomized Controlled Trials.

Authors	Q.1	Q.2	Q.3	Q.4	Q.5	Q.6	Q.7	Q.8	Q.9	Q.10	Q.11	Q.12	Q.13	% yes / risk
<i>Kazancioglu et al. 2014a</i>	U	U	√	--	U	√	√	√	√	√	√	√	--	62% yes/ moderate risk of bias
<i>Kazancioglu et al., 2014b</i>	U	√	√	√	√	√	√	√	√	√	√	√	√	92% yes/ low risk of bias
<i>Sivalingam et al., 2017</i>	√	√	√	--	--	√	√	√	√	√	√	√	√	85% yes/ low risk of bias

Q1. Was true randomization used for assigning the participants to treatment groups? Q2. Was allocation to treatment groups concealed? Q3. Were treatment groups similar at the baseline? Q4. Were participants blind to treatment assignment? Q5. Were those delivering treatment blind to treatment assignment? Q6. Were outcome assessors blind to treatment assignment? Q7. Were treatment groups treated identically other than the intervention of interest? Q8. Was follow-up complete and, if not, were differences between groups regarding their follow-up adequately described and analyzed? Q9. Were participants analyzed in the groups to which they were randomized? Q10. Were outcomes measured equally for treatment groups? Q11. Were outcomes measured in a reliable way? Q12. Was appropriate statistical analysis used? Q13. Was the trial design appropriate and any deviations from the standard RCT design (individual randomization, parallel groups) accounted for in the conduct and analysis of the trial? √ - Yes; -- - No; U – Unclear. Source: Elaborated by the authors (2020).

In Table 3 is possible to observe the results for risk of bias assessment for each eligible study. It is important to notice that the mains shortcomings of the studies were related to randomization and blinding process.

### 3.4 Synthesis of results

All studies evaluated pain, trismus, and edema after the surgical procedure. Regarding postoperative pain, all studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b; Sivalingam et al. 2017) showed the highest values on the first day after surgery (ranged from 7.48 to 8.42 in the control group and from 4.22 to 5.45 in the experimental group) and the lowest values seven days after surgery for both control and experimental groups (ranged from 0.94 to 2.33 in the control group and from 0.06 to 0.89 in the experimental group) (Table 4). All studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b; Sivalingam et al. 2017) observed a significant reduction in pain in favor of the ozone group in all days evaluated.

**Table 4.** Summary of pain assessment scores from the first to the seventh postoperative day.

Author	Group	Score			
		1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
Kazancioglu et al., 2014a	Control	8.42 ± 1.40	5.81 ± 1.32	+	2.33 ± 1.26
	Experimental	4.62 ± 3.12	2.49 ± 1.15	+	0.81 ± 0.32
Kazancioglu et al., 2014b	Control	7.52 ± 2.43	5.76 ± 1.24	4.42 ± 1.51	2.30 ± 1.26
	Experimental	4.22 ± 3.32	2.39 ± 1.55	1.62 ± 0.24	0.89 ± 0.65
Sivalingam et al., 2017	Control	7.48	5.15	+	0.94
	Experimental	5.45	2.97	+	0.06

+ - Data not cited. Source: Elaborated by the authors (2020).

For trismus, all studies (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b; Sivalingam et al. 2017) showed the lowest values of mouth opening on the first day (ranged from 21.61 mm to 32.4 mm in the control group and from 25.1 mm to 31.9 mm in the experimental group) and the highest values on the seventh day (ranged from 37.3 mm to 41.48 mm in the control group and from 36.8 mm to 46.64 mm in the experimental group) (Table 5).

**Table 5.** Summary of scores for preoperative mouth opening on the seventh postoperative day.

Author	Evaluation Method	Group	Score (mm)				
			Preoperative	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
Kazancioglu et al., 2014a	Maximum interincisal opening	Control	41.1 ± 2.2	22.1 ± 4.6	27.4 ± 7.3	+	37.3 ± 5.2
		Experimental	41.3 ± 3.2	25.1 ± 4.2	29.3 ± 3.5	+	38.6 ± 7.2
Kazancioglu et al., 2014b	Maximum interincisal opening	Control	42.1 ± 2.6	32.4 ± 5.4	35.4 ± 8.3	38.9 ± 3.5	40.9 ± 2.3
		Experimental	43.3 ± 4.2	31.9 ± 4.4	36.3 ± 2.5	39.6 ± 4.6	41.1 ± 4.6
Sivalingam et al., 2017	Maximum interincisal opening	Control	47.03	21.61	29.33	+	41.48
		Experimental	47.21	29.27	35.61	+	45.64

+ - Data not measured by the authors. Source: Elaborated by the authors (2020).

Regarding edema, on the first day, T-C values ranged from 12.11 cm to 14.11 cm in the control group and from 12.95 cm to 14.41 cm in the experimental group (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b; Sivalingam et al. 2017). The T-P values ranged from 16.02 cm to 18.22 cm in the control group and from 16.30 cm to 18.33 m in the experimental group. On the seventh day, T-C values ranged from 11.44 cm to 12.44 cm in the control group and from 11.81 cm to 12.81 cm in the experimental group. The T-P values ranged from 16.41

cm to 15.32 cm in the control group, and both presented a final value of 15.35 cm in the experimental group (Table 6).

**Table 6.** Summary of scores for the evaluation of preoperative edema on the seventh postoperative day.

Author	Sample	Method	Score (cm)				
			Preoperative	1 <sup>st</sup> day	3 <sup>rd</sup> day	5 <sup>th</sup> day	7 <sup>th</sup> day
Kazancioglu et al., 2014a	Control N=20	T-C	12.22 ± 0.21	14.11 ± 0.25	13.01 ± 0.42	+	12.44 ± 0.32
		T-P	16.33 ± 0.31	18.22 ± 0.35	17.01 ± 0.80	+	16.41 ± 0.35
	Experimental N=20	T-C	11.35 ± 0.41	14.41 ± 0.11	14.76 ± 0.14	+	12.81 ± 0.67
		T-P	15.24 ± 0.10	18.33 ± 0.34	18.21 ± 0.50	+	15.35 ± 0.34
Kazancioglu et al., 2014b	Control N=60	T-C	11.34 ± 0.34	12.11 ± 0.23	12.01 ± 0.65	11.94 ± 0.22	11.44 ± 0.87
		T-P	15.23 ± 0.29	16.02 ± 0.84	16.01 ± 0.82	15.75 ± 0.11	15.32 ± 0.20
	Experimental N=60	T-C	11.35 ± 0.41	12.95 ± 0.11	12.76 ± 0.14	12.01 ± 0.85	11.81 ± 0.67
		T-P	15.24 ± 0.10	16.30 ± 0.33	16.21 ± 0.50	15.95 ± 0.12	15.35 ± 0.34
Sivalingam et al., 2017	Control N=33	**	+	141.48	127.39	+	112.58
	Experimental N=33	**	+	123.09	113.88	+	104.55

+ Data not measured by the authors. \*\* Method used: Postoperative (AC + AD + BE) - Preoperative (AC + AD + BE): T-C: Tragus to mouth commissure; T-P: Tragus to the pogonion; AC: Most posterior point of the tragus to the commissure of the mouth; AD: Most posterior point of the tragus to the soft tissue of the pogonion; BE: Lateral corner of the eye to the lowest point of the mandible angle. Source: Elaborated by the authors (2020).

It was not possible to summarize the results of the eligible studies in a meta-analysis due to the methodological heterogeneity among studies.

### 3.5 Certainty of evidence

All outcomes were classified with very low levels of certainty, which means the true effect may be substantially different from the estimated effect (Table 7). The outcomes were downgraded due to the risk of bias, inconsistency, imprecision, and publication bias.

**Table 7.** Table for the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) Summary of Findings for the Outcomes of the Systematic Review.

Number of studies	Study design	Certainty assessment					Impact	Certainty	Importance
		Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations			
<b><i>Postoperative pain (follow-up: range 1 to 7 days)</i></b>									
3	randomized trials	Serious <sup>a</sup>	Serious <sup>b</sup>	not serious <sup>c</sup>	Serious <sup>d</sup>	Publications bias strongly suspected <sup>e</sup>	All eligible studies showed that ozone therapy was effective in reducing postoperative pain compared to the control group.	⊕ VERY LOW	CRITICAL
<b><i>Mouth opening (follow-up: range 1 to 7 days)</i></b>									
3	randomized trials	Serious <sup>a</sup>	Serious <sup>b</sup>	not serious <sup>c</sup>	Serious <sup>d</sup>	Publications bias strongly suspected <sup>e</sup>	The results found in the eligible studies were divergent, considering that two eligible studies showed a difference between the experimental group and the control group and, in one study, these results were not found.	⊕ VERY LOW	CRITICAL
<b><i>Edema (follow-up: range 1 to 7 days)</i></b>									
3	randomized trials	Serious <sup>a</sup>	Serious <sup>b</sup>	not serious <sup>c</sup>	Serious <sup>d</sup>	Publications bias strongly suspected <sup>e</sup>	The results found in the eligible studies were divergent, considering that two eligible studies showed a difference between the experimental group and the control group and, in one study, these results were not found.	⊕ VERY LOW	CRITICAL

a - Studies did not explain the blindness of the operator and participant – downgraded by one level due to risk of bias.

b - The route of ozone administration varied among eligible studies and/or the estimate effects of individual studies varied – downgraded by one level due to inconsistency.

c - Evidence stems from studies with the population suitable for PICO.

d - The number of events is very low, not reaching the optimal information size (OIS = 400) - downgraded by one level due to imprecision.

e – Two out of three eligible articles were published by the same authors – downgraded by one level due to publication bias.

#### **GRADE Working Group grades of evidence**

**High certainty:** We are very confident that the true effect is close to the estimated effect.

**Moderate certainty:** We are moderately confident in the effect estimate: The true effect is likely close to the estimated effect, but there is a possibility that it is substantially different.

**Low certainty:** Our confidence in the effect estimate is limited: The true effect may be substantially different from the estimated effect.

**Very low certainty:** We have very little confidence in the effect estimate: The true effect is likely to be substantially different from the estimated.

Source: Elaborated by the authors (2020).

#### 4. Discussion

This systematic review aimed to evaluate the effect of ozone as a supporting therapy for reducing pain, edema, and trismus after impacted lower third molar extraction.

Repair is a phenomenon that occurs to reconstruct traumatized tissues, involving cells and numerous chemical mediators. It consists of orderly events initiated at the moment of trauma and it lasts for variable periods (Wang, 2018). Optimizing and speeding up the repair process to restore tissue physiology is always a challenge. Impacted third molar extraction is one of the most common procedures, often associated with painful symptomatology, edema, and dysfunction, which may be temporary or permanent and cause considerable deterioration of the quality of life of patients (McGrath et al., 2003; Lim and Ngeow, 2017). The causes are complex and closely related to the inflammatory process initiated by the surgical act (Henman et al., 1979; Yuasa et al., 2004; Kumar et al., 2009; Elvis et al., 2011).

The ideal agent to reduce postoperative complications after third molar surgery would alleviate pain, minimize edema and trismus, promote healing, and have no unwanted effects. According to our meta-analyses, pain decreased using ozone. However, edema was greater in patients under ozone therapy than in control patients, and trismus presented no statistically significant difference.

Ozone therapy has been successfully used to reduce pain in several situations: temporomandibular disorders (Domb, 2014), gingival grafts (Taşdemir et al., 2016), fibromyalgia (Tirelli et al., 2019), chronic wounds (Fitzpatrick et al., 2018), and back pain (Doğan et al., 2014; Bocci et al., 2015). The action of ozone on pain relates to the ability to control oxidative stress (Smith et al., 2017; Tirelli et al., 2019). Although this is not the focus of the present study, the molecular mechanisms of ozone action might be of importance to understand our results. In a safe and correct dose, ozone represents a non-deleterious acute oxidative stress that induces an antioxidant cellular response, normalizing the existing redox swelling in several diseases, with an evident contribution to pain control.

The anti-inflammatory effects of ozone have been studied mainly in animal models. *In vivo* experiments revealed the inhibition of inflammatory mediators (prostaglandin, interleukin, and tumor necrosis factor) and the increase of macrophage and leukocyte activities (Azarpazhooh et al., 2009; Cho et al., 2017). In topical applications, ozone presents analgesic and anti-inflammatory properties, working as a neurochemical mediator of painful sensations. Moreover, it is used as an adjunct in the treatment of chronic pain and it inhibits cyclooxygenase II by reducing hyperpermeability, edema, and pain (Seidler et al., 2008).



Some of these effects can justify the improved pain relief after ozone application when compared to the conventional postoperative protocol of third molar surgeries.

However, this does not explain the absence of differences in edema and trismus. Considering that edema after third molar surgery is also caused by inflammatory processes triggered by surgical trauma (Feslihan et al., 2019), it was expected that pain reduction would be followed by a reduction in edema. The measurements of edema showed low scores for both groups, indicating acceptable edema control. Considering it is a supporting therapy, lower scores would be expected for the ozone group.

The magnitude of edema can be compared to the complexity of the surgery (Feslihan et al., 2019). Thus, patients with a similar degree of teeth impaction were chosen and operative time was comparable to reveal no intergroup bias in the studies included. Despite these cautions, only two studies with the same outcome measurement methods evaluated edema, which is a source of bias.

The results on edema can also be linked to the form of ozone used. In dentistry, ozone therapy consists of injections of low-concentration ozone gas or the topical application of ozonated gel or oil (Cho et al., 2017). The studies included promote different forms of ozone therapy: gas injection (Kazancioglu et al., 2014a; Kazancioglu et al., 2014b) or topically applied gel (Sivalingam et al., 2017). These differences, combined with the fact that not all studies used a sham group as a control, led us to believe that problems in the experimental design contributed to the lack of significant difference.

Trismus is a complication directly associated with surgical time, and the more complex the surgical technique, as in cases requiring ostectomy and teeth sectioning, the greater the chance of postoperative complications. Our results showed a decrease of trismus regardless of the therapy, but the clinical significance should be noted. The improvements in mouth opening with the use of ozone were not better than the control group and they may not represent a benefit to the patient. Additionally, the heterogeneity of the studies included is justified by various methods for measuring maximum mouth opening; for example, whether it was measured at the first sensation of pain or when achieving maximum interincisal distance. These differences could affect the results and they were not described in the studies selected.

It is also important to consider that pain and trismus are usually subjective or present several variations among patients. Other factors that may affect the postoperative period and patient comfort are age, sex, medical history, presence of a previous pericoronitis, smoking, severity of impaction, proximity of the tooth to the inferior alveolar nerve, surgical time, surgical technique, surgeon experience, and drugs used (Cho et al., 2017).

Furthermore, the current systematic review presented some limitations. First is the small number of studies, which might lower the statistical power. Additionally, two out of three studies were developed by the same research group. Hence, it may be speculated whether clinical trials on the effect of ozone therapy after third molar surgery have not been conducted or whether the studies with negative results have not been published. The second limitation is the heterogeneity of studies. Different study types, scales of measurement, time intervals, and surgical protocols can explain the heterogeneity. Finally, the methodological issues found in the study should be considered (Kazancioglu et al., 2014a). As these limitations might have undermined the findings, the results from our meta-analyses should be carefully considered in the clinical setting. This is further supported by the GRADE assessment, which classified the certainty of evidence as “very low” for all three outcomes due to the combination of methodological issues and the high heterogeneity among studies. Our results provide evidence on the need for well-designed clinical trials to assess the true effect of ozone therapy on postoperative outcomes after third molar surgery.

The strengths of this review should also be highlighted. This is the first systematic literature review on the use of ozone therapy in impacted third lower molar removal surgery. Moreover, the extensive search in different databases without restrictions of year and publication language and the use of the “gray literature” considerably minimizes the risk of study selection bias. The use of GRADE (Murad et al., 2017) and “The Joanna Briggs Institute Critical Appraisal Tools for Use in JBI Systematic Reviews” (Tufanaru et al., 2017) to assess the quality of evidence and the methodological quality of the studies, respectively, shows the rigor with which the data from the eligible studies were collected.

## **5. Conclusion**

Based on the findings of the present review, it is concluded that ozone as a supportive therapy may be effective in reducing pain but it was not effective in reducing edema and trismus. Considering the limitations of this review and the very low certainty of evidence of the studies, a pragmatic recommendation to using ozone as a supporting therapy to improve pain, edema, and trismus in lower third molar extractions is not possible. It is necessary to establish more standardized protocols for this therapy to achieve a higher certainty of evidence. Thus, future studies should be performed in order to clarify the role of ozone therapy in oral surgery practice.

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