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Carina Michelon

INFLUÊNCIA DA OSTEOPOROSE E DO ÁCIDO ZOLEDRÔNICO NO DESENVOLVIMENTO DE PERIODONTITE APICAL INDUZIDA EM RATAS

Santa Maria, RS

2017

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Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Odontológicas, Área de Concentração em Odontologia, Ênfase em Endodontia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do grau de Doutora **em Ciências Odontológicas**.

Orientador: Prof. Dr. Carlos Alexandre Souza Bier

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Santa Maria, RS
2017

Dedicatória

Dedico este trabalho especialmente a minha família, verdadeira razão do meu viver, base sólida sobre o qual edifico todos os meus sonhos...

"Não é sobre ter
Todas as pessoas do mundo pra si
É sobre saber que em algum lugar
Alguém zela por ti
É sobre cantar e poder escutar
Mais do que a própria voz
É sobre dançar na chuva de vida
Que cai sobre nós

É saber se sentir infinito
Num universo tão vasto e bonito
É saber sonhar
E, então, fazer valer a pena cada verso
Daquele poema sobre acreditar

Não é sobre chegar no topo do mundo
E saber que venceu
É sobre escalar e sentir
Que o caminho te fortaleceu
É sobre ser abrigo
E também ter morada em outros corações
E assim ter amigos contigo
Em todas as situações

A gente não pode ter tudo
Qual seria a graça do mundo se fosse assim?
Por isso, eu prefiro sorrisos
E os presentes que a vida trouxe
Pra perto de mim..."

Trem Bala
Ana Vilela

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“Ninguém pode voltar atrás e fazer um novo começo. Mas qualquer um pode recomeçar e fazer um novo fim.”

Chico Xavier

RESUMO

INFLUÊNCIA DA OSTEOPOROSE E DO ÁCIDO ZOLEDRÔNICO NO DESENVOLVIMENTO DE PERIODONTITE APICAL INDUZIDA EM RATAS

AUTORA: Carina Michelon
ORIENTADOR: Carlos Alexandre Souza Bier

A osteoporose é uma condição sistêmica caracterizada pela redução da densidade mineral óssea e pela deterioração da microarquitetura do tecido ósseo, levando ao aumento da fragilidade óssea e, consequentemente, ao maior risco de fratura. Atualmente, o Ácido Zoledrônico (ZOL) é um dos medicamentos mais utilizados para tratamento dessa doença sistêmica, pois atua diretamente no processo de remodelação óssea. Entretanto, os efeitos da osteoporose e da terapia com ZOL na progressão da periodontite apical não estão bem esclarecidos. Assim, os objetivos deste estudo foram (1) avaliar a influência da osteoporose induzida em ratas na progressão da periodontite apical, por meio de uma revisão sistemática com metanálise; (2) avaliar o efeito da osteoporose e do ZOL no desenvolvimento de lesões periapicais em ratas usando a Microtomografia Computadorizada (Micro-CT). Na revisão sistemática, as bases de dados PubMed, Embase, Web of Science, SCOPUS e LILACS foram analisadas até junho de 2017. Dos 446 potencialmente elegíveis, 10 estudos foram incluídos na revisão sistemática. Os desfechos incluídos foram o tamanho da lesão periapical e as expressões TRAP, RANKL e OPG. A metanálise foi realizada para o tamanho da lesão periapical obtido nas análises histométrica e radiográfica. Para o estudo experimental, 40 ratas *Wistar* foram randomizadas em 4 grupos (n=10): SHAM – cirurgia Sham e tratamento com solução salina; SHAM-ZOL – cirurgia Sham e tratamento com ZOL; OVX – ovariectomia e tratamento com solução salina; OVX-ZOL – ovariectomia e tratamento com ZOL. A lesão periapical foi induzida no primeiro molar direito inferior de todos os animais e a solução salina e o ZOL foram administrados via intraperitoneal. O tamanho da lesão periapical foi mensurada usando raio x e Micro-CT. O Conteúdo Mineral Ósseo (CMO) foi avaliado no fêmur e na mandíbula usando tomografia computadorizada. De acordo com os resultados da metanálise para dados histométricos, lesões periapicais induzidas em ratas com osteoporose são maiores que em animais saudáveis ($P < 0.05$), tanto na análise global quanto nas análises de subgrupo para o tempo de indução de lesão periapical e para a linhagem de ratas. Na metanálise para os dados radiográficos (mm^2), ratas com osteoporose também apresentaram lesões periapicais maiores ($P < 0.05$). Quanto maior as lesões periapicais mais elevadas às expressões TRAP e RANKL. A OPG variou nos grupos OVX e SHAM de acordo com o tempo de indução de lesão periapical, porém, não houve um padrão de atividade. Os resultados do estudo experimental mostraram que não houve diferença estatística na área e volume das lesões periapicais entre os grupos ($P > 0.05$). O CMO no côndilo do fêmur foi estatisticamente menor para o grupo OVX do que para o grupo SHAM ($P < 0.05$). Para CMO da mandíbula não houve diferença estatística entre os grupos ($P > 0.05$). Em conclusão, os resultados deste estudo sugerem que a osteoporose tem um papel importante no desenvolvimento da periodontite apical e, que o tempo de indução das lesões periapicais é principal fator a ser considerado nesta relação. O ZOL influencia na CMO sistêmico, porém parece ter pouco efeito nos ossos mandibulares e na progressão da periodontite apical em dose utilizada para tratamento da osteoporose.

Palavras-Chaves: Bisfosfonatos. Densidade óssea. Osteoporose. Periodontite periapical.

ABSTRACT

INLFLUENCE OF OSTEOPOROSIS AND ZOLEDRONIC ACID IN DEVELOPMENT OF APICAL PERIODONTITIS INDUCED IN RATS

AUTHOR: Carina Michelon
ADVISOR: Carlos Alexandre Souza Bier

Osteoporosis is a systemic condition characterized by the reduction of bone mineral density and deterioration of the microarchitecture of the bone tissue, leading to an increase of the bone fragility and, consequently, a greater risk of fracture. Currently, zoledronic acid (ZOL) is one of the drugs most used to treat this systemic disease, since it acts directly on the process of bone remodeling. However, the effects of osteoporosis and systemic therapy with ZOL on the progression of apical periodontitis are not well understood. Thus, the objectives of this study were (1) to evaluate the influence of induced osteoporosis in rats on the progression of apical periodontitis through a systematic review and meta-analysis; (2) to evaluate the effect of osteoporosis and ZOL on the development of periapical lesions in rats using micro-computed tomography (micro-CT). In the systematic review, PubMed, Embase, Web of Science, SCOPUS and LILACS databases were searched up to June 2017. Of 446 potentially eligible, 10 were included in the systematic review. The included outcomes were periapical lesion size and TRAP, RANKL and OPG expressions. Meta-analysis was performed with histometric and radiographic data of the periapical lesion size. For experimental study, 40 female Wistar rats were randomized into four groups (n=10): SHAM – Sham surgery and saline treatment; SHAM-ZOL – SHAM surgery and ZOL treatment; OVX – ovariectomy surgery and saline treatment; OVX-ZOL – OVX surgery and ZOL treatment. The apical periodontitis was inducted on the right mandibular first molar in all animals and saline and ZOL were administered intraperitoneally on the same day. The periapical lesion was measured by radiography and micro-CT. Bone mineral content (BMC) of the femur and mandible was analyzed by computed tomography. According meta-analysis results, induced periapical lesions in rats with osteoporosis are larger than in healthy rats ($P < 0.05$), both in the global analysis and in the subgroup analyzes for periapical lesion induction time and lineage of rats. In the meta-analysis for x-ray data (mm^2), rats with osteoporosis also had larger periapical lesions ($P < 0.05$). Larger periapical lesions tend to have more elevated expression of TRAP and RANKL. OPG changed in the OVX and SHAM groups according time of induction of periapical lesion; however, there was no pattern of activity. The results of the experimental study showed that there were no statistical difference among the groups in the area and volume of periapical lesions ($P > 0.05$). BMC in femur's condyle was significantly lower in the OVX group than in SHAM group ($P < 0.05$). BMC of the mandible was similar among groups ($P > 0.05$). In conclusion, the results of this study suggest that osteoporosis plays an important role on the development of apical periodontitis; and that the time of induction of the periapical lesions is the main factor to be considered in this relation. ZOL influences on the systemic BMC, but it appears to have little effect on mandibular bones and on the progression of the periapical lesions at doses used for osteoporosis treatment.

Key-words: Bisphosphonates. Bone density. Osteoporosis. Periapical periodontitis.

LISTA DE ILUSTRAÇÕES

ARTIGO 1 – INFLUENCE OF OSTEOPOROSIS ON THE PROGRESSION OF APICAL PERIODONTITIS INDUCED IN RATS: A SYSTEMATIC REVIEW AND META-ANALYSES

ARTIGO 2 – EFFECT OF OSTEOPOROSIS AND ZOLEDRONIC ACID ON THE DEVELOPMENT OF APICAL PERIODONTITIS: ANALYSIS BY MICRO-CT

LISTA DE TABELAS

ARTIGO 1 – INFLUENCE OF OSTEOPOROSIS ON THE PROGRESSION OF APICAL PERIODONTITIS INDUCED IN RATS: A SYSTEMATIC REVIEW AND META-ANALYSES

ARTIGO 2 – EFFECT OF OSTEOPOROSIS AND ZOLEDRONIC ACID ON THE DEVELOPMENT OF APICAL PERIODONTITIS: ANALYSIS BY MICRO-CT

SUMÁRIO

1 INTRODUÇÃO

O aumento da expectativa de vida e a redução das taxas de mortalidade e fertilidade resultaram em um crescente aumento da população mundial de idosos e das doenças crônico-degenerativas, tais como a osteoporose (NATIONAL OSTEOPOROSIS FOUNDATION, 2014). Na Europa, Estados Unidos da América e Japão, aproximadamente, 75 milhões de pessoas tem osteoporose (HERNLUND et al., 2013; NATIONAL OSTEOPOROSIS FOUNDATION, 2014). No Brasil, a prevalência de osteoporose nas mulheres varia de 15% a 33% (PINHEIRO et al., 2010 a; PINHEIRO et al., 2010 b; BRUTTOS et al., 2011; BACCARO et al., 2013) e este número tende a aumentar se levarmos em conta os dados estatísticos do último censo do Instituto Brasileiro de Geografia e Estatística (IBGE). Estima-se, segundo os dados do censo 2010, que as pessoas com mais de 50 anos de idade representam cerca de 20% (40 milhões) da população brasileira. Além disso, a expectativa de vida subiu de 52 anos em 1952 para 71 anos em 2010 e projeta-se que chegue a 80 anos em 2050, sendo que 37% (96 milhões) da população terá mais de 50 anos (CENSO DEMOGRÁFICO 2010).

A osteoporose é uma doença esquelética sistêmica caracterizada pela diminuição da massa óssea e deterioração da microarquitetura do osso, com consequente aumento da fragilidade do tecido ósseo e do risco de desenvolver fratura (NATIONAL OSTEOPOROSIS FOUNDATION, 2014). A osteoporose ocorre principalmente em mulheres após a menopausa devido à perda da função ovariana e redução dos níveis de estrogênio. Em geral, há um excelente equilíbrio entre a reabsorção do tecido ósseo pelos osteoclastos e a subsequente restauração do osso pelos osteoblastos. Porém, a deficiência deste hormônio resulta em altas taxas de remodelação óssea, sendo que a reabsorção excede a formação do osso, levando à redução da massa óssea corporal (RIGGS et al., 1998).

A perda de massa óssea não causa sintomas. No entanto, quando ocorre uma fratura, pode haver dor, perda de função e deformidades, além do risco de ocorrência de novas fraturas (NATIONAL OSTEOPOROSIS FOUNDATION, 2014). Fraturas e suas complicações são as principais consequências clínicas da osteoporose, pois limitam as atividades diárias e comprometem a qualidade de vida dessas pessoas. Todos os tipos de fratura estão associados à morbidade; entretanto, a fratura de quadril é a que apresenta maior taxa de mortalidade e de deficiências permanentes (COOPER, 1997). Para diminuir esses riscos, tentar predizer o risco de ocorrência de fratura e auxiliar nas recomendações do tratamento, a Organização Mundial da Saúde criou em 2008 uma Ferramenta para Avaliação

do Risco de Fratura (FRAX® – do inglês, *fracture risk assessment model*). O FRAX® foi desenvolvido para calcular a probabilidade que uma pessoa tem de desenvolver uma fratura de quadril em 10 anos e a probabilidade, em 10 anos, de ocorrer uma grande fratura por causa da osteoporose (definida como fratura vertebral, de quadril, antebraço ou proximal do úmero), levando em consideração a Densidade Mineral Óssea (DMO) do colo do fêmur e fatores clínicos de risco, tais como ocorrência prévia de fratura, artrite reumatoide e uso de glicocorticoides (WATTS, 2011).

Uma vez que a osteoporose é uma doença silenciosa, a redução dos níveis de estrogênio e/ou a ocorrência de uma fratura em uma pessoa com mais de 50 anos de idade, devem ser consideradas como eventos importantes para a identificação e posterior tratamento da osteoporose (NATIONAL OSTEOPOROSIS FOUNDATION, 2014). O diagnóstico de osteoporose é estabelecido pela mensuração da DMO ou pela ocorrência de fraturas no quadril ou nas vertebras, na idade adulta sem a ocorrência de traumatismo grave (NATIONAL OSTEOPOROSIS FOUNDATION, 2014). Clinicamente, a DMO é avaliada por meio da Densitometria Óssea (DEXA); um indivíduo com osteoporose apresenta a DMO de 2,5 ou mais desvio padrão abaixo da média de uma população adulta jovem de referência (NATIONAL OSTEOPOROSIS FOUNDATION, 2014).

Em condições de normalidade, há um excelente equilíbrio no processo de remodelação e a homeostase óssea é garantida. De forma geral, a remodelação óssea inicia quando o Ligante do Receptor Ativador do fator Nuclear Kappa (RANKL – do inglês, *receptor activator of nuclear fator kappa- β ligand)*, secretado por células da linhagem osteoblástica, liga-se ao seu receptor RANK, localizado na membrana dos pré-osteoclastos (ROUX et al., 2002), estimulando a diferenciação do precursor do osteoclasto em osteoclasto maduro (NAKAGAWA et al., 1998; CLARKE, 2008). Em contraste, a Osteoprotegerina (OPG) produzida pelo osteoblasto, bloqueia a formação de osteoclasto, ligando-se ao RANKL, com o qual tem grande afinidade, impedindo que RANKL se ligue ao RANK e inibindo a osteoclastogênese (ROUX et al., 2002). Entretanto, na presença da deprivação de estrogênio, ocorre um desequilíbrio nesse mecanismo, pois a produção de OPG fica comprometida e um maior número de receptores RANK será ativado. Deste modo, o processo de reabsorção será maior que a formação óssea levando ao início das primeiras alterações.

Já o tratamento da osteoporose pode ser realizado com vários medicamentos incluindo terapia hormonal, bisfosfonatos, denosumab, moduladores seletivos de receptores de estrogênio e hormônios análogos ao paratormônio. Dentre esses, os bisfosfonatos são os fármacos de primeira escolha devido ao seu excelente custo-benefício e sua capacidade de

inibir o processo de remodelação óssea (CHRISTENSON et al., 2012; REGINSTER et al., 2014). O Ácido Zoledrônico (ZOL) é um potente bisfosfonato injetável de terceira geração que tem mostrado bons resultados para prevenção e tratamento da osteoporose induzida pela pós-menopausa (REID et al., 2002; DEVOGELAER et al., 2007; MCCLUNG et al., 2007; BLACK et al., 2007), osteoporose induzida por glicocorticóides (REID et al., 2009), no pós-operatório de fraturas de quadril (LYLES et al., 2007) e também para o tratamento de pacientes osteoporóticos do gênero masculino (BOONEN et al., 2012). O ZOL melhora a densidade óssea e reduz o risco de desenvolver fraturas, além de ter viabilidade de administração quando comparado aos medicamentos de uso oral (BLACK et al., 2007; LAMBRINOUDAKI et al., 2008; REID et al., 2009; WAALEN, 2010; REGINSTER et al., 2014).

Interessantemente, um número crescente de evidências clínicas sugere que existe uma relação positiva entre a osteoporose e a presença de alterações na cavidade oral. Um estudo transversal recente, comparando mulheres na pós-menopausa saudáveis e com osteoporose, mostrou associação entre a presença de osteoporose e a ocorrência de doença periodontal (RICHA et al., 2016). Outro estudo transversal, também com mulheres na pós-menopausa, verificou que aquelas que possuíam baixa DMO apresentavam menor número de dentes na cavidade bucal (KIM et al., 2015). Já um ensaio clínico multicêntrico mostrou uma correlação positiva entre a DMO da maxila e a estabilidade de implantes em mulheres acima de 60 anos de idade (MERHEB et al., 2016).

Na área endodôntica, a relação da osteoporose e do seu tratamento farmacológico com a presença e o desenvolvimento da periodontite apical também tem sido investigada. Em um estudo transversal foi observado que não houve diferença estatística no número de lesões periapicais entre mulheres saudáveis, com osteopenia e com osteoporose na pós-menopausa. Entretanto, os autores observaram que a baixa DMO estava correlacionada com a presença de lesões periapicais em pelo menos um elemento dentário (LÓPEZ-LÓPEZ et al., 2015). Segundo HSIAO et al. (2009), pacientes que fazem uso de bisfosfonatos orais e que foram submetidos ao tratamento e retratamento endodôntico apresentam reparo ósseo alveolar semelhante à pacientes saudáveis. Além disso, estudos em modelo animal mostram que o uso de medicamentos como ZOL, alendronato e raloxifeno minimizam a reabsorção óssea periapical em dentes de ratas com osteoporose induzida (XIONG et al., 2007; WAYAMA et al., 2015; GOMES-FILHO et al., 2015).

Tendo em vista que a perda de tecido ósseo periapical é uma das principais consequências da periodontite apical não tratada, que pacientes com osteoporose apresentam

altas taxas de reabsorção óssea e que o ZOL inibe o processo de remodelação óssea, os objetivos do presente estudo foram: (1) avaliar os efeitos da osteoporose na progressão de lesões periapicais induzidas em ratas, por meio de uma revisão sistemática; (2) avaliar o efeito da osteoporose e do tratamento sistêmico com ZOL no desenvolvimento de periodontite apical em ratas, usando a Microtomografia Computadorizada (Micro-CT). Estes objetivos estão apresentados como os artigos 1 e 2 desta Tese, respectivamente.

2 ARTIGO 1 – INFLUENCE OF OSTEOPOROSIS ON THE PROGRESSION OF APICAL PERIODONTITIS INDUCED IN RATS: A SYSTEMATIC REVIEW AND META-ANALYSES

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INFLUENCE OF OSTEOPOROSIS ON THE PROGRESSION OF APICAL PERIODONTITIS INDUCED IN RATS: A SYSTEMATIC REVIEW AND META-ANALYSIS

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Running title: Osteoporosis and apical periodontitis

ABSTRACT

This systematic review investigated the influence of osteoporosis on the progression of apical periodontitis induced in female rats. Two reviewers independently conducted a comprehensive search in the PubMed, Embase, Web of Science, SCOPUS, and LILACS databases for articles published up to June 5-6, 2017. The grey literature and bibliographies of all relevant articles were also searched. Among the 846 records retrieved, 446 were potentially eligible and 10 were included after the full-text analysis. The outcomes were periapical lesion size (histometry, x-ray, or micro-CT) and the expression of TRAP, RANKL, or OPG. Meta-analysis was performed with the histometric and radiographic data. Subgroup analysis and meta-regression were also performed to investigate heterogeneity. According to the meta-analysis of the histometric and x-ray (in mm^2) findings, periapical lesions induced in osteoporotic rats are larger than in healthy rats ($P < 0.05$). The subgroup analysis of the histometric findings maintained the statistical difference found in the global meta-analysis ($P < 0.05$), suggesting that periapical lesion induction time is an important factor to determining the influence of osteoporosis on the progression of such lesions. The meta-regression demonstrated that rat lineage together with the ovariectomy period and the time of periapical lesion induction explained 80.38% of the heterogeneity in the global meta-analysis. Osteoporotic rats tend to have higher TRAP and RANKL activity than healthy rats. This systematic review and meta-analysis suggests that osteoporosis exerts an influence on the progression of periapical lesions and that apical periodontitis induction time is an important factor to consider in this relationship.

Keywords: Apical periodontitis; estrogen deficiency; osteoporosis; ovariectomy; periapical lesion.

INTRODUCTION

Osteoporosis is a systemic condition characterized by a reduction in bone mineral density (BMD) and deterioration of the microarchitecture of the bone tissue, which leads to an increase in trabecular spaces, greater bone frailty, and a consequent increase in the risk of fractures (Cosman *et al.* 2014). Estrogen deficiency in the postmenopausal period is suggested to be the predominant cause of osteoporosis (Han *et al.* 2015).

Low estrogen concentration in plasma produces changes in the bone pattern of the body, including bones of the oral cavity (Duarte *et al.* 2004, Liu *et al.* 2015, Penoni *et al.* 2016). In patients with periodontal disease, osteoporosis is considered a risk factor to its progression (Juluri *et al.* 2015). However, the relationship between osteoporosis and apical periodontitis remains unproven due to the scarcity of clinical studies. López-López *et al.* (2015) conducted a cross-sectional study involving postmenopausal women and found a borderline association between the presence of periapical radiolucency and low BMD.

On the other hand, many studies with animal models have been performed to clarify alterations in the skeletal architecture caused by osteoporosis and its influence on the progression of periapical lesions. The literature has long demonstrated that estrogen deficiency induced in rats could be related to changes in the development pattern of apical periodontitis (Gilles *et al.* 1997). However, while some studies have recently reported that the alveolar bone is affected by estrogen deficiency in the presence of endodontic infection (Wayama *et al.* 2015, Qian *et al.* 2016, Brasil *et al.* 2017), others have indicated that periapical lesion size (Zhang *et al.* 2011) and bone resorption markers (Xiong *et al.* 2007) are similar between healthy and osteoporotic animals.

The reviewed literature also showed considerable methodological variability among these studies, such as differences in rat lineage, time of ovariectomy, and periapical lesion induction time. Thus, the aim of this study was to conduct a systematic review to evaluate the effect of osteoporosis on the progression of apical periodontitis induced in rats and perform a critical analysis of the methodological characteristics of the studies selected.

MATERIALS AND METHODS

This study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement (Moher *et al.* 2009) and is registered in the Collaborative Approach to Meta-Analysis and Review of Animal Data from

Experimental Studies (CAMARADES) (http://www.dcn.ed.ac.uk/camarades/research.html#protocols).

Formulation of the Review Question

Does estrogen deficiency-induced osteoporosis in rats affect the progression of apical periodontitis and bone resorption markers when compared to healthy animals? Based on this research question, the following PICO framework was developed:

- *Population:* female rats;
- *Intervention:* ovariectomy for induction of osteoporosis (OVX group);
- *Comparison:* simulation of ovariectomy surgery (SHAM group);
- *Outcomes:* measurement of periapical lesion size using X-ray, micro-CT, or histometry as the primary outcome; tartrate-resistant acid phosphatase (TRAP), receptor activator of nuclear factor kappa-B ligand (RANKL), or osteoprotegerin (OPG) expression in the periapical lesion as secondary outcomes.

Search Strategy and Study Selection

The MEDLINE/PubMed, Web of Science, EMBASE, Scopus, and LILACS databases were searched. To search for potentially unidentified publications, Google Scholar, OpenGrey, and the Thesis Bank of the Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) were also considered. The electronic searches were initially conducted in January 2017 and again on June 5 and 6, 2017 to identify more recent relevant literature (Table S1). The searches were performed using a combination of controlled vocabulary and key words (Table S2). Manual searches were also performed the reference lists of all included articles to determine the existence of any study not found in the electronic search. The searches were conducted with no language restrictions by two independent reviewers (CM and MDCB).

Eligibility Criteria

Studies were pre-selected based on a reading of the title and abstract using the following inclusion criteria:

1. Animal model studies;
2. Studies using female rat as animal model;
3. Ovariectomy surgery for osteoporosis induction;
4. Induction of periapical lesion through pulp exposure;

5. Studies with any segment time, and;
6. Description of rat lineage.

Records from the searches were entered in the Mendeley desktop software. Among the 846 records, 446 remained after the duplicates were removed. Full texts were obtained for studies that appeared to be relevant or had insufficient data in the title or abstract to make a clear decision. The following exclusion criteria were considered during the full-text analyses:

1. Studies that did not have a SHAM surgery group as the control;
2. Studies that did not have a OVX group without systemic treatment, and;
3. Studies did not leave the pulp chamber open to the oral environment after pulp exposure.

The evaluation of relevant articles and final appraisal of eligibility were performed by two independent reviewers (CM and MDCB). Interexaminer agreement in the screening process was measured using the Kappa test. The results of which were K = 0.95 for titles and abstracts and K = 0.98 for full texts. Papers fulfilling the selection criteria were processed for data extraction. Disagreements between reviewers were resolved by discussion. If a disagreement persisted, the judgment of a third reviewer (JM) was considered to be decisive.

Data Extraction and Data Analysis

Two reviewers (CM and MDCB) independently collected the following data from eligible studies: publication year; country of the study; animal lineage; animal age; number of animals per group; period between ovariectomy and periapical lesion induction (OVX–PL); period between periapical lesion induction and euthanasia (PL–EUT); total experimental period (OVX–EUT); analysis unit for each outcome; tooth exposed to the oral environment; method for detecting osteoporosis (uterus weight, estradiol levels, or bone mineral content [BMC]); periapical lesion size in X-ray, micro-CT, or histometry; and TRAP, RANKL, or OPG expression.

Statistical measurement units were extracted from the following outcomes included in this review: periapical lesion size in X-ray, micro-CT, or histometry; and TRAP, RANKL, or OPG activity. When papers provided insufficient data for inclusion in the analysis, the corresponding author was contacted three times via e-mail to determine whether additional data could be provided (answer rate = 20%). When an answer was not provided and data graphs and figures were inadequately labeled, the WebPlotDigitizer software (version 3.11, 2017; http://arohatgi.info/WebPlotDigitizer) was used to identify precise values.

Quality Assessment (Risk of Bias)

The quality of the studies selected was appraised by two reviewers (CM and MDCB) (Interexaminer agreement k = 0.96) using the SYRCLE checklist for the risk of bias assessment with minor adaptations (Hooijmans *et al.* 2014). The criteria were categorized into selection bias, performance bias, detection bias, attrition bias, reporting bias, and other bias. The analysis of selection bias considered sample randomization and allocation concealment. The analysis of performance bias considered the blinding of the operator for each outcome during experimental phase. The analysis of detection bias considered the blinding of the examiner for each outcome of the study. The analysis of attrition bias considered the sample loss rate and whether this loss was similar between groups for each outcome. The analysis of reporting bias considered whether the findings of the study were in accordance with the methodology presented. The analysis of other bias considered the calibration of the examiner for the outcome assessment.

For each study, these categories were rated as exhibiting a low, high or unclear (no information or uncertain) risk of bias. A high risk of bias was considered when the category had one or more responses that compromised the quality; an unclear risk of bias was considered when one or more pieces of information could not be determined; a low risk of bias was defined when all criteria of the each category were adequate and did not compromise the quality of the study. For the final classification of the risk of bias, disagreements between reviewers were resolved by consensus.

Strategy for Data Synthesis

Data analyses were performed in accordance with the Cochrane statistical guidelines (Higgins *et al.* 2011) using the meta and metafor packages in R version 3.6-0 (R Foundation for Statistical Computing, Vienna, Austria). Meta-analysis was performed on histometric and radiographic data to compare periapical lesion size between the test (OVX) and control (SHAM) groups. The mean difference (MD) between groups was calculated using a random effects model. A forest plot was created to illustrate the effects of the global estimation of the meta-analysis results. Statistical significance was defined as $P < 0.05$.

Assessment of Heterogeneity and Sensitivity Analyses

The extent of heterogeneity (interpreted based on the total percentage of variation among the studies concerned) was examined using the inconsistency test (I^2). I^2 statistics of 0

to 40% are interpreted as unimportant and those > 40% suggest the presence of moderate to considerable heterogeneity (Higgins *et al.* 2011).

For the histometric data, subgroup analysis was performed for rat lineage (Wistar and Sprague-Dawley) and experimental time, which was divided into OVX–PL (studies ≤ 21 days / > 21 days), PL–EUT (7 days / 21 days / 30 days), and OVX–EUT (28 days / 40 days / 100 days) periods.

Weighted random effects meta-regression analysis was performed to investigate the reason behind the variation in periapical lesion size in different studies and quantify how much of the heterogeneity in the estimates of periapical lesion size was explained by rat lineage and different experimental periods (OVX–PL, PL–EUT, and OVX–EUT).

Publication Bias

Publication bias was assessed for histometric and radiographic data using Egger's test and by visualizing the funnel plot. $P < 0.05$ indicated such bias.

RESULTS

Selection and Characteristics of Studies

Among 446 potentially eligible studies, 15 were selected for full-text analysis, 10 were included in the systematic review, and five were considered in the meta-analysis (Figure 1). Table 1 displays the main characteristics of the selected studies.

Measurement of periapical lesion size

Eight of the 10 studies included in the present systematic review evaluated periapical lesion size. Table 2 lists the characteristics and outcomes of these studies. In six of the eight studies, periapical lesion size was determined using radiographs (Xiong *et al.* 2007, Liu *et al.* 2010b, Zhang *et al.* 2011, Gomes-Filho *et al.* 2015b, Mello 2016, Brasil *et al.* 2017) and the radiographic exam was digital in three of these studies (Zhang *et al.* 2011, Mello 2016, Brasil *et al.* 2017). The area of the periapical lesions was measured using the ImageJ (Mello 2016, Brasil *et al.* 2017), Sigma Pro Scan (Zhang *et al.* 2011), SPOT RT v3.5 (Liu *et al.* 2010b), and Image-Pro (Xiong *et al.* 2007) software programs.

Only one study evaluated periapical lesion size using micro-CT (Wayama *et al.* 2015). However, the area of the periapical lesion (in mm^2) was calculated in the sagittal, coronal, and axial sections and volume was not measured as expected.

Five of the ten studies used histometric analysis to determine periapical lesion size (in mm^2) in sections obtained through histological processing (Xiong *et al.* 2007, Liu *et al.* 2010b, Gomes-Filho *et al.* 2015b, Wayama *et al.* 2015, Qian *et al.* 2016).

TRAP, RANKL, and OPG expression

Table 3 lists the characteristics and outcomes of the studies that evaluated TRAP, RANKL, and OPG expression. Several studies included in this systematic review evaluated TRAP activity in the periapical lesions (Xiong *et al.* 2007, Zhang *et al.* 2007, Liu *et al.* 2010b, Gomes-Filho *et al.* 2015a, Wayama *et al.* 2015, Qian *et al.* 2016) and three studies (Zhang *et al.* 2007, Gomes-Filho *et al.* 2015a, Qian *et al.* 2016) performed immunohistochemical analysis to determine RANKL and OPG expression in the periapical region.

Methodological Quality and Risk of Bias Assessment

Judgments on the risk of bias in the studies included are presented in Table S3. Most studies demonstrated a high risk of selection bias (Zhang *et al.* 2007, Liu *et al.* 2010b, Gomes-Filho *et al.* 2015a, Gomes-Filho *et al.* 2015b, Wayama *et al.* 2015, Qian *et al.* 2016). All studies had an unclear risk of performance bias. The outcomes of all studies demonstrated an uncertain or low risk of detection bias. Most studies demonstrated an uncertain risk of attrition bias (Zhang *et al.* 2007, Zhang *et al.* 2011, Gomes-Filho *et al.* 2015a, Gomes-Filho *et al.* 2015b, Wayama *et al.* 2015, Qian *et al.* 2016, Brasil *et al.* 2017) and a low risk of reporting bias (Xiong *et al.* 2007, Zhang *et al.* 2007, Liu *et al.* 2010b, Wayama *et al.* 2015, Brasil *et al.* 2017). One study demonstrated a low risk of other bias (calibration of the examiner for the outcome assessment) (Mello 2016).

Results of Meta-Analysis and Sensitivity Analysis

Due to the heterogeneity in the measurement units and methods among the studies included in the present systematic review, meta-analysis was possible only for the histometric and radiographic data. For the histometric data, five studies were included in the meta-analysis and two of these studies were considered twice, because apical lesion size was determined after two induction periods (Gomes-Filho *et al.* 2015b, Wayama *et al.* 2015). Based on the results of the meta-analysis, periapical lesions were significantly larger in the OVX group than the SHAM group (MD = 0.48 mm^2, n = 7, $P < 0.0001$, 95% confidence interval [CI]: 0.32 to 0.63). However, the global meta-analysis demonstrated high

heterogeneity (I^2 = 96.5%, 95% CI: 94.6 to 97.7) (Figure 2). The subgroup analysis for rat lineage showed that OVX rats had larger periapical lesions than SHAM rats considering both Wistar (MD = 0.71 mm^2, n = 4, $P < 0.0001$, 95% CI: 0.31 to 1.12, I^2 = 98%) and Sprague-Dawley (MD = 0.30 mm^2, n = 3, $P < 0.0001$, 95% CI: 0.18 to 0.42, I^2 = 88%) rats (Figure 2). Moreover, no statistically significant difference was found in mean periapical lesion size between Wistar and Sprague-Dawley rats ($P = 0.05$).

Table 4 displays the meta-analysis findings for subgroups according to experimental periods. The subgroup analysis revealed a statistically significant difference in periapical lesion size between groups with regard to the PL-EUT period ($P < 0.0001$). For this period, heterogeneity reduced (I^2 = 88.1 %) at 21 days and was null (I^2 = 0 %) at seven and 30 days.

Meta-regression was performed for rat lineage and experimental periods for the histometric findings and demonstrated that rat lineage together with the OVX-PL and PL-EUT periods explained 80.38% of the heterogeneity in the global meta-analysis (Table 5).

Meta-analysis was also performed for the radiographic findings. Considering periapical lesion size in mm^2, the OVX group had larger periapical lesions than the SHAM group (MD = 0.20mm^2, n = 3, $P = 0.0418$, 95% CI: 0.007 to 0.407, I^2 = 81.6%, 95% CI: 43.0 to 94.1). For data in pixels, no difference in periapical lesion size was found between OVX and SHAM groups (MD = 11.46 pixels, n = 4, $P = 0.1675$, 95% CI: -4.82 to 27.74, I^2 = 88.9%, 95% CI: 74.4 to 95.2).

Publication Bias

Despite the trend toward publication bias based on the visual exam of the funnel plot (Figure 3), the result of Egger's test demonstrated no publication bias among the articles with regard to the histometric and radiographic analyses ($P > 0.05$).

DISCUSSION

The present systematic review was conducted to investigate the influence of osteoporosis on periapical lesion size and markers of bone remodeling in female rats with apical periodontitis. Global meta-analysis of the histometric findings revealed that osteoporotic rats have larger periapical lesions compared to healthy animals. The subgroup analysis carried out for periapical lesion induction time maintained the statistically significant difference between the SHAM and OVX groups and reduced the heterogeneity among the studies. Based on these results, osteoporosis seems to affect mandibular bone metabolism to

the point of accelerating the progression of apical periodontitis. Systemically, a low estrogen concentration decreases bone mass and alters bone microarchitecture, thereby increasing the frailty of bone tissue (Cosman *et al.* 2014). The effects of osteoporosis on the mandible are unclear. However, some studies have shown that BMC in alveolar bone may be affected by estrogen deficiency (Dai *et al.* 2014, Liu *et al.* 2015, Xu *et al.* 2015), which may explain the development of larger periapical lesions in the presence of osteoporosis. Moreover, a longer induction time for apical periodontitis was associated with larger periapical lesion size and a larger mean difference between the SHAM and OVX groups. Thus, one may infer that time plays an important role in the progression of apical periodontitis, especially in the presence of osteoporosis. In healthy animals, alterations in periapical tissues are found within one week after pulp exposure and these bone changes are much more evident after one month (Tagger & Massler 1975).

The subgroup analysis of the histometric data for rat lineage also showed that both Wistar and Sprague-Dawley rats with osteoporosis had larger periapical lesions than healthy animals, with no statistically significant difference in mean periapical lesion size between lineages. However, the data analysis indicated a borderline result with regard to statistical significance. The authors believe that if a larger number of studies were analyzed, a possible statistically significant difference could be observed between rat lineages. It has been reported that Sprague-Dawley and Wistar rats are not equivalent models for the study of postmenopausal osteoporosis, since Sprague-Dawley rats exhibit significantly greater bone loss in the lumbar area following ovariectomy than Wistar rats (Fang *et al.* 2015). Moreover, the meta-regression revealed that rat lineage helped explain the high heterogeneity across the studies in the global meta-analysis; and, that the rat lineage plus the OVX–PL and PL–EUT periods together explained more than 80% of the heterogeneity among the studies submitted to meta-analysis. That is, rat lineage and period of estrogen deficiency until apical periodontitis induction added to the time of progression of the periapical pathology exert considerable influence on the variation in periapical lesion size.

The heterogeneity of the results in a meta-analysis may also be related to publication bias. Studies that do not reveal statistically significant differences among tested groups tend to not be published, which contributes to the risk of such bias. In the present review, Egger's test revealed no publication bias, but this finding was probably due to the small number of studies included in the meta-analysis, which decreases the power to detect possible publication bias. Moreover, the visual exam of the funnel plot indicated a tendency toward bias in the meta-analysis.

Unlike the histometric findings, the results of the X-ray measurements were not unanimous in demonstrating the influence of osteoporosis on the progression of periapical lesions. For data measured in mm^2, meta-analysis showed that rats with osteoporosis had larger periapical lesions than healthy animals. When the data were expressed in pixels, however, no significant difference in periapical lesion size was found between groups. Meta-analysis for the radiographic results was performed separately for units of measurement because a calibration reference is necessary for converting pixels into mm^2, which should ideally be determined in each analysis. The use of values established in the literature, as provided by two studies (Xiong *et al.* 2007, Cintra *et al.* 2014), could lead to biased results, which could compromise the outcome of the meta-analysis. Moreover, it was not possible to compare the radiographic and histometric results due to the weak correlation between x-ray imaging and histopathological results (Bornstein *et al.* 2015). In histometric analysis, the area of a periapical lesion is generally associated with only one of the roots and the determination of lesion size can be influenced by the method employed for cutting the slices. It is also difficult to include the entire extension of the lesion in the analysis, even when considering several slices per sample. In our understanding, X-rays enable a more reliable measurement than histological analysis, since the entire extension of the lesion can be measured, which has practical and clinical relevance, despite the two-dimensional shape. Thus, three-dimensional analysis, such as micro-CT, seems to be the best method for evaluating periapical lesion size.

Micro-CT analysis has been widely used to study bone metabolism in animal models (Xu *et al.* 2015, Kalatzis-Sousa *et al.* 2017, Wu *et al.* 2017). This is a non-destructive technique that offers high-resolution images on three dimensional microscales and is a very precise method that can detect slight changes in alveolar bone loss (Feldkamp *et al.* 1989, Park *et al.* 2007). Thus, micro-CT is a sensible alternative for evaluating the microstructure of alveolar bone when compared to conventional methods (Bouxsein *et al.* 2010), besides offering a high standard of accuracy for measuring periapical lesions in animal models (De Oliveira *et al.* 2015). Although micro-CT enables the volumetric evaluation of periapical lesions, (Wayama *et al.* 2015) performed two-dimensional analysis of the periapical lesion in the sagittal, coronal and axial sections, just as in a radiographic measurement.

With regard to TRAP and RANKL expression, the OVX groups had a higher number of positive cells than the SHAM groups (Xiong *et al.* 2007, Zhang *et al.* 2007, Liu *et al.* 2010b, Gomes-Filho *et al.* 2015a, Gomes-Filho *et al.* 2015b, Wayama *et al.* 2015, Qian *et al.* 2016). OPG activity had a tendency toward being increased in the OVX groups, but remained similar between groups in the majority of the periapical lesion induction times (Zhang *et al.*

2007, Gomes-Filho *et al.* 2015a, Qian *et al.* 2016). TRAP is a well-known biochemical marker of osteoclast function (Minkin 1982), while the RANK/RANKL/OPG system is an important factor that influences the production of osteoclasts (Pitari *et al.* 2015, Sağlam *et al.* 2015). When RANKL expression is up-regulated, OPG activity is generally down-regulated or not induced to the same degree as RANKL, such that the RANKL/OPG ratio changes in favor of osteoclastogenesis (Kearns *et al.* 2008). However, in the early stage of estrogen deficiency, OPG expression can be high, what may indicate a reactive response of the organism to resist bone resorption (Zhang *et al.* 2007). The findings of the studies included in the present review suggest that larger periapical lesions tend to have higher TRAP and RANKL expression, but the results remain unclear with regard to OPG activity.

Unlike other studies included in this review, Zhang *et al.* (2011) placed bacterial lipopolysaccharide (LPS) into the pulp chamber to promote inflammation. The periapical lesion was induced for 28 days in Sprague-Dawley rats and no statistically significant difference in periapical lesion size was found between the OVX and SHAM groups. A critical analysis of these results and comparisons with other studies were not performed because the authors used LPS and there was insufficient methodological information. Furthermore, the authors did not report the time between ovariectomy surgery and periapical lesion induction or the lesion area measured by radiographic analysis.

The use of rats as the animal model enables the evaluation of conditions and clinical situations that is sometimes not viable to perform on humans. Rats have advantages over other animals, such as the cost benefit, easy handling, easy standardization, and the control of many variables (Wronski *et al.* 1989). Moreover, rats have similarities with humans in many aspects, such as the presence of cortical and cancellous bone, and bone changes are similar to those observed in menopause and ageing (Wronski *et al.* 1989). The development of periapical lesions is also well established in the literature as equivalent to human counterparts (Kakehashi *et al.* 1965, Yu & Stashenko 1987). However, the greatest disadvantage of studies using animal models is the difficulty in translating the results to clinical practice.

The bilateral removal of the ovaries is recognized in the literature for the induction of estrogen deficiency in rats (Wronski *et al.* 1989, Kalu 1991). All articles included in the present literature review that evaluated estrogen levels found a reduction in this hormone in the group submitted to ovariectomy surgery (Xiong *et al.* 2007, Zhang *et al.* 2007, Liu *et al.* 2010b Zhang *et al.* 2011 Gomes-Filho *et al.* 2015a, Gomes-Filho *et al.* 2015b, Mello 2016, Qian *et al.* 2016, Brasil *et al.* 2017). Moreover, uterus weight was lower in OVX rats compared to SHAM animals (Gomes-Filho *et al.* 2015a, Gomes-Filho *et al.* 2015b, Wayama

et al. 2015, Mello 2016), which also proves the osteoporosis model. The presentation of this information in the primary studies ensured the comparison of healthy rats to hypoestrogenic rats.

The assessment of BMC is another way of confirming the osteoporosis induction model. In humans, dual-energy x-ray absorptiometry (DXA) is the main measure used to detect a reduction in BMC in the lumbar spine and femur and indicate the occurrence of osteoporosis (Kanis 1997, Cosman *et al.* 2014). Among the studies included in the present review, only Zhang *et al.* (2007) evaluated BMC in the femur using DXA. The authors found lower BMC in OVX rats than healthy rats, corroborating clinical findings in postmenopausal women. In a similar analysis, Mello (2016) evaluated BMC in the femur using computed tomography and also found lower mineral content in the OVX group than the SHAM group.

Examiner training and the determination of both inter-examiner and intra-examiner agreement are important basic research principles, because such procedures decrease the possibility of measurement bias and increase the reproducibility of the results (Chilton & Fleiss 1986). These principles were only reported in three studies (Zhang *et al.* 2011, Wayama *et al.* 2015, Mello 2016), in which the examiners were calibrated and trained to perform the radiographic or histometric analyses. However, only one study reported the reproducibility index (Mello 2016). The remaining studies did not offer an explicit description of these steps, which could compromise the validity of the findings. Moreover, several studies failed to report the blinding of the examiner, which is necessary to avoid biasing the analysis (Zhang *et al.* 2007, Gomes-Filho 2015a, Wayama *et al.* 2015, Qian *et al.* 2016).

The results of the present review should be interpreted with caution due to limitations with regard to the meta-analysis. It is important to note that the study by Qian *et al.* (2016), which was included in the meta-analysis, did not clarify the period in which the histometric analysis was performed (7, 14 or 21 days after pulp exposure) or whether the results represented a compilation of measurements obtained at different induction times of the periapical lesion. For the meta-analysis, we considered 21 days of pulp exposure and n = 15; we decided to maintain this study, because heterogeneity was not improved after excluding it. The study by Gomes-Filho *et al.* (2015b) presented confusing data in relation to the radiographic analysis. In the methods section, the authors state that the area of the periapical lesion was evaluated in pixels and converted into mm^2. However, the data on the radiographic density of the periapical lesion were expressed in pixels. For the meta-analysis, we considered data in pixels. On the other hand, the study by Zhang *et al.* (2011) was not included in the meta-analysis due to insufficient information regarding the radiographic analysis. Another

limitation of the present review is that most studies were unclear with regard to the experimental unit in the different evaluations; although the number of rats per group was reported, the fact that the researchers performed pulp exposure in more than one tooth per animal raises doubts about the sample size in some analyses. Attempts were made to contact the authors to clarify this issue. When no response was obtained, the number of rats per group was considered to be the experimental unit for the meta-analysis. Moreover, considering all studies included in this review, only three reported the rate of sample loss during the experiment (Xiong *et al.* 2007, Liu *et al.* 2010b, Mello 2016), most studies had an unclear risk of bias for the criteria analyzed. The reasons that contributed to this risk of bias were related to the lack of a detailed description of exposure variables and measurement quality.

No standardization was found in the execution of the studies or drafting of the manuscripts included in the present review. It is imperative for studies involving animal models to follow the ARRIVE guidelines, which presents essential points to be detailed in articles. Descriptions should be offered on characteristics of the sample, such as the age of the rats and the number of animals in each experimental group as well as the sample number in each analysis (e.g., number of teeth). Moreover, articles should clarify the units of measure, statistical units, sample number in each group and exposure time of the variable of interest. The calibration and blinding of the evaluators is another point that must be adequately described, since it exerts a direct influence on the methodological quality of studies.

CONCLUSIONS

In summary, the results of the present meta-analysis suggest a relationship between osteoporosis and the progression of apical periodontitis, as osteoporotic rats had larger periapical lesions than healthy animals. Moreover, the sub-group analysis revealed that periapical lesion induction time plays an important role in the progression of apical periodontitis, especially in the presence of osteoporosis. The results of the meta-regression analysis showed that rat lineage and experimental time help explain the variability in periapical lesion size. Nonetheless, the few reports involving animal models for this subject, the considerable variability in the outcomes, and the methodological limitations of the primary studies underscore the need for further well-delineated studies to answer our research question accurately.

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LIST OF FIGURES

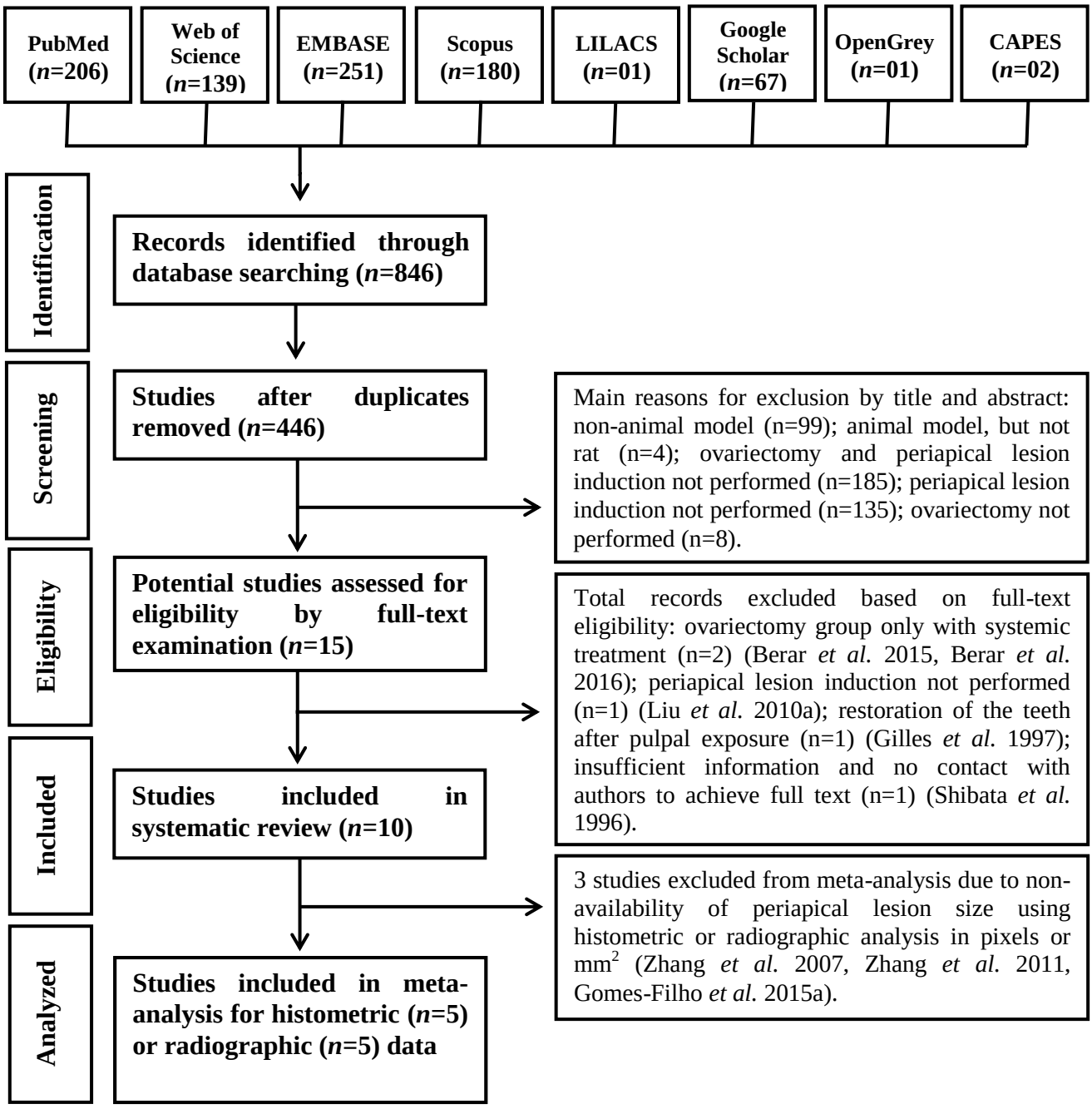

Figure 1. Flow diagram of study selection process according to PRISMA statement

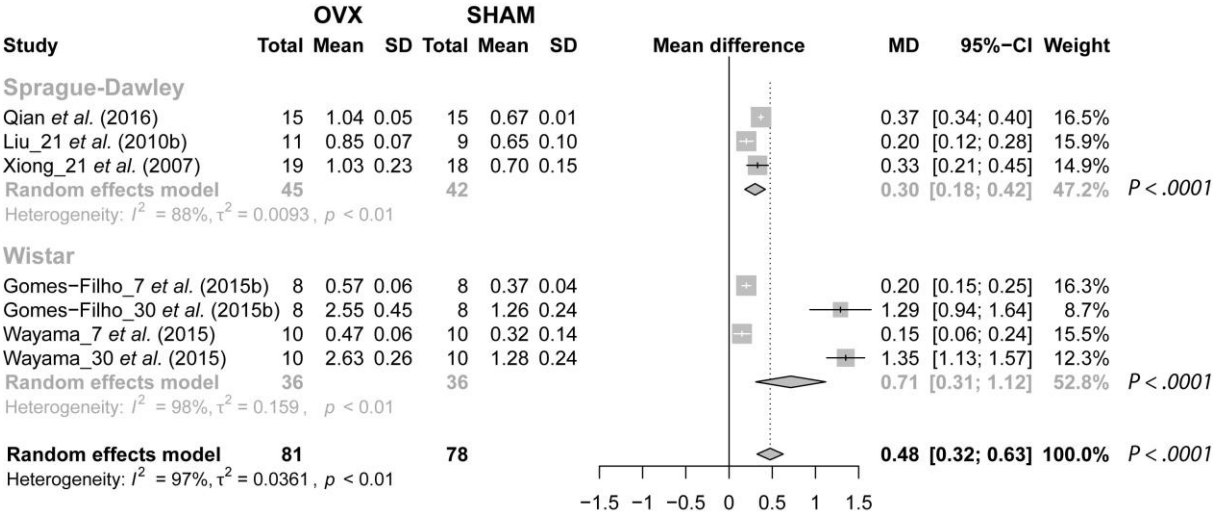

Figure 2. Global and subgroup (rat lineage) meta-analysis for periapical lesion size by histometric analysis.

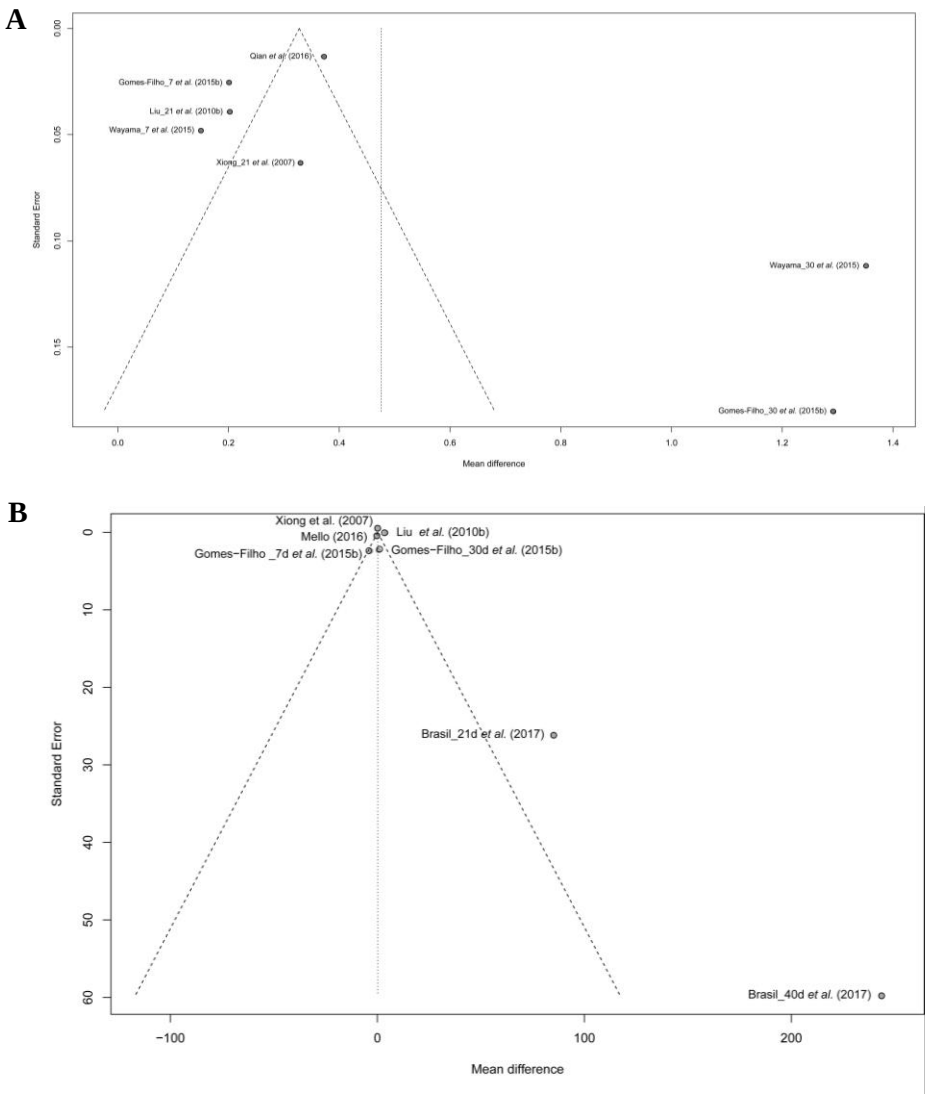

Figure 3. Publication bias assessment. (a) Histometric analysis. (b) Radiographic analysis in both mm^2 and pixels.

LIST OF TABLES

Table 1. Main characteristics of the studies included in systematic review

Study	Country	Rat lineage	Age	n value (rats/group)	OVX – PL period	PL – EUT period	OVX – EUT period	Teeth	Confirmation of osteoporosis model	Main outcomes
Brasil *et al.* 2017	Brazil	Wistar	90 d	6	120 d	21 d / 40 d	141 d / 160 d	Left mandibular first molar	Estrogen (RIA)	X-ray
Mello 2016	Brazil	Wistar	70 d	10	21 d	35 d	56 d	Right mandibular first molar	CT, uterus weight and estrogen (CHL)	X-ray
Qian *et al.* 2016	China	Sprague-Dawley	84 d	5	7 d	7 d / 14 d / 21 d	14 d / 21 d / 28 d	Mandibular first molars	Estrogen (RIA)	Hist, TRAP, RANKL, OPG
Gomes-Filho *et al.* 2015a	Brazil	Wistar	180 d	8	70 d / 93 d	7 d / 30 d	100 d	Mandibular first molars	Uterus weight and estradiol (RIA)	RANKL, OPG
Gomes-Filho *et al.* 2015b	Brazil	Wistar	180 d	8	70 d / 93 d	7 d / 30 d	100 d	Right and left mandibular first molars	Uterus weight and estrogen (RIA)	X-ray, Hist, TRAP
Wayama *et al.* 2015	Japan	Wistar	180 d	5	10 d / 33 d	7 d / 30 d	40 d	Mandibular first molars	Uterus weight	Micro-CT, Hist, TRAP
Zhang *et al.* 2011	USA	Sprague-Dawley	U	8 / 13	U	28 d	U	Right first and second maxillary molars	Estrogen (ELISA)	X-ray
Liu *et al.* 2010b	China	Sprague-Dawley	90-120 d	6 / 7	7 d	21 d	28 d	Right and left mandibular first molars	Estrogen (RIA)	X-ray, Hist, TRAP
Zhang *et al.* 2007	China	Sprague-Dawley	U	5	7 d	0 / 7 d / 14 d / 21 d / 28 d	7 d / 14 d / 21 d / 28 d / 35 d	Mandibular first molars	DXA of femur and estrogen (RIA)	TRAP, RANKL, OPG
Xiong *et al.* 2007	China	Sprague-Dawley	U	10	21 d	21 d	42 d	Right and left mandibular first molars	Estrogen (RIA)	X-ray, Hist, TRAP

OVX, ovariectomy surgery; PL, periapical lesion; EUT, euthanasia; X-ray, radiographic analysis; TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, osteoprotegerin; Hist, histometric analysis; Micro-CT, micro-computed tomography; CT, multislice computed tomography; DXA, dual energy x-ray absorptiometry; RIA, radioimmunoassay; CHL, chemiluminescence; ELISA, Enzyme Linked Immunosorbent Assay; d, day; U, unclear.

Table 2. Results from studies that evaluated periapical lesion size

	X-ray				Histometry				Micro-CT			
Study	**n value (teeth/ group)**	**Unit of measure**	**Results**		**n value (teeth/ group)**	**Unit of measure**	**Results**		**n value (teeth/ group)**	**Unit of measure**	**Results**	
Brasil *et al.* 2017	6[§]	Pixel (mean±SD)	SHAM-21d: 268.5±40.98 OVX-21d: 353.7±49.31 SHAM-40d: 304.8±36.98 OVX-40d: 548.4±141.7	OVX-40d exhibited larger PLs than SHAM-21d and SHAM-40d ($P < 0.05$)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Mello 2016	7-9[†]	mm^2 (mean±SE)	SHAM: 1.653±0.356 OVX: 1.403±0.322	No significant difference between OVX and SHAM groups ($P > 0.05$)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Qian *et al.* 2016	n/a	n/a	n/a	n/a	U	mm^2 (mean±SD)	SHAM*: 0.67±0.02 OVX*: 1.05±0.05	OVX group had larger PLs than SHAM group ($P < 0.05$)	n/a	n/a	n/a	n/a
Gomes-Filho *et al.* 2015b	U	Pixel (?)	SHAM-7d: 82.84±4.49 OVX-7d: 82.58±5.03 SHAM-30d: 77.04±5.24 OVX-30d: 77.05±5.13	No significant difference in radiographic density of PLs between OVX and SHAM groups at same time point ($P > 0.05$)	U	mm^2 (?)	SHAM-7d: 0.37±0.04 OVX-7d: 0.57±0.06 SHAM-30d: 1.26±0.24 OVX-30d: 2.55±0.45	At both time points, OVX group exhibited larger PLs than SHAM groups ($P < 0.05$)	n/a	n/a	n/a	n/a

Continuation of Table 2:

Wayama *et al.* 2015	n/a	n/a	n/a	n/a	U	mm^2 (mean±SD)	SHAM-7d: 0.32±0.04 OVX-7d: 0.47±0.06 SHAM-30d: 1.28±0.24 OVX-30d: 2.63±0.26	At both time points, OVX group had larger PLs than SHAM groups ($P < 0.05$)	U	mm^2 (mean±SD)	**AXIAL** SHAM-7d 0.98±0.18 OVX-7d: 1.15±0.05 SHAM-30d: 1.07±0.04 OVX-30d: 1.34±0.05 **CORONAL** SHAM-7d: 0.90±0.22 OVX-7d: 1.13±0.10 SHAM-30d: 1.04±0.05 OVX-30d: 1.24±0.08 **SAGITAL** SHAM-7d: 0.06±0.01 OVX-7d: 0.13±0.02 SHAM-30d: 0.18±0.07 OVX-30d: 0.37±0.06	At both time points, bone loss in all sections was greater in OVX group than SHAM group ($P < 0.05$)
Zhang *et al.* 2011	U	μm^2 (?)	U	No significant difference between OVX and SHAM groups ($P > .05$)	n/a	n/a	n/a	a	n/a	n/a	n/a	n/a
Liu *et al.* 2010b	SHAM: 9† OVX: 11†	mm^2 (mean±SD)	SHAM*: 0.64±0.08 OVX*: 0.77±0.06	OVX group exhibited larger PLs than SHAM group ($P < 0.05$)	SHAM: 9† OVX: 11†	mm^2 (mean±SD)	SHAM*: 0.65±0.1 OVX*: 0.85±0.07	OVX group exhibited larger PLs than SHAM group ($P < 0.05$)	n/a	n/a	n/a	n/a
Xiong *et al*, 2007	SHAM:18† OVX: 19†	mm^2 (mean±SD)	SHAM: 0.583±0.149 OVX: 0.925±0.206	OVX group exhibited larger PLs than SHAM group ($P < 0.05$)	SHAM:12† OVX: 11†	mm^2 (mean±SD)	SHAM: 0.707±0.158 OVX: 1.036±0.231	OVX group exhibited larger PLs than SHAM group ($P < 0.05$)	n/a	n/a	n/a	n/a

X-ray, radiographic analysis; Micro-CT, micro-computed tomography; OVX, ovariectomy; SHAM, ovariectomy simulation; PLs, periapical lesions; mm^2, square millimeter; μm^2, square micrometer; d, day; SD, standard deviation; SE, standard error; n/a, not available; * Graphically imputed values by WebPlotDigitizer software; U, unclear; ?, unreported statistical measure units; § sample loss rate not available; † including sample loss rate.

Table 3. Results fromstudies that evaluated TRAP, RANKL, and OPG expression

	TRAP				RANKL				OPG			
Study	**n value (teeth/ group)**	**Unit of measure**	**Results**		**n value (teeth/ group)**	**Unit of measure**	**Results**		**n value (teeth/ group)**	**Unit of measure**	**Results**	
Qian *et al.* 2016	U	cells/hpf (mean±SD)	SHAM-7d*: 2.86±0.34 OVX-7d*: 4.22±0.54 SHAM-14d*: 4.60±0.48 OVX-14d*: 9.17±1.36 SHAM-21d*: 4.77±0.75 OVX-21d*: 8.80±1.29	At all time points, number of osteoclasts in OVX group was greater than in SHAM group ($P < 0.05$)	U	cells/hpf (mean±SD)	SHAM-7d*:7. 14±0.74 OVX-7d*: 12.35±0.89 SHAM-14d*: 18.45±1.63 OVX-14d*: 27.97±1.64 SHAM-21d*: 6.84±0.45 OVX-21d*: 11.75±0.75	In all time points, number of RANKL-positive cells in OVX group was greater than in SHAM group ($P < 0.05$)	U	cells/hpf (mean±SD)	SHAM-7d*: 5.92±0.69 OVX-7d*: 8.62±0.69 SHAM-14d*: 8.90±0.87 OVX-14d*: 13.16±0.63 SHAM-21d*: 5.57±0.63 OVX-21d*: 6.89±0.75	On 14th day, number of OPG-positive cells in OVX group was greater than in SHAM group ($P < 0.05$)
Gomes-Filho *et al.* 2015a	n/a	n/a	n/a	n/a	U	Scores 0 = absence of IR 1= low IR pattern 2 = moderate IR pattern 3 = high IR pattern	In SHAM group, score 1 and score 2 prevailed on days 7 and 30, respectively. In OVX group, score 3 and score 2 prevailed on days 7 and 30, respectively.	OVX group exhibited higher IR pattern when compared to SHAM group on day 7 ($P < 0.05$).	U	Scores 0 = absence of IR 1= low IR pattern 2 = moderate IR pattern 3 = high IR pattern	Score 1 prevailed in SHAM and OVX groups at all time points.	No significant differences between any experimental groups at either time point ($P > 0.05$).
Gomes-Filho *et al.* 2015b	U	cells/mm^2 (?)	SHAM-7d: 4.48±1.60 OVX-7d: 7.68±1.34 SHAM-30d: 5.55±2.07 OVX-30d: 9.63±5.15	At both time points, OVX group exhibited greater number of TRAP-positive cells than SHAM group ($P < 0.05$)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Wayama *et al.* 2015	U	cells/mm^2 (mean±SD)	SHAM-7d: 3.42±1.43 OVX-7d: 6.61±1.32 SHAM-30d:4.59±1.47 OVX-30d: 8.67±1.69	At both time points, OVX group exhibited greater number of TRAP-positive cells than SHAM group ($P < 0.05$)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

Continuation of Table 3:

Liu *et al.* 2010b	SHAM: 9[†] OVX: 11[†]	cells/hpf (mean±SD)	SHAM*: 3.13±0.27 OVX*: 4.05±0.50	OVX group exhibited greater number of osteoclasts than SHAM group ($P < 0.05$)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a
Zhang *et al*, 2007	U	cells/hpf (mean±SD)	SHAM-0d: 0 (zero) OVX-0: 4.00±1.58 SHAM-7d: 2.40±0.55 OVX-7d*: 7.81±1.11 SHAM-14: 10.60±1.81 OVX-14d*: 12.00±1.01 SHAM-21: 4.40±1.14 OVX-21: 6.00±1.22 SHAM-28: 1.20±1.09 OVX-28: 4.40±1.95	In days 0, 7, and 28, OVX group exhibited greater number of TRAP-positive cells ($P < 0.05$)	U	cells/hpf (mean±SD)	SHAM-0d*: 0.39±0.89 OVX-0d*: 0.79±0.91 SHAM-7d*: 4.00±1.08 OVX-7d*: 6.60±1.16 SHAM-14d*: 10.04±0.68 OVX-14d*: 10.83±1.30 SHAM-21d*: 3.40±0.58 OVX-21d*: 3.22±0.27 SHAM-28d*: 0.80±0.91 OVX-28d*: 0.64±0.68	On days 7, 21, and 28, RANKL activity in OVX groups was greater than in SHAM group ($P < 0.05$)	U	cells/hpf (mean±SD)	SHAM-0d*: 0.81±1.08 OVX-0d*: 2.00±1.18 SHAM-7d*: 12.00±2.01 OVX-7d*: 15.78±2.27 SHAM-14d*: 23.54±3.39 OVX-14d*: 26.92±2.62 SHAM-21d*: 7.54±1.54 OVX-21d*: 10.88±1.07 SHAM-28d*: 1.46±2.11 OVX-28d*: 3.73±1.16	On day 7, OPG values were greater in OVX group than SHAM group ($P < 0.05$)
(Xiong *et al.*, 2007)	SHAM:18[†] OVX: 19[†]	cells/hpf (mean±SD)	SHAM: 4.5±0.9 OVX: 5.0±1.1	No significant difference in number of osteoclast between OVX and SHAM groups ($P > 0.05$)	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a

TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, osteoprotegerin; OVX, ovariectomy; SHAM, ovariectomy simulation; cells/hpf, cells per high-power field; cells/mm^2, cells per square millimeter; IR, immunoreactivity; d, day; SD, standard deviation; n/a, not available; * Graphically imputed values by WebPlotDigitizer software; U, unclear; ?, unreported statistical measure units; [†] including sample loss rate.

Table 4. Subgroup analysis for periapical lesion size measured by histometry according to experimental period

	Category	Time	n	MD	CI	I^2-statistic (%)	*p*-value
OVX–PL	0	≤ 21 d	4	0.52	0.29–0.76	96.9	0.62
	1	> 21 d	3	0.43	0.16–071	94.7	
PL–EUT	0	7 d	2	0.18	0.14–0.23	0	
	1	21 d	3	0.30	0.18–0.42	88.1	< 0.0001
	2	30 d	2	1.33	1.14–1.51	0	
OVX–EUT	0	28 d	2	0.29	0.12–0.45	94	
	1	40 d	3	0.73	0.05–1.14	97.9	0.42
	2	100 d	3	0.73	-0.33–1.79	97.2	

OVX, ovariectomy; PL, periapical lesion; EUT, euthanasia; MD, mean difference; CI, confidence interval; d, days. *P*-value, *P*-value between categories

Table 5. Meta-regression for histometric findings

	%
OVX–PL	0
PL–EUT	19
OVX–PL + OVX–EUT	47
RAT LINEAGE	68
RAT LINEAGE + OVX–PL + OVX–EUT	80.38

OVX, ovariectomy; PL, periapical lesion; EUT, euthanasia

Table S1. Results of searches of electronic databases

Electronic Databases	Date/Time	Results	Duplicates
MEDLINE/PubMed	June 5, 2017 (21:40)	206	No
Web of Science	June 6, 2017 (10:52)	139	No
Scopus	June 6, 2017 (09:48)	180	No
EMBASE	June 6, 2017 (10:37)	251	3
LILACS	June 5, 2017 (21:55)	1	No
Google Scholar	June 6, 2017 (08:58)	67	No
OpenGrey	June 6, 2017 (08:32)	1	No
CAPES	June 6, 2017 (11:30)	1	No

Table S2. Search strategy used in electronic databases.

	PubMed/MEDLINE - Search terms
No. 16	No. 3 AND No. 7 AND No. 15
No. 15	No. 8 OR No. 9 OR No. 10 OR No. 11 OR No. 12 OR No. 13 OR No.14
No. 14	All fields: “lesion periapical” OR “periapical, lesion” OR “apical lesion” OR “lesion apical” OR “lesions periapical” OR “periapical, lesions” OR “apical lesions” OR “lesions, apical” OR “apical periodontitis lesion” OR “periapical inflammation” OR “endodontic treatment” OR “oral infection process” OR “oral infectious process” OR “oral infection” OR “endodontic infection” OR “endodontic pathology” OR “periradicular lesion” OR “periradicular lesions” OR “root canal infection” OR “alveolar bone loss” OR “oral bone resorption” OR “alveolar bone resorption”
No. 13	All fields: “periapical disease” [MeSH Terms] OR “disease, periapical” OR “diseases, periapical” OR “periapical disease”
No. 12	All fields: “jaw diseases” [MeSH Terms] OR “jaw diseases” OR “jaw disease” OR “disease, jaw” OR “diseases, jaw”
No. 11	All fields: “radicular cyst” [MeSH Terms] OR “cyst, radicular” OR “cysts, radicular” OR “periapical cyst” OR “cyst, periapical” OR “periapical cyst” OR “periodontal cyst, apical” OR “apical periodontal cyst” OR “apical periodontal cysts” OR “cyst, apical periodontal” OR “cysts, apical periodontal” OR “ periodontal cysts, apical”
No. 10	All fields: “periapical abscess” [MeSH Terms] OR “periapical abscess” OR “dento alveolar abscess, apical” OR “abscess, apical dento alveolar” OR “abscesses, apical dento alveolar” OR “apical dento alveolar abscess” OR “apical dento alveolar abscesses” OR “dento alveolar abscesses, apical” OR “periodontitis, apical, suppurative” OR “Periapical periodontitis, suppurative” OR “periapical periodontitides, suppurative” OR “periodontitides, suppurative periapical” OR “periodontitis, suppurative periapical” OR “suppurative periapical periodontitides” OR “suppurative periapical periodontitis” OR “alveolar abscess, apical” OR “abscess, apical alveolar” OR “abscesses, apical alveolar” OR “alveolar abscesses, apical” OR “apical alveolar abscess” OR “apical alveolar abscesses” OR “abscess, periapical” OR “abscesses, periapical” OR “periapical abscesses” OR “ radicular cyst”
No. 9	All fields: “granuloma” [MeSH Terms] OR “granuloma” OR “granulomas” OR “periapical granuloma” [MeSH Terms] OR “periapical granuloma” OR “periapical granulomas” OR “granuloma, periapical” OR “granulomas, periapical” OR “periapical periodontitis, chronic nonsuppurative” OR “periodontitis, apical, chronic nonsuppurative” OR “dental granulomas” OR “granulomas, dental” OR “dental granuloma” OR “granuloma, dental”
No. 8	All fields: "periapical periodontitis" [MeSH Terms] OR "periapical periodontitis” OR “periapical periodontitides” OR “periodontitides, periapical” OR “periodontitis, periapical” OR “periodontitis, apical” OR “apical periodontitides” OR “apical periodontitis” OR “periodontitides, apical” OR “periodontitis, acute nonsuppurative” OR “acute nonsuppurative periodontitides” OR “acute nonsuppurative periodontitis” OR “nonsuppurative periodontitides, acute” OR “nonsuppurative periodontitis, acute” OR “periodontitides, acute nonsuppurative”
No. 7	No. 4 OR No. 5 OR No. 6
No. 6	All fields: “ovariectomy” [MeSH Terms] OR “ovariectomy” OR “ovariectomies” OR “oophorectomy” OR “oophorectomies” OR “castration, female” OR

	"castrations, female" OR "female castration" OR "female castrations" OR "bilateral ovariectomy" OR "bilateral ovariectomies" OR "ovariectomies, bilateral" OR "ovariectomy, bilateral" OR "ovariectomized" OR "ovariectomization" OR "hysterectomy" OR "ovariohysterectomy" OR "estrogen deficiency" OR "estrogen-deficiency" OR "oestrogen deficiency"
No. 5	All fields: "osteoporosis, postmenopausal" [MeSH Terms] OR "osteoporosis, postmenopausal" OR "bone loss, postmenopausal" OR "bone losses, postmenopausal" OR "postmenopausal bone losses" OR "postmenopausal osteoporosis" OR "osteoporoses, postmenopausal" OR "postmenopausal osteoporoses" OR "perimenopausal bone loss" OR "postmenopausal bone loss" OR "bone loss, perimenopausal" OR "bone losses, perimenopausal" OR "perimenopausal bone losses" OR "osteoporosis, post-menopausal" OR "osteoporoses, post-menopausal" OR "osteoporosis, post menopausal" OR "post-menopausal osteoporoses" OR "post-menopausal osteoporosis" OR "post-menopausal" OR "menopause"
No. 4	All fields: "osteoporosis" [MeSH Terms] OR "osteoporosis" OR "osteoporosis, post-traumatic" OR "osteoporosis, post traumatic" OR "post-traumatic osteoporosis" OR "post-traumatic, osteoporosis" OR "osteoporosis, senile" OR "osteoporoses, senile" OR "senile osteoporosis" OR "senile osteoporoses" OR "osteoporosis, involutional" OR "osteoporosis, age-related" OR "osteoporosis, age related" OR "bone loss, age-related" OR "age-related bone loss" OR "age-related bone losses" OR "bone loss, age related" OR "bone losses, age-related" OR "age-related osteoporosis" OR "age related osteoporosis" OR "age-related osteoporosis" OR "osteoporoses, age-related" OR "osteoporotic" OR "osteopenia"
No. 3	No. 1 OR No. 2
No. 2	All fields: "mice" [MeSH Terms] OR "mice" OR "mice, laboratory" OR "laboratory mice" OR "rodents" OR "rodent"
No. 1	All fields: "rat" [Mesh Terms] OR "rats" OR "rattus" OR "rat" OR "rats, laboratory" OR "laboratory rat" OR "laboratory rats" OR "rat, laboratory" OR "rattus norvegicus" OR "rats, norway"

	Embase - Search terms
No. 4	No. 1 and No. 2 and No. 3
No. 3	All fields: "periapical periodontitis" OR "periapical periodontitis" OR "periapical periodontitides" OR "periodontitides, periapical" OR "periodontitis, periapical" OR "periodontitis, apical" OR "apical periodontitides" OR "apical periodontitis" OR "periodontitides, apical" OR "periodontitis, acute nonsuppurative" OR "acute nonsuppurative periodontitides" OR "acute nonsuppurative periodontitis" OR "nonsuppurative periodontitides, acute" OR "nonsuppurative periodontitis, acute" OR "periodontitides, acute nonsuppurative" OR "lesion periapical" OR "periapical, lesion" OR "apical lesion" OR "lesion apical" OR "lesions periapical" OR "periapical, lesions" OR "apical lesions" OR "lesions, apical" OR "granuloma" OR "granulomas" OR "periapical granuloma" OR "periapical granulomas" OR "granuloma, periapical" OR "granulomas, periapical" OR "periapical periodontitis, chronic nonsuppurative" OR "periodontitis, apical, chronic nonsuppurative" OR "Dental Granulomas" OR "apical periodontitis lesion" OR "granulomas, dental" OR dental granuloma" OR "Granuloma, Dental" OR "apical periodontitis lesion" OR "periapical inflammation" OR "endodontic treatment" OR "oral infection process" OR "oral infectious process" OR "oral infection" OR "endodontic infection" OR "endodontic pathology" OR "periradicular lesion" OR "periradicular lesions" OR "root canal infection" OR "alveolar bone loss"" OR "oral bone resorption" OR "alveolar bone resorption" OR "periapical abscess" OR "Dento alveolar

	Abscess, Apical" OR "Abscess, Apical Dento alveolar" OR "Abscesses, Apical Dento alveolar" OR "Apical Dento alveolar Abscess" OR "Apical Dento alveolar Abscesses" OR "Dento alveolar Abscesses, Apical" OR "Periodontitis, Apical, Suppurative" OR "Periapical Periodontitis, Suppurative" OR "Periapical Periodontitides, Suppurative" OR "Periodontitides, Suppurative Periapical" OR "Periodontitis, Suppurative Periapical" OR "Suppurative Periapical Periodontitides" OR "Suppurative Periapical Periodontitis" OR "Alveolar Abscess, Apical" OR "Abscess, Apical Alveolar" OR "Abscesses, Apical Alveolar" OR "Alveolar Abscesses, Apical" OR "Apical Alveolar Abscess" OR "Apical Alveolar Abscesses" OR "Abscess, Periapical" OR "Abscesses, Periapical" OR "Periapical Abscesses" OR "radicular cyst" OR "cyst, radicular" OR "cysts, radicular" OR "periapical cyst" OR "cyst, periapical" OR "periapical cyst" OR "periodontal cyst, apical" OR "apical periodontal cyst" OR "apical periodontal cysts" OR "cyst, apical periodontal" OR "cysts, apical periodontal" OR " periodontal cysts, apical" OR "jaw diseases" OR "jaw disease" OR "disease, jaw" OR "diseases, jaw" OR "periapical disease" OR "disease, periapical" OR "diseases, periapical" OR "periapical disease")
No. 2	All fields: "osteoporosis" OR "osteoporosis" OR "osteoporosis, post-traumatic" OR "osteoporosis, post traumatic" OR "post-traumatic osteoporosis" OR "post-traumatic, osteoporosis" OR "osteoporosis, senile" OR "osteoporoses, senile" OR "senile osteoporosis" OR "senile osteoporoses" OR "osteoporosis, involutional" OR "osteoporosis, age-related" OR "osteoporosis, age related" OR "bone loss, age-related" OR "age-related bone loss" OR "age-related bone losses" OR "bone loss, age related" OR "bone losses, age-related" OR "age-related osteoporosis" OR "age related osteoporosis" OR "age-related osteoporosis" OR "osteoporoses, age-related" OR "osteoporotic" OR "osteopenia" OR "osteoporosis, postmenopausal" OR "osteoporosis, postmenopausal" OR "bone loss, postmenopausal" OR "bone losses, postmenopausal" OR "postmenopausal bone losses" OR "postmenopausal osteoporosis" OR "osteoporoses, postmenopausal" OR "postmenopausal osteoporoses" OR "perimenopausal bone loss" OR "postmenopausal bone loss" OR "bone loss, perimenopausal" OR "bone losses, perimenopausal" OR "perimenopausal bone losses" OR "osteoporosis, post-menopausal" OR "osteoporoses, post-menopausal" OR "osteoporosis, post menopausal" OR "post-menopausal osteoporoses" OR "post-menopausal osteoporosis" OR "post-menopausal" OR "menopause" OR "ovariectomy" OR "ovariectomy" OR "ovariectomies" OR "oophorectomy" OR "oophorectomies" OR "castration, female" OR "castrations, female" OR "female castration" OR "female castrations" OR "bilateral ovariectomy" OR "bilateral ovariectomies" OR "ovariectomies, bilateral" OR "ovariectomy, bilateral" OR "ovariectomized" OR "ovariectomization" OR "hysterectomy" OR "ovariohysterectomy" OR "estrogen deficiency" OR "estrogen-deficiency" OR "oestrogen deficiency"
No. 1	All fields: "rat" OR "rats" OR "rattus" OR "rat" OR "rats, laboratory" OR "laboratory rat" OR "laboratory rats" OR "rat, laboratory" OR " rattus norvegicus" OR "rats, norway" OR "mice" OR "mice" OR "mice, laboratory" OR "laboratory mice" OR "rodents" OR "rodent"

	Web of Science - Search terms
No. 4	No. 1 and No. 2 and No. 3
No. 3	Topic: ("periapical periodontitis" OR "periapical periodontitis" OR "periapical periodontitides" OR "periodontitides, periapical" OR "periodontitis, periapical" OR "periodontitis, apical" OR "apical periodontitides" OR "apical periodontitis" OR "periodontitides, apical" OR "periodontitis, acute nonsuppurative" OR "acute nonsuppurative periodontitides" OR "acute nonsuppurative periodontitis" OR "nonsuppurative periodontitides, acute" OR "nonsuppurative periodontitis, acute"

	OR “periodontitides, acute nonsuppurative” OR “lesion periapical” OR “periapical, lesion” OR “apical lesion” OR “lesion apical” OR “lesions periapical” OR “periapical, lesions” OR “apical lesions” OR “lesions, apical” OR “granuloma” OR “granulomas” OR “periapical granuloma” OR “periapical granulomas” OR “granuloma, periapical” OR “granulomas, periapical” OR “periapical periodontitis, chronic nonsuppurative” OR “periodontitis, apical, chronic nonsuppurative” OR “Dental Granulomas” OR “apical periodontitis lesion” OR “granulomas, dental” OR dental granuloma” OR “Granuloma, Dental” OR “apical periodontitis lesion” OR “periapical inflammation” OR “endodontic treatment” OR “oral infection process” OR “oral infectious process” OR “oral infection” OR “endodontic infection” OR “endodontic pathology” OR “periradicular lesion” OR “periradicular lesions” OR “root canal infection” OR “alveolar bone loss”” OR “oral bone resorption” OR “alveolar bone resorption” OR “periapical abscess” OR “Dento alveolar Abscess, Apical” OR “Abscess, Apical Dento alveolar” OR “Abscesses, Apical Dento alveolar” OR “Apical Dento alveolar Abscess” OR “Apical Dento alveolar Abscesses” OR “Dento alveolar Abscesses, Apical” OR “Periodontitis, Apical, Suppurative” OR “Periapical Periodontitis, Suppurative” OR “Periapical Periodontitides, Suppurative” OR “Periodontitides, Suppurative Periapical” OR “Periodontitis, Suppurative Periapical” OR “Suppurative Periapical Periodontitides” OR “Suppurative Periapical Periodontitis” OR “Alveolar Abscess, Apical” OR “Abscess, Apical Alveolar” OR “Abscesses, Apical Alveolar” OR “Alveolar Abscesses, Apical” OR “Apical Alveolar Abscess” OR “Apical Alveolar Abscesses” OR “Abscess, Periapical” OR “Abscesses, Periapical” OR “Periapical Abscesses” OR “radicular cyst” OR “cyst, radicular” OR “cysts, radicular” OR “periapical cyst” OR “cyst, periapical” OR “periapical cyst” OR “periodontal cyst, apical” OR “apical periodontal cyst” OR “apical periodontal cysts” OR “cyst, apical periodontal” OR “cysts, apical periodontal” OR “ periodontal cysts, apical” OR “jaw diseases” OR “jaw disease” OR “disease, jaw” OR “diseases, jaw” OR “periapical disease” OR “disease, periapical” OR “diseases, periapical” OR “periapical disease”)
No. 2	Topic: (“osteoporosis” OR “osteoporosis” OR “osteoporosis, post-traumatic” OR “osteoporosis, post traumatic” OR “post-traumatic osteoporosis” OR “post-traumatic, osteoporosis” OR “osteoporosis, senile” OR “osteoporoses, senile” OR “senile osteoporosis” OR “senile osteoporoses” OR “osteoporosis, involutional” OR “osteoporosis, age-related” OR “osteoporosis, age related” OR “bone loss, age-related” OR “age-related bone loss” OR “age-related bone losses” OR “bone loss, age related” OR “bone losses, age-related” OR “age-related osteoporosis” OR “age related osteoporosis” OR “age-related osteoporosis” OR “osteoporoses, age-related” OR “osteoporotic” OR “osteopenia” OR “osteoporosis, postmenopausal” OR “osteoporosis, postmenopausal” OR “bone loss, postmenopausal” OR “bone losses, postmenopausal” OR “postmenopausal bone losses” OR “postmenopausal osteoporosis” OR “osteoporoses, postmenopausal” OR “postmenopausal osteoporoses” OR “perimenopausal bone loss” OR “postmenopausal bone loss” OR “bone loss, perimenopausal” OR “bone losses, perimenopausal” OR “perimenopausal bone losses” OR “osteoporosis, post-menopausal” OR “osteoporoses, post-menopausal” OR “osteoporosis, post menopausal” OR “post-menopausal osteoporoses” OR “post-menopausal osteoporosis” OR “post-menopausal” OR “menopause” OR “ovariectomy” OR “ovariectomy” OR “ovariectomies” OR “oophorectomy” OR “oophorectomies” OR “castration, female” OR “castrations, female” OR “female castration” OR “female castrations” OR “bilateral ovariectomy” OR “bilateral ovariectomies” OR “ovariectomies, bilateral” OR “ovariectomy, bilateral” OR “ovariectomized” OR “ovariectomization” OR “hysterectomy” OR “ovariohysterectomy” OR “estrogen deficiency” OR “estrogen-deficiency” OR “oestrogen deficiency”)
No. 1	Topic: (“rat” OR “rats” OR “rattus” OR “rat” OR “rats, laboratory” OR “laboratory rat” OR “laboratory rats” OR “rat, laboratory” OR “ rattus norvegicus” OR “rats, norway” OR “mice” OR “mice” OR “mice, laboratory” OR “laboratory mice” OR

	"rodents" OR "rodent")

	SCOPUS - Search terms
No. 4	No. 1 AND No. 2 AND No. 3
No. 3	TITLE-ABS-KEY ("periapical periodontitis" OR "periapical periodontitis" OR "periapical periodontitides" OR "periodontitides, periapical" OR "periodontitis, periapical" OR "periodontitis, apical" OR "apical periodontitides" OR "apical periodontitis" OR "periodontitides, apical" OR "periodontitis, acute nonsuppurative" OR "acute nonsuppurative periodontitides" OR "acute nonsuppurative periodontitis" OR "nonsuppurative periodontitides, acute" OR "nonsuppurative periodontitis, acute" OR "periodontitides, acute nonsuppurative" OR "lesion periapical" OR "periapical, lesion" OR "apical lesion" OR "lesion apical" OR "lesions periapical" OR "periapical, lesions" OR "apical lesions" OR "lesions, apical" OR "granuloma" OR "granulomas" OR "periapical granuloma" OR "periapical granulomas" OR "granuloma, periapical" OR "granulomas, periapical" OR "periapical periodontitis, chronic nonsuppurative" OR "periodontitis, apical, chronic nonsuppurative" OR "Dental Granulomas" OR "apical periodontitis lesion" OR "granulomas, dental" OR dental granuloma" OR "Granuloma, Dental" OR "apical periodontitis lesion" OR "periapical inflammation" OR "endodontic treatment" OR "oral infection process" OR "oral infectious process" OR "oral infection" OR "endodontic infection" OR "endodontic pathology" OR "periradicular lesion" OR "periradicular lesions" OR "root canal infection" OR "alveolar bone loss"" OR "oral bone resorption" OR "alveolar bone resorption" OR "periapical abscess" OR "Dento alveolar Abscess, Apical" OR "Abscess, Apical Dento alveolar" OR "Abscesses, Apical Dento alveolar" OR "Apical Dento alveolar Abscess" OR "Apical Dento alveolar Abscesses" OR "Dento alveolar Abscesses, Apical" OR "Periodontitis, Apical, Suppurative" OR "Periapical Periodontitis, Suppurative" OR "Periapical Periodontitides, Suppurative" OR "Periodontitides, Suppurative Periapical" OR "Periodontitis, Suppurative Periapical" OR "Suppurative Periapical Periodontitides" OR "Suppurative Periapical Periodontitis" OR "Alveolar Abscess, Apical" OR "Abscess, Apical Alveolar" OR "Abscesses, Apical Alveolar" OR "Alveolar Abscesses, Apical" OR "Apical Alveolar Abscess" OR "Apical Alveolar Abscesses" OR "Abscess, Periapical" OR "Abscesses, Periapical" OR "Periapical Abscesses" OR "radicular cyst" OR "cyst, radicular" OR "cysts, radicular" OR "periapical cyst" OR "cyst, periapical" OR "periapical cyst" OR "periodontal cyst, apical" OR "apical periodontal cyst" OR "apical periodontal cysts" OR "cyst, apical periodontal" OR "cysts, apical periodontal" OR " periodontal cysts, apical" OR "jaw diseases" OR "jaw disease" OR "disease, jaw" OR "diseases, jaw" OR "periapical disease" OR "disease, periapical" OR "diseases, periapical" OR "periapical disease")
No. 2	TITLE-ABS-KEY ("osteoporosis" OR "osteoporosis" OR "osteoporosis, post-traumatic" OR "osteoporosis, post traumatic" OR "post-traumatic osteoporosis" OR "post-traumatic, osteoporosis" OR "osteoporosis, senile" OR "osteoporoses, senile" OR "senile osteoporosis" OR "senile osteoporoses" OR "osteoporosis, involutional" OR "osteoporosis, age-related" OR "osteoporosis, age related" OR "bone loss, age-related" OR "age-related bone loss" OR "age-related bone losses" OR "bone loss, age related" OR "bone losses, age-related" OR "age-related osteoporosis" OR "age related osteoporosis" OR "age-related osteoporosis" OR "osteoporoses, age-related" OR "osteoporotic" OR "osteopenia" OR "osteoporosis, postmenopausal" OR "osteoporosis, postmenopausal" OR "bone loss, postmenopausal" OR "bone losses, postmenopausal" OR "postmenopausal bone losses" OR "postmenopausal osteoporosis" OR "osteoporoses, postmenopausal" OR "postmenopausal osteoporoses" OR "perimenopausal bone loss" OR "postmenopausal bone loss" OR "bone loss, perimenopausal" OR "bone losses, perimenopausal" OR "perimenopausal bone losses" OR "osteoporosis, post-menopausal" OR "osteoporoses, post-menopausal" OR "osteoporosis, post

	menopausal" OR "post-menopausal osteoporoses" OR "post-menopausal osteoporosis" OR "post-menopausal" OR "menopause" OR "ovariectomy" OR "ovariectomy" OR "ovariectomies" OR "oophorectomy" OR "oophorectomies" OR "castration, female" OR "castrations, female" OR "female castration" OR "female castrations" OR "bilateral ovariectomy" OR "bilateral ovariectomies" OR "ovariectomies, bilateral" OR "ovariectomy, bilateral" OR "ovariectomized" OR "ovariectomization" OR "hysterectomy" OR "ovariohysterectomy" OR "estrogen deficiency" OR "estrogen-deficiency" OR "oestrogen deficiency")
No. 1	TITLE-ABS-KEY ("rat" OR "rats" OR "rattus" OR "rat" OR "rats, laboratory" OR "laboratory rat" OR "laboratory rats" OR "rat, laboratory" OR " rattus norvegicus" OR "rats, norway" OR "mice" OR "mice" OR "mice, laboratory" OR "laboratory mice" OR "rodents" OR "rodent")

	LILACS, PROSPERO and Open Grey - Search terms
No. 4	No.1 AND No. 2 AND No. 3
No.3	"periapical periodontitis" OR "apical periodontitis" OR "periapical periodontitides" OR "apical periodontitides" OR "periapical lesion" OR "apical lesion" OR "apical periodontitis lesion" OR "periapical granuloma" OR "apical periodontitis lesion" OR "periapical inflammation" OR "radicular cyst" OR "diseases, periapical" OR "periradicular lesion" OR "periapical abscess"
No. 2	Osteoporosis OR osteoporotic OR osteopenia OR "osteoporosis, senile" OR "osteoporosis, age-related" OR "osteoporosis, postmenopausal" OR "bone loss, postmenopausal" OR ovariectomy OR "estrogen deficiency"
No. 1	Rats OR rat OR rattus OR mice OR rodents OR rodent

In the LILACS database, the terms were also used in the Portuguese and Spanish languages to set up the search strategy. For LILACS, PROSPERO and Open Grey, the terms of population, intervention and outcome were combined of different ways.

	Schoolar Google - Search terms
All words	Ovariectomy osteoporosis rat mice periodontitis periapical lesion estrogen

Table S3. Risk of bias of studies included in systematic review

Study	Selection bias Random sequence generation	Performance bias Blinding of performance operator	Detection bias Blinding of outcome assessment	Attrition bias Incomplete outcome data	Reporting bias Selective reporting	Other bias Calibration of outcome assessment
Brasil *et al.* 2017	U	U*	L*	U*	L	U
Mello 2016	U	U*	L*	L*	L	L
Qian *et al.* 2016	H	U*	TRAP/RANKL/OPG = U Hist = L	TRAP/RANKL/OPG = U Hist = L	H	U
Gomes-Filho e*t al.* 2015a	H	U*	U*	U*	L	U
Gomes-Filho *et al.* 2015b	H	U*	Hist/TRAP = L X-ray = U	U*	H	U
Wayama *et al.* 2015	H	U*	U*	U*	L	H
Zhang *et al.* 2011	U	U*	L*	U*	H	H
Liu *et al.* 2010b	H	U*	L*	L*	L	U
Zhang *et al.* 2007	H	U*	L*	U*	L	U
Xiong *et al.* 2007	U	U*	L*	X-ray/TRAP = L Hist = H	L	U

TRAP, tartrate-resistant acid phosphatase; RANKL, receptor activator of nuclear factor kappa-B ligand; OPG, osteoprotegerin; Hist, histometry; L, low; U, unclear; H, High; U, unclear; * Risk of bias presented for all outcomes of study

3 ARTIGO 2 – EFFECT OF OSTEOPOROSIS AND ZOLEDRONIC ACID ON THE DEVELOPMENT OF APICAL PERIODONTITIS: ANALYSIS BY MICRO-CT

Este artigo foi submetido à publicação no periódico *Journal of Endodontics*, Fator de impacto = 2.788; Qualis A1, no dia 23 de maio de 2017 e aguarda parecer do Editor Chefe.
As normas para publicação estão descritas no Anexo B.

EFFECT OF OSTEOPOROSIS AND ZOLEDRONIC ACID ON THE DEVELOPMENT OF APICAL PERIODONTITIS: ANALYSIS BY MICRO-CT

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ABSTRACT

Introduction: The aim of this study was to evaluate the effects of osteoporosis and systemic treatment with zoledronic acid (ZOL) on the development of apical periodontitis in rats using micro-computed tomography (micro-CT). **Methods:** Forty female Wistar rats were divided into 4 experimental groups (n=10): sham surgery and saline treatment (SHAM); sham surgery and ZOL treatment (SHAM-ZOL); ovariectomy surgery and saline treatment (OVX); ovariectomy surgery and ZOL treatment (OVX-ZOL). Twenty-one days after surgery, all animals were submitted to apical periodontitis induction on the right mandibular first molar. ZOL was administered intraperitoneally in a single application. Periapical lesions were measured by radiography (X-ray) and micro-CT. Bone mineral content (BMC) of the femur and mandible was analyzed by computed tomography (CT). The data were analyzed by two-way analysis of variance (ANOVA) and Bonferroni tests ($P < .05$). **Results:** X-ray and micro-CT analyses showed no statistically significantly difference among the groups in the area and volume of periapical lesions ($P > .05$), respectively. BMC in the femur condyles of OVX group (cancellous and cortical bone) was significantly lower than that of the SHAM group ($P < .05$). There was no statistical difference among groups in the BMC of the mandible ($P >.05$). **Conclusions:** Osteoporosis induction caused a decrease in the BMC of femur, but it was not able to cause significant changes in the BMC of the mandible and in the progression of the periapical lesions. A single dose of ZOL did not prevent volumetric changes in the periapical lesion of SHAM and OVX groups.

Keywords: Apical periodontitis; bisphosphonate; bone metabolism; estrogen deficiency; micro-computed tomography; osteoporosis.

INTRODUCTION

Apical periodontitis is an inflammatory disease caused by bacterial infection of the root canal system, and it is characterized by destruction of periapical tissues (1). Recently, studies have reported that systemic conditions, such as diabetes mellitus, hypertension, cardiovascular disease, and osteoporosis, may influence the development of endodontic periapical infection (2-5). Although these conditions do not cause apical periodontitis, they are considered modulating factors because they can influence the development, progression, and severity of the periapical diseases as well as in its prevalence (2, 3, 6, 7).

Osteoporosis is one of the most common and relevant bone disorders. It is characterized by reduction in bone mass, decreased bone strength, and micro-architectural deterioration of bone tissue (8). Osteoporosis is known to progress with increasing age, and the primary cause is thought to be derived from an imbalance between bone formation and bone resorption due mainly to postmenopausal estrogen deficiency (8, 9). Hypoestrogenism can aggravate oral conditions, such as periodontitis (10), tooth movement (11), and apical periodontitis progression (7, 12).

There are many therapies available for the treatment of osteoporosis, including estrogen therapy, denosumab, and bisphosphonates (8). Zoledronic acid (ZOL) is a potent nitrogen-containing bisphosphonate that acts on the cells of the bone remodeling process, decreasing bone turnover and inhibiting the inflammatory mediators related to the development of bone lesions (13-15). In a recent study, ZOL treatment decreased the size of periapical lesions in osteoporotic rats compared to untreated osteoporotic animals (16).

Considering this, the aim of this study was to evaluate the effect of osteoporosis and the systemic treatment with ZOL on the development of apical periodontitis in rats using micro-computed tomography (micro-CT). The null hypotheses of this study were: (1) osteoporosis has no influence on the progression of apical periodontitis in rats; (2) ZOL treatment has no influence on the development of apical periodontitis in rats with osteoporosis.

MATERIALS AND METHODS

Forty female Wistar rats (N = 40) (70 days of age, 150-200 g) from the Center for Biological Models and Experiments (CeMBE) were used in this study. The animals were housed in a microisolator (4 animals per cage) in a temperature- and humidity-controlled room (22±1°C and 60±5% relative humidity), with a 12-h light/dark cycle and free access to

food and water. The experimental procedures were approved by the Ethics Committee on Animal Use of the Pontifical Catholic University of Rio Grande do Sul (CEUA-PUCRS Registration 15/00436) in compliance with the applicable ethical guidelines and regulations of the International Guiding Principles for Biomedical Research Involving Animals (17).

Experimental Design

Forty female Wistar rats were equally randomized into 4 groups (n = 10): SHAM – simulation of ovariectomy surgery (sham surgery) and saline solution treatment; SHAM-ZOL – sham surgery and ZOL treatment; OVX – ovariectomy surgery and saline solution treatment; OVX-ZOL – ovariectomy surgery and ZOL treatment.

Ovariectomy

The animals were anesthetized intraperitoneally with xylazine (10 mg/kg; Anasedan®, Sespo Industry, and LTD Trade, Paulínia, Brazil) and ketamine (100 mg/kg; Dopalen®, Sespo Industry, and LTD Trade, Paulínia, Brazil). Bilateral ovariectomy (18) was performed in 20 animals. The remaining rats were subjected to sham surgery. To eliminate any discomfort, paracetamol was administered (80 mg/kg) by gavage for 48 hours after surgery (CeMBE/PUCRS protocol).

Induction of Periapical Lesion

Twenty-one days after ovariectomy and sham surgery, animals were anesthetized as described in the previous section. The endodontic access of the right mandibular first molar was performed in all rats in order to induce periapical lesion (19). Dental pulps were exposed by drilling cavities on the central portion of the occlusal surface with a 1011 HL round bur at high speed (KG Sorensen, Cotia, Brazil) to a depth nearly equal to the half-diameter of the bur (0.5 mm). A #10 endodontic file (Dentsply-Maillefer, Ballaigues, Switzerland) was used to explore the root canal and remove remnants of pulp tissue. The teeth were left open to the oral environment throughout the experimental period.

Zoledronic Acid

ZOL (Zometa®, Novartis, Basel, Switzerland) was administered intraperitoneally in a single application (100 μg/kg body weight) (20) on the same day of the induction of periapical lesions (SHAM-ZOL and OVX-ZOL groups). An intraperitoneal dose of saline solution (0.1 mg/kg body weight) was administered in the control groups (SHAM and OVX groups) (20).

Sample Collection

After 35 days, the euthanasia was performed by deep anesthesia with sevoflurane (Sevorane®, Abbott Laboratories Ltda Brazil, São Paulo, SP, Brazil). The uteri of the rats was removed and weighed. Blood samples were obtained for analysis of estradiol levels. The mandible and femur were removed, dissected, and placed in formalin solution (10%) for tissue fixation. The femur and the left hemimandible were submitted to computed tomography (CT). The right side of the mandible was used to perform radiographic (X-ray) and micro-CT analyses.

Uterus Weight and Serum Estradiol Level

Uterus weight was determined using a precision scale (Mettler Toledo, Barueri, Brazil). Blood samples were collected by cardiac puncture vacutainer device (BD Vacutainer® Blood Collection Eclipse ™ Needle) and centrifuged for 12 min to obtain the serum. The estradiol level was verified by chemiluminescence (Estradiol IMMULITE, Diagnostic Products Corp., Los Angeles, USA). Procedures were performed according to the manufacturer's instructions. The minimum detectable dose of estradiol was 11.0 pg/mL.

Bone Mineral Content (BMC) Analysis

The femur and left hemimandible were submitted to CT (BrightSpeed, GE Healthcare, Little Chalfont, UK) to determine the cortical and cancellous bone mineral content (BMC). The images were analyzed by a trained radiologist, who was blinded to the comparison groups, with the auxiliary of the OsiriX software (Prixmeo SARL, Geneva, Switzerland). The measurement of BMC in Hounsfield units (HU) was based on the study of Kuroda et al (21), with minor adaptations. The BMC of the mandible was observed in the condyle and periapical region near the mesial root of the first molar. In the femur, 3 measurements were carried out: the first measurement was obtained in the condyle, at 2 mm from the growth plate; the second and third measurements were performed in the epiphysis to 8.5 mm and 17 mm from the cartilage, respectively. All measurements were calculated for the cortical and cancellous bone except for the mandibular condyle, in which it was only possible to identify the cortical bone.

X-Ray Analysis

The right hemimandible was X-rayed in a standardized manner using a digital sensor (Digora, Soredex, Orion Corporation, Helsinki, Finland) and X-ray machine (Dabi Atlante,

Ribeirão Preto, Brazil). The samples were positioned perpendicularly at a distance of 15 cm from the mandible/film with an exposure time of 0.63 seconds (70 kVp, 10 mA). The area of periapical lesions associated with the apex of the mesial root of the first molar were measured and quantified in square millimeters (mm^2) using the ImageJ software (National Institutes of Health, Washington, DC). An endodontist calibrated and blinded to the experimental groups performed the measurements of the lesion area. The intraclass correlation coefficient was calculated to determine the intra-reproducibility (ICC = 0.97).

Micro-CT Scanning and Analysis

The scanning procedure was performed using a desktop X-ray microfocus CT Scanner (SkyScan 1172, Bruker-microCT, Kontich, Belgium) at 89 kV, 112 µA, rotational angle of 180° and with an isotropic resolution of 12.8 µm, resulting in 700 to 800 slices per root. The images obtained from the scan were reconstructed to show two-dimensional (2D) slices of the root structures with the NRecon 1.6.9.3 software (Bruker-microCT, Kontich, Belgium). The CTAn 1.11.10 software (Bruker-microCT, Kontich, Belgium) was used for measuring the volume of the periapical lesion (mm^3). The analysis was performed by one calibrated and blinded observer (ICC = 0.96).

Statistical Analysis

The results were expressed as mean ± standard error (SE) for each experimental group. Comparative analysis of the data was performed by parametric two-way analysis of variance (ANOVA) and Bonferroni multiple comparisons tests. Data analyses were performed using STATA 12.0 software (Stata Corp. College Station, Texas, USA). The level of statistical significance was set at 5% ($P < .05$).

RESULTS

Uterus Weight and Serum Estradiol Level

The uterus weight revealed a significant main effect of ovariectomy, treatment, and ovariectomy × treatment interaction [F (1, 27) = 195.47, $P < .001$; 10.02, $P = .003$; and 9.88, $P = .004$, respectively]. Bonferroni tests showed that the uteri of the OVX groups weighed less than those of the SHAM groups ($P < .05$) (Figure 1A).

For serum estradiol levels, there was also a main effect of ovariectomy, treatment, and ovariectomy × treatment [F (1, 27) = 136.71, $P < .001$; 77.46, $P < .001$; and 55.47, $P < .001$,

respectively]. OVX and OVX-ZOL groups demonstrated significantly lower levels of estradiol when compared to SHAM groups ($P < .05$) (Figure 1B).

BMC Analysis

The results of BMC of the femur and mandible are described in Table 1. For femur-2 mm, BMC analysis of the cortical bone revealed a main effect of treatment and ovariectomy × treatment interaction [$F(1, 27) = 13.71$, $P = .001$; and 8.74, $P = .006$, respectively] and the cancellous bone showed a main effect of ovariectomy, treatment, and ovariectomy × treatment [$F (1, 27) = 7.40$, $P = .011$; 53.27, $P < .001$; and 9.31, $P = .005$, respectively].

BMC analysis of the cancellous bone of the femur-8.5 mm revealed a main effect of treatment [$F (1, 27) = 13.81$, $P < .001$]. The cancellous bone of the femur-17 mm showed a main effect of ovariectomy, treatment, and ovariectomy × treatment interaction [$F (1, 27) = 4.21$, $P = .049$; 9.79, $P = .004$; and 4.52, $P = .042$, respectively].

In the mandible, the mineral content analysis of the cancellous bone in the periapical region revealed a main effect of ovariectomy × treatment interaction [$F (1, 22) = 5.22$, $P = .032$]. In the condyle, a main effect of ovariectomy × treatment [$F (1, 22) = 6.27$, $P = .018$] was observed.

The OVX group showed statistically lesser BMC than the other groups in the condyle of the femur (2 mm), for both cortical ($P < .05$) and cancellous bone ($P < .05$). The BMC of the cancellous bone of the femur was statistically increased in OVX rats treated with ZOL ($P < .05$). There were no statistical differences among groups for BMC of the mandible ($P > .05$).

X-ray and micro-CT analysis

Two-way ANOVA of the periapical lesion size from X-ray and micro-CT analyses revealed that there are no main effects of the ovariectomy, treatment, and ovariectomy × treatment interaction (Table 2). Figures 2 and 3 show the periapical lesion associated with the right mandibular first molar in the X-ray and micro-CT images, respectively.

DISCUSSION

Postmenopausal osteoporosis is a significant health problem in elderly women. Bone loss caused by osteoporosis has been proven to be associated with the reduction of estrogen levels (6, 22). Estrogen deficiency results in changes in the bone remodeling process, in which

the resorption exceeds the formation, leading to the loss of bone mass. The imbalance in bone turnover can negatively affect oral conditions (6, 10-12).

The literature suggests a possible relationship between osteoporosis and endodontic alterations, which has encouraged animal model studies. However, these studies present conflicting results on the effects that estrogen deficiency and osteoporosis treatment have in the progression of apical periodontitis (7, 12, 23-27). Thus, the objective of this study was to simulate the initial effects of postmenopausal osteoporosis and to verify the influence of ZOL on apical periodontitis.

In the present study, the OVX groups had lower uterus weight and estradiol levels than SHAM surgery rats, confirming the efficacy of the osteoporosis induction model. The bilateral ovarian removal results in suppression of estrogen. This deficiency leads to a reduction in the uterus weight due to atrophy of the organ and promotes changes in the bone remodeling patterns (18, 25).

X-ray and micro-CT analyses showed no significant differences in the area and volume of the periapical lesion among the groups after 35 days of pulp exposure, indicating that neither osteoporosis nor ZOL treatment influenced the development of apical periodontitis. Therefore, the null hypotheses were accepted. However, studies using X-rays have shown significantly greater periapical lesions in OVX rats compared to control groups after 20 days of apical periodontitis induction (12, 24). The lineages of rats used as the experimental model in the cited studies may explain the inconsistency between their results and ours. Sprague-Dawley and Wistar rats are not equivalent models for the study of post-menopausal osteoporosis since Sprague-Dawley rats have shown significantly greater bone loss in the lumbar area after ovariectomy than Wistar rats (28).

The hypoestrogenism period before periapical lesion induction can also influence on the progression of the apical periodontitis because the longer this period, the more intense are the systemic alterations at the moment of pulp exposure. Ovariectomized rats present progressive bone loss, mainly in the cancellous bone (29). This loss is evident as early as 2 weeks after ovariectomy; however, its intensity increases considerably until 100 days after surgery (29). Our study used 21 days of estrogen deprivation until pulp exposure and did not observe a statistically significant difference in the size of the periapical lesion among the groups. Nonetheless, a recent study with a longer estrogen deficiency period (120 days) showed that OVX rats had significantly greater periapical lesions than SHAM rats (7).

The literature examination revealed that there is only one study evaluating the size of the periapical lesion in osteoporotic rats using micro-CT (16). The micro-CT allows a high-

resolution noninvasive analysis, three-dimensional (3D) quantitative evaluation, and detection of slight changes in alveolar bone loss (30, 31), but it is not yet a clinically viable technique (32). Although the micro-CT allows 3D analysis, the authors performed 2D evaluation of the periapical lesion areas in the axial, coronal and sagittal sections. Therefore, the comparison of the micro-CT results with our results is not possible. Notwithstanding, Wayama et al (16) showed that ZOL treatment decreased the size of the periapical lesion in osteoporotic rats, at both 7 and 30 days after periapical lesion induction. These findings differ from our 2D analysis, probably because of the differences in the ZOL administration protocols. In the reported study, the ZOL was applied intravenously once a week for 4 weeks. Here, a single dose of ZOL was used, which is consistent with the recommended treatment for osteoporosis in postmenopausal women (33).

In the current study, the ovariectomized rats showed a decrease in BMC values in the femur, indicating the presence of systemic changes resulting from osteoporosis induction. However, mandibular bone did not present this pattern. This result can also explain the absence of differences in the periapical lesion size among the groups. The mandibular bone is less susceptible to estrogen deficiency than the femur, and it needs a longer hypoestrogenism period to develop changes in bone microarchitecture (21). In a recent study, with a longer period of estrogen deficiency between ovariectomy and euthanasia, it was possible to observe that OVX rats had larger periapical lesions than rats in the SHAM group (7).

We also showed that administration of ZOL increased the femur's BMC mainly in the cancellous bone. ZOL is an injectable bisphosphonate, which is an alternative option to oral bisphosphonates. A single annual application of ZOL significantly improves the bone mineral density and decreases the risk of bone fracture (33). The high potency and affinity of ZOL for hydroxyapatite and the highest bioavailability afforded by the application form contribute to its prolonged action (13, 14). Surprisingly, we observed that SHAM rats treated with ZOL presented higher estrogen levels. However, if the mechanism of bone loss prevention is in part attributed to its action on the estrogen hormone, this is not known yet.

Some methodological considerations are needed in this study. Even though the dual energy x-ray absorptiometry (DXA) is considered the reference standard for mineral density analyses, it was not used in this study because changes in bone mineral density in rat mandibles are not detectable with this method (21), and no T-scores have been defined for mandible bones (34). CT has the highest contrast resolution and uses combinations of many X-ray images taken from different angles to produce cross-sectional images, allowing the examiner to see inside the bone without cutting (35). Moreover, it is believed that

osteoporosis development leads to skeletal-site specific effects, including different functions for cortical and cancellous bone, justifying its analyses separately (35).

In summary, the current study has shown that osteoporosis induction caused a decrease in the BMC of the femur. Nevertheless, BMC of the mandible and the development of periapical lesions were not affected by estrogen deficiency. A single dose of ZOL increases BMC in the femur, but did not prevent volumetric changes in the periapical lesions of SHAM and OVX groups. We suggest that further animal model and clinical studies should be performed. The influence of the different periods of estrogen deficiency and ZOL administration to treat osteoporosis on the progression of apical periodontitis should be clarified in order to assist in the treatment of osteoporotic patients, especially postmenopausal women.

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LIST OF FIGURES

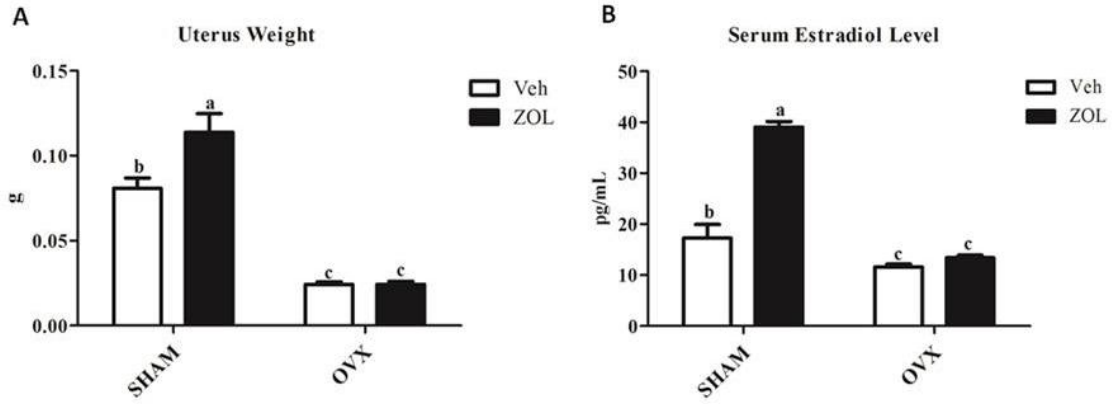

Figure 1. (A) Uterus weight and (B) serum estradiol level. Columns represent the mean of 7–9 experiments. Different letters indicate statistical difference ($P < .05$).

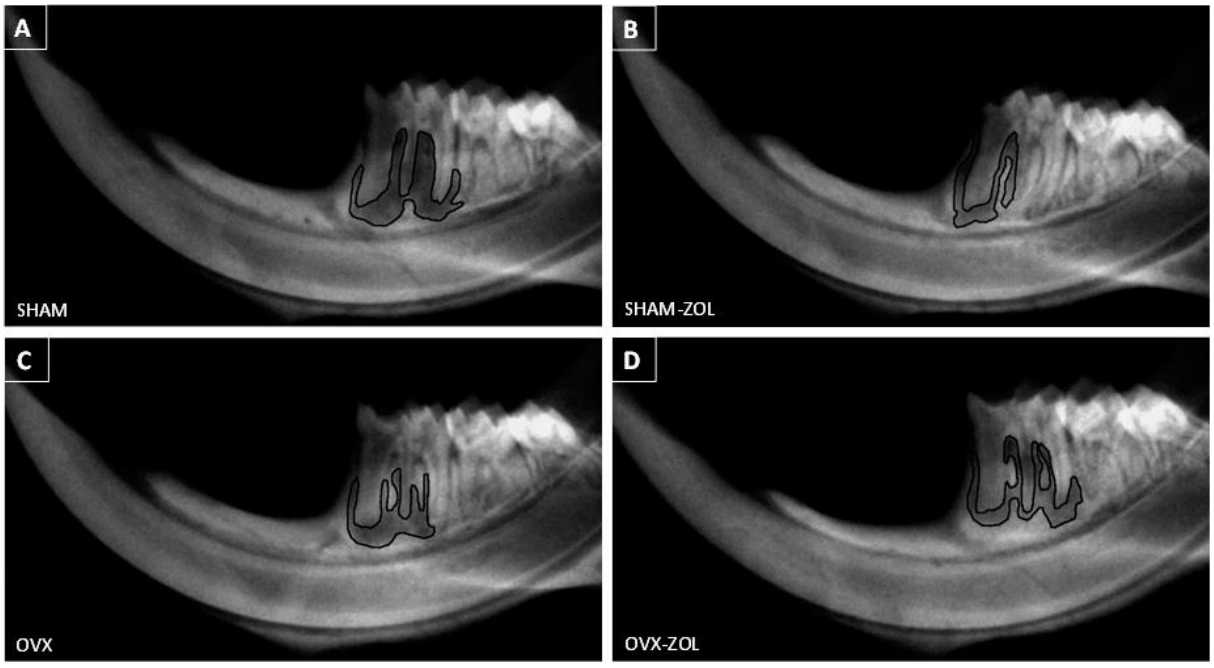

Figure 2. Representative radiographic images of the delimitation of the periapical lesion area in the SHAM (A), SHAM-ZOL (B), OVX (C), and OVX-ZOL (D) groups.

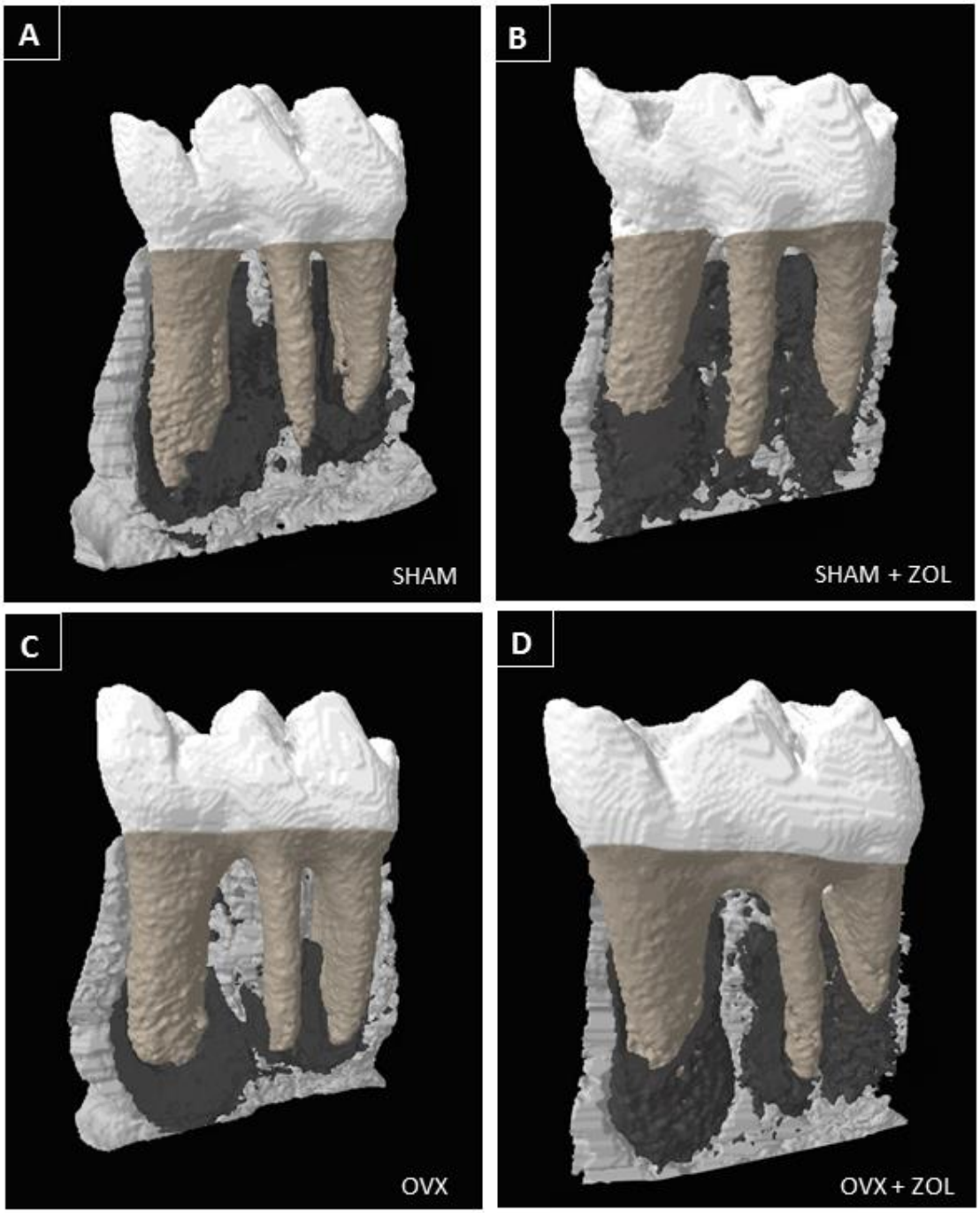

Figure 3. Representative three-dimensional (3D) images of the periapical lesion volume in the SHAM (A), SHAM-ZOL (B), OVX (C), and OVX-ZOL (D) groups.

LIST OF TABLES

Table 1. Mean ± standard error (SE) of the bone mineral content (BMC) in Hounsfield units (HU).

	Cortical bone			
	SHAM	**SHAM-ZOL**	**OVX**	**OVX-ZOL**
Femur				
2 mm	1476.39 ± 63.10[a]	1520.25 ± 48,35[a]	1211.80 ± 70.61[b]	1640.64 ± 60.62[a]
8.5 mm	1448.96 ± 79.59	1462.83 ± 56.70	1451.03 ± 79.01	1672.22 ± 72.14
17 mm	1777.14 ± 85.43	1711.17 ± 67.82	1690.14 ± 54.74	1889.83 ± 75.73
Mandible				
Periapical	2022.00 ± 87.20	1941.20 ± 136.70	1999.00 ± 83.34	2164.18 ± 74.76
Condyle	1526.40 ± 87.83	1334.39 ± 61.10	1404.23 ± 87.30	1584.44 ± 43.50

	Cancellous bone			
	SHAM	**SHAM-ZOL**	**OVX**	**OVX-ZOL**
Femur				
2 mm	875.68 ± 95.02[a]	1153.12 ± 62.55[ac]	498.50 ± 52.40[b]	1174.69 ± 48.94[c]
8,5 mm	779.82 ± 119.14[b]	971.41 ± 129.89[ab]	666.55 ± 89.62[b]	1237.36 ± 79.52[a]
17 mm	766.00 ± 92.52[b]	887.92 ± 142.62[b]	756.94 ± 90.22[b]	1397.08 ± 143.03[a]
Mandible				
Periapical	1701.41 ± 69.14	1561.70 ± 121.95	1535.68 ± 66.95	1764.89 ± 70.08

In a single line, different superscript letters indicate statistical difference among groups ($P < .05$) (7–9 experiments per group).

Table 2. Mean ± standard error (SE) of the extension of periapical lesions in the experimental groups.

	Periapical Lesion	
	Area (mm^2)	**Volume (mm^3)**
SHAM	1.653 ± 0.35	5.121 ± 0.97
SHAM-ZOL	0.699 ± 0.23	2.969 ± 0.62
OVX	1.312 ± 0.30	3.887 ± 0.42
OVX-ZOL	1.332 ± 0.34	4.550 ± 0.69

The sample number was 7–9 animals per group.

4 DISCUSSÃO

A periodontite apical é caracterizada como um processo inflamatório causado por agentes etiológicos de origem endodôntica que levam à destruição dos tecidos perirradiculares (KAKEHASHI; STANLEY; FITZGERALD, 1965). A polpa dental necrótica e infectada serve como um ambiente favorável para a proliferação microbiana, a qual avança e invade a região periapical. Frente a essa agressão, o mecanismo de defesa do organismo estimula os mediadores químicos da inflamação que ativam os osteoclastos e levam à destruição dos tecidos em torno do ápice radicular com o objetivo de conter a infeção microbiana, ocasionando as lesões ósseas periapicais (NAIR, 2004).

A literatura tem demonstrado que a ocorrência e a progressão da periodontite apical podem ser influenciadas pela presença de doenças sistêmicas (LÓPEZ-LÓPEZ et al., 2011; MAROTTA et al., 2012; WILLERSHAUSEN et al., 2014; JULURI et al., 2015; LÓPEZ-LÓPEZ et al., 2015; AN et al., 2016; GOMES et al., 2016). A relação das desordens que afetam o metabolismo ósseo, como a osteoporose, com as patologias endodônticas tem despertado um interesse especial dos pesquisadores. A periodontite apical é uma doença que tem reflexo direto no tecido ósseo alveolar e a osteoporose ocorre nos ossos causando alterações significativas na microarquitetura óssea, o que pode sugerir a existência de uma possível associação entre essas duas doenças.

Nesse sentido, o primeiro estudo desta Tese apresenta uma revisão sistemática com metanálise cujo objetivo foi avaliar a influência da osteoporose no desenvolvimento da periodontite apical em estudos de modelo animal usando ratas. Os resultados da metanálise, realizada para o tamanho da lesão periapical obtido por meio da análise histométrica, mostraram que as ratas com osteoporose apresentam lesões periapicais maiores que os animais saudáveis. A análise global apresentou grande heterogeneidade, porém, na análise de subgrupo para os períodos experimentais, observou-se que para o tempo de indução de lesão periapical, a heterogeneidade diminuiu e a diferença estatística entre os grupos se manteve. Na análise de subgrupo para a linhagem das ratas também observou-se que os animais com osteoporose apresentaram lesões periapicais maiores que àqueles saudáveis, tanta para as ratas Wistar quanto para as ratas Sprague-Dawley. Com o objetivo de verificar a razão da variação do tamanho da lesão periapical nos estudos e quantificar o quanto da heterogeneidade foi explicada pela linhagem das ratas e pelos períodos experimentais, a meta-regressão foi realizada. Constatou-se que a linhagem das ratas somada ao tempo de ovariectomia até a

indução da lesão periapical e ao período de indução da lesão até a eutanásia explicam mais de 80% da heterogeneidade da metanálise global.

Já a metanálise para os dados do tamanho da lesão periapical obtidos no exame radiográfico apresentou resultados controversos. Enquanto que a metanálise para os valores em mm^2 mostrou que os animais com osteoporose apresentaram lesões periapicais maiores que as ratas em que foi realizada a simulação da ovariectomia, a análise realizada com os dados em pixels mostrou que não houve diferença estatística entre os grupos.

A deficiência de estrogênio, principalmente em mulheres pós-menopausa, altera a microarquitetura do tecido ósseo, levando a diminuição da qualidade e fragilidade óssea (COSMAN et al, 2014). No osso mandibular, os efeitos do hipoestrogenismo ainda não estão bem claros. Entretanto, segundo os resultados da revisão sistemática, a presença da osteoporose induzida em ratas parece afetar o metabolismo ósseo ao ponto de acelerar a progressão da periodontite apical, aumentando o tamanho das lesões periapicais.

A avaliação dos marcadores de formação e reabsorção óssea nos estudos incluídos na revisão indicou que, na presença da osteoporose, as lesões ósseas periapicais tendem a apresentar um número maior de células positivas para os marcadores de reabsorção óssea, tais como Fosfatase Ácida Resistente ao Tartarato (TRAP – do inglês, *tartrate-resistant acid phosphatase*) e RANKL. Já quanto à identificação da OPG, um marcador de formação óssea, os grupos de ratas ovariectomizadas e controle apresentaram quantidades similares na maioria dos tempos experimentais. A identificação destes marcadores é importante para a melhor compreensão do mecanismo de reabsorção óssea periapical na presença da osteoporose, pois, o sistema RANK/RANKL/OPG desempenha um papel essencial no processo de remodelação óssea, já que o balanço entre a produção de RANKL e OPG determina a quantidade de osso que é reabsorvida (NAKAGAWA et al., 1998; ROUX et al., 2002).

A realização desta revisão sistemática, sobre a influência da osteoporose na progressão da periodontite apical em ratas, permitiu mostrar que não há padronização na execução dos procedimentos experimentais nem na escrita dos estudos. Diferenças metodológicas, tais como, linhagem das ratas, unidades de medida, tempo de hipoestrogenismo e de indução da lesão periapical podem ser indicadas como as principais deficiências encontradas nesses estudos.

Além da osteoporose, também tem sido questionado se os medicamentos utilizados para inibir o processo de reabsorção óssea podem interferir no desenvolvimento da periodontite apical. Com o objetivo de responder a esta dúvida e esclarecer os resultados divergentes da literatura a respeito da influência do hipoestrogenismo na periodontite apical, o

segundo artigo desta Tese avaliou a influência da osteoporose e do ZOL no desenvolvimento de lesões periapicais induzidas em ratas, por meio da análise radiográfica e da Micro-CT.

Os resultados deste estudo mostraram que tanto a osteoporose quanto a administração de ZOL para seu tratamento não foram capazes de influenciar o desenvolvimento das lesões periapicais. Guardadas as devidas proporções, este resultado vai ao encontro do único estudo clínico identificado na literatura, até o presente momento, que verificou o efeito da deficiência de estrogênio na periodontite apical. Segundo Lopéz-Lopéz et al. (2015) não há diferença no número de lesões periapicais entre pacientes saudáveis e com osteoporose, indicando que talvez a osteoporose não seja capaz de induzir a alterações significativas no tecido ósseo alveolar. Corroborando com estes resultados, alguns relatos na literatura também não identificaram relação entre a deprivação de estrogênio e outras intervenções e alterações ósseas na cavidade oral. Marjanovic et al. (2013) verificaram, em um estudo transversal com mulheres, que não há relação entre a severidade da doença periodontal e a osteoporose. Chow et al. (2016) mostraram, em um estudo prospectivo, que a presença de osteoporose não foi uma contraindicação para a realização de implantes e que a redução da DMO em idosos não foi associada com aumento da perda óssea marginal ao redor de implantes.

Em relação ao papel que o ZOL desempenha no desenvolvimento da periodontite apical, o estudo de WAYAMA et al. (2015) mostrou que o tratamento de ratas osteoporóticas com ZOL diminui o tamanho das lesões periapicais quando comparado àquelas não tratadas. Este resultado está em desacordo com os achados do segundo artigo desta Tese, o qual mostrou que o bisfosfonato não influencia na progressão da periodontite apical. A diferença nesses resultados pode ser explicada pela diferença nos protocolos de administração do ZOL utilizados nos estudos. Entretanto, considerações mais aprofundadas sobre o papel deste fármaco na periodontite apical não são possíveis, pois ao meu conhecimento, até o presente momento, não há outros estudos experimentais em modelo animal, bem como estudos clínicos nesta linha de pesquisa.

O ZOL faz parte da classe de medicamentos chamados de bisfosfonatos. Os bisfosfonatos são as drogas mais prescritas para o tratamento da osteoporose devido seu potente efeito anti-reabsortivo, que resulta em maior densidade óssea e menor risco de fratura (CARBONARE et al., 2010; ERIKSEN; DÍEZ-PÉREZ; BOONEN, 2014). Entretanto, o ZOL difere dos demais fármacos desse grupo, principalmente, devido à forma de administração. Para tratamento da osteoporose, o ZOL é administrado anualmente via intravenosa, enquanto que os demais bisfosfonatos são de uso oral e, geralmente, administrados semanalmente (NATIONAL OSTEOPOROSIS FOUNDATION, 2014). A biodisponibilidade dos

bisfosfonatos de uso oral é muito baixa, aproximadamente 1%, enquanto que para as formulações intravenosas a biodisponibilidade é de 100% (CARBONARE et al., 2010). Além da biodisponibilidade, o fato do ZOL ter grande afinidade com a hidroxiapatita garante seu efeito prolongado (NANCOLLAS et al., 2006). Apesar das vantagens do uso do ZOL, seu mecanismo de ação ainda não está bem esclarecido, o que pode gerar dúvidas a respeito da real influencia que ele exerce sobre o tecido ósseo alveolar, principalmente na presença de lesões patológicas ósseas como na periodontite apical.

Além disso, o segundo estudo desta Tese mostrou, como já esperado, que o ZOL aumentou o conteúdo mineral do fêmur das ratas com osteoporose. Entretanto, nos ossos mandibulares o conteúdo mineral não diferiu entre os grupos. É conhecido que com a deficiência de estrogênio, as primeiras alterações no conteúdo mineral ósseo acontecem nos ossos longos e nas vértebras (KURODA et al., 2003). Assim, a mandíbula necessitaria de um período maior de deprivação de estrogênio para que apresentasse alteração no padrão ósseo mineral. Entretanto, é provável que o constante estresse mecânico derivado da oclusão funcional sofrido pela mandíbula tenha ajudado a evitar a perda óssea alveolar nos animais com osteoporose (INMAN et al., 1999).

Embora a administração dos bisfosfonatos seja o tratamento de escolha para a osteoporose, o seu uso tem como grande preocupação clínica a possibilidade de ocorrência de osteonecrose dos ossos mandibulares. Tipicamente, a osteonecrose ocorre após um procedimento invasivo, como extração dental ou algum procedimento cirúrgico, mas também pode ocorrer espontaneamente (RUGGIERO et al., 2014). De todos os bisfosfonatos, o ZOL é o medicamento que mais tem sido associado com a presença de osteonecrose (HANSEN et al., 2012; SILVA et al., 2015). Entretanto, quase a totalidade desses estudos usam altas doses de ZOL em períodos curtos de tempo, o qual condiz com os protocolos utilizados para tratamento de doenças cancerígenas ósseas e não da osteoporose. A osteonecrose dos ossos mandibulares foi relatada em 0,7 - 6,7% dos pacientes com câncer em que foi prescrito 4 mg de ZOL a cada 3 semanas (RUGGIERO et al., 2014). Por outro lado, apenas 0,017- 0,04% das pessoas com osteoporose em tratamento com ZOL (5 mg/ano) apresentaram osteonecrose; além disso, o risco para osteonecrose foi semelhante ao dos pacientes com osteoporose tratados com placebo ou dos pacientes com osteoporose tratados com bisfosfonatos orais (RUGGIERO et al., 2014). Um estudo clínico com pacientes que faziam uso de bisfosfonatos e que realizaram tratamento e retratamento endodôntico mostrou que não houve casos de osteonecrose após a intervenção endodôntica (HSIAO et al., 2009). Portanto, com base no exposto, podemos considerar que o ZOL é um medicamento seguro para tratamento da

osteoporose do ponto de vista da ocorrência da osteonecrose nos ossos mandibulares quando de intervenções endodônticas.

É importante ressaltar a eficácia do modelo de indução da osteoporose realizado nesta Tese. A ovariectomia é um modelo já consagrado na literatura para deprivação de estrogênio e desenvolvimento da osteoporose (WRONSKI et al., 1989; KALU, 1991). A remoção bilateral dos ovários leva à supressão dos níveis de estrogênio e, consequentemente, à perda de massa óssea, similarmente ao que ocorre nas mulheres no período pós-menopausa (KALU, 1991). A redução do peso do útero e do nível de estrogênio, além da diminuição do conteúdo mineral ósseo no fêmur das ratas ovariectomizadas comprovou o sucesso deste modelo experimental no presente estudo.

5 CONCLUSÃO

A osteoporose é uma doença esquelética sistêmica caracterizada pela diminuição da massa óssea e deterioração da microarquitetura do osso. As alterações causadas pela osteoporose podem influenciar a ocorrência e a progressão de desordens na cavidade bucal como a periodontite apical.

Em resumo, os achados do presente estudo mostram que a osteoporose desempenha um papel importante no desenvolvimento da periodontite apical. Segundo a revisão sistemática, ratas com osteoporose apresentam lesões periapicais maiores que os animais saudáveis; além disso, quanto maiores são as lesões periapicais maior a quantidade de marcadores de reabsorção óssea.

Embora a literatura tenha uma tendência a sugerir que a deficiência de estrogênio afeta a periodontite apical, o estudo experimental realizado nesta Tese mostrou que ratas com osteoporose apresentam lesões periapicais de tamanho semelhante às ratas não osteoporóticas. Os animais com osteoporose apresentaram uma redução do conteúdo mineral ósseo no fêmur, indicando os efeitos sistêmicos da doença; porém, na mandíbula não houve diferença no conteúdo mineral entre ratas saudáveis e osteoporóticas. Já o tratamento farmacológico da osteoporose com ZOL parece não interferir no conteúdo mineral ósseo dos ossos mandibulares e, consequentemente, na progressão da periodontite apical.

Entretanto, a presença de poucos estudos sobre o assunto, a variabilidade dos protocolos experimentais e a inconsistência dos resultados apresentados dificultam um parecer conclusivo sobre a influência da osteoporose e do ZOL no desenvolvimento da periodontite apical. Ressalta-se a importância de que mais estudos a cerca do tema sejam realizados, principalmente, estudos clínicos com mulheres no período pós-menopausa.

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ANEXO A – NORMAS PARA PUBLICAÇÃO DE ARTIGO CIENTÍFICO NO PERIÓDICO *INTERNATIONAL ENDODONTIC JOURNAL*.

International Endodontic Journal

Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Manuscript Submission Procedure, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

Useful Websites: Submission Site, Articles published in *International Endodontic Journal*, Author Services, Wiley's Ethical Guidelines, Guidelines for Figures

The journal to which you are submitting your manuscript employs a plagiarism detection system. By submitting your manuscript to this journal you accept that your manuscript may be screened for plagiarism against previously published works.

1. GENERAL

International Endodontic Journal publishes original scientific articles, reviews, clinical articles and case reports in the field of Endodontology; the branch of dental sciences dealing with health, injuries to and diseases of the pulp and periradicular region, and their relationship with systemic well-being and health. Original scientific articles are published in the areas of biomedical science, applied materials science, bioengineering, epidemiology and social science relevant to endodontic disease and its management, and to the restoration of root-treated teeth. In addition, review articles, reports of clinical cases, book reviews, summaries and abstracts of scientific meetings and news items are accepted.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *International Endodontic Journal*. Authors are encouraged to visit Wiley Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

International Endodontic Journal adheres to the below ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper do so on the understanding that the manuscript has been read and approved by all authors and that all authors agree to the submission of the manuscript to the Journal.

International Endodontic Journal adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, authorship criteria should be based on 1) substantial contributions to conception and design of, or acquisiation of data or analysis and interpretation of data, 2) drafting the article or

revising it critically for important intellectual content and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

Acknowledgements: Under acknowledgements please specify contributors to the article other than the authors accredited. Please also include specifications of the source of funding for the study and any potential conflict of interests if appropriate. Please find more information on the conflict of interest form in section 2.6.

2.2. Ethical Approvals

Experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study. The authors MUST upload a copy of the ethical approval letter when submitting their manuscript. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

2.3 Clinical Trials

The International Endodontic Journal asks that authors submitting manuscripts reporting from a clinical trial to register the trials in any of the following public clinical trials registries: www.clinicaltrials.gov, https://www.clinicaltrialsregister.eu/, http://isrctn.org/.

Other primary registries if named in the WHO network will also be considered acceptable. The clinical trial registration number and name of the trial register should be included in the Acknowledgements at the submission stage.

2.3.1 Randomised control clinical trials

Randomised control clinical trials should be reported using the guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

2.3.2 Epidemiological observational trials

Submitting authors of epidemiological human observations studies are required to review and submit a 'strengthening the reporting of observational studies in Epidemiology' (STROBE) checklist and statement. Compliance with this should be detailed in the materials and methods section. (www.strobe-statement.org)

2.4 Systematic Reviews

Systematic reviews should be reported using the PRISMA guidelines available at http://prisma-statement.org/. A PRISMA checklist and flow diagram (as a Figure) should also be included in the submission material.

2.5 DNA Sequences and Crystallographic Structure Determinations

Papers reporting protein or DNA sequences and crystallographic structure determinations will not be accepted without a Genbank or Brookhaven accession number, respectively. Other supporting data sets must be made available on the publication date from the authors directly.

2.6 Conflict of Interest and Source of Funding

International Endodontic Journal requires that all authors (both the corresponding author and co-authors) disclose any potential sources of conflict of interest. Any interest or relationship, financial or otherwise that might be perceived as influencing an author's objectivity is considered a potential source of conflict of interest. These must be disclosed when directly relevant or indirectly related to the work that the authors describe in their manuscript. Potential sources of conflict of interest include but are not limited to patent or stock ownership, membership of a company board of directors, membership of an advisory board or committee for a company, and consultancy for or receipt of speaker's fees from a company. If authors are unsure whether a past or present affiliation or relationship should be disclosed in the manuscript, please contact the editorial office at iejeditor@cardiff.ac.uk. The existence of a conflict of interest does not preclude publication in this journal.

The above policies are in accordance with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals produced by the International Committee of Medical Journal Editors (http://www.icmje.org/).

It is the responsibility of the corresponding author to have all authors of a manuscript fill out a conflict of interest disclosure form, and to upload all forms individually (do not combine the forms into one file) together with the manuscript on submission. The disclosure statement should be included under Acknowledgements. Please find the form below:

Conflict of Interest Disclosure Form

2.7 Appeal of Decision

The decision on a paper is final and cannot be appealed.

2.8 Permissions

If all or parts of previously published illustrations are used, permission must be obtained from the copyright holder concerned. It is the author's responsibility to obtain these in writing and provide copies to the Publishers.

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3. OnlineOpen

OnlineOpen is available to authors of primary research articles who wish to make their article available to non-subscribers on publication, or whose funding agency requires grantees to archive the final version of their article. With OnlineOpen, the author, the author's funding agency, or the author's institution pays a fee to ensure that the article is made available to non-subscribers upon publication via Wiley Online Library, as well as deposited in the funding agency's preferred archive. For the full list of terms and conditions, see http://wileyonlinelibrary.com/onlineopen#OnlineOpen_Terms
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Prior to acceptance there is no requirement to inform an Editorial Office that you intend to publish your paper OnlineOpen if you do not wish to. All OnlineOpen articles are treated in the same way as any other article. They go through the journal's standard peer-review process and will be accepted or rejected based on their own merit.

3.1 MANUSCRIPT SUBMISSION PROCEDURE

Manuscripts should be submitted electronically via the online submission site http://mc.manuscriptcentral.com/iej. The use of an online submission and peer review site enables immediate distribution of manuscripts and consequentially speeds up the review process. It also allows authors to track the status of their own manuscripts. Complete instructions for submitting a paper is available online and below. Further assistance can be obtained from iejeditor@cardiff.ac.uk.

3.2. Getting Started

• Launch your web browser (supported browsers include Internet Explorer 5.5 or higher, Safari 1.2.4, or Firefox 1.0.4 or higher) and go to the journal's online Submission Site: http://mc.manuscriptcentral.com/iej
• Log-in, or if you are a new user, click on 'register here'.
• If you are registering as a new user.
- After clicking on 'register here', enter your name and e-mail information and click 'Next'. Your e-mail information is very important.
- Enter your institution and address information as appropriate, and then click 'Next.'

- Enter a user ID and password of your choice (we recommend using your e-mail address as your user ID), and then select your areas of expertise. Click 'Finish'.
• If you are registered, but have forgotten your log in details, please enter your e-mail address under 'Password Help'. The system will send you an automatic user ID and a new temporary password.
• Log-in and select 'Author Centre'

3.3. Submitting Your Manuscript

• After you have logged into your 'Author Centre', submit your manuscript by clicking on the submission link under 'Author Resources'.
• Enter data and answer questions as appropriate. You may copy and paste directly from your manuscript and you may upload your pre-prepared covering letter.
• Click the 'Next' button on each screen to save your work and advance to the next screen.
• You are required to upload your files.
- Click on the 'Browse' button and locate the file on your computer.
- Select the designation of each file in the drop down next to the Browse button.
- When you have selected all files you wish to upload, click the 'Upload Files' button.
• Review your submission (in HTML and PDF format) before completing your submission by sending it to the Journal. Click the 'Submit' button when you are finished reviewing.

3.4. Manuscript Files Accepted

Manuscripts should be uploaded as Word (.doc) or Rich Text Format (.rft) files (not write-protected) plus separate figure files. GIF, JPEG, PICT or Bitmap files are acceptable for submission, but only high-resolution TIF or EPS files are suitable for printing. The files will be automatically converted to HTML and PDF on upload and will be used for the review process. The text file must contain the abstract, main text, references, tables, and figure legends, but no embedded figures or Title page. The Title page should be uploaded as a separate file. In the main text, please reference figures as for instance 'Figure 1', 'Figure 2' etc to match the tag name you choose for the individual figure files uploaded. Manuscripts should be formatted as described in the Author Guidelines below.

3.5. Blinded Review

Manuscript that do not conform to the general aims and scope of the journal will be returned immediately without review. All other manuscripts will be reviewed by experts in the field (generally two referees). International Endodontic Journal aims to forward referees´ comments and to inform the corresponding author of the result of the review process. Manuscripts will be considered for fast-track publication under special circumstances after consultation with the Editor.

International Endodontic Journal uses double blinded review. The names of the reviewers will thus not be disclosed to the author submitting a paper and the name(s) of the author(s) will not be disclosed to the reviewers.

To allow double blinded review, please submit (upload) your main manuscript and title page as separate files.

Please upload:

• Your manuscript without title page under the file designation 'main document'
• Figure files under the file designation 'figures'

• The title page and Acknowledgements where applicable, should be uploaded under the file designation 'title page'

All documents uploaded under the file designation 'title page' will not be viewable in the html and pdf format you are asked to review in the end of the submission process. The files viewable in the html and pdf format are the files available to the reviewer in the review process.

3.6. Suspension of Submission Mid-way in the Submission Process

You may suspend a submission at any phase before clicking the 'Submit' button and save it to submit later. The manuscript can then be located under 'Unsubmitted Manuscripts' and you can click on 'Continue Submission' to continue your submission when you choose to.

3.7. E-mail Confirmation of Submission

After submission you will receive an e-mail to confirm receipt of your manuscript. If you do not receive the confirmation e-mail after 24 hours, please check your e-mail address carefully in the system. If the e-mail address is correct please contact your IT department. The error may be caused by some sort of spam filtering on your e-mail server. Also, the e-mails should be received if the IT department adds our e-mail server (uranus.scholarone.com) to their whitelist.

3.8. Manuscript Status

You can access ScholarOne Manuscripts any time to check your 'Author Centre' for the status of your manuscript. The Journal will inform you by e-mail once a decision has been made.

3.9. Submission of Revised Manuscripts

To submit a revised manuscript, locate your manuscript under 'Manuscripts with Decisions' and click on 'Submit a Revision'. Please remember to delete any old files uploaded when you upload your revised manuscript.

4. MANUSCRIPT TYPES ACCEPTED

Original Scientific Articles: must describe significant and original experimental observations and provide sufficient detail so that the observations can be critically evaluated and, if necessary, repeated. Original Scientific Articles must conform to the highest international standards in the field.

Review Articles: are accepted for their broad general interest; all are refereed by experts in the field who are asked to comment on issues such as timeliness, general interest and balanced treatment of controversies, as well as on scientific accuracy. Reviews should generally include a clearly defined search strategy and take a broad view of the field rather than merely summarizing the authors´ own previous work. Extensive or unbalanced citation of the authors´ own publications is discouraged.

Mini Review Articles: are accepted to address current evidence on well-defined clinical, research or methodological topics. All are refereed by experts in the field who are asked to comment on timeliness, general interest, balanced treatment of controversies, and scientific rigor. A clear research question, search strategy and balanced synthesis of the evidence is expected. Manuscripts are limited in terms of word-length and number of figures.

Clinical Articles: are suited to describe significant improvements in clinical practice such as the report of a novel technique, a breakthrough in technology or practical approaches to

recognised clinical challenges. They should conform to the highest scientific and clinical practice standards.

Case Reports: illustrating unusual and clinically relevant observations are acceptable but they must be of sufficiently high quality to be considered worthy of publication in the Journal. On rare occasions, completed cases displaying non-obvious solutions to significant clinical challenges will be considered. Illustrative material must be of the highest quality and healing outcomes, if appropriate, should be demonstrated.

Supporting Information: *International Endodontic Journal* encourages submission of adjuncts to printed papers via the supporting information website (see submission of supporting information below). It is encouraged that authors wishing to describe novel procedures or illustrate cases more fully with figures and/or video may wish to utilise this facility.

Letters to the Editor: are also acceptable.

Meeting Reports: are also acceptable.

5. MANUSCRIPT FORMAT AND STRUCTURE

5.1. Format

Language: The language of publication is English. It is preferred that manuscript is professionally edited. A list of independent suppliers of editing services can be found at http://authorservices.wiley.com/bauthor/english_language.asp. All services are paid for and arranged by the author, and use of one of these services does not guarantee acceptance or preference for publication.

Presentation: Authors should pay special attention to the presentation of their research findings or clinical reports so that they may be communicated clearly. Technical jargon should be avoided as much as possible and clearly explained where its use is unavoidable. Abbreviations should also be kept to a minimum, particularly those that are not standard. The background and hypotheses underlying the study, as well as its main conclusions, should be clearly explained. Titles and abstracts especially should be written in language that will be readily intelligible to any scientist.

Abbreviations: International Endodontic Journal adheres to the conventions outlined in Units, Symbols and Abbreviations: A Guide for Medical and Scientific Editors and Authors. When non-standard terms appearing 3 or more times in the manuscript are to be abbreviated, they should be written out completely in the text when first used with the abbreviation in parenthesis.

5.2. Structure

All manuscripts submitted to *International Endodontic Journal* should include Title Page, Abstract, Main Text, References and Acknowledgements, Tables, Figures and Figure Legends as appropriate

Title Page: The title page should bear: (i) Title, which should be concise as well as descriptive; (ii) Initial(s) and last (family) name of each author; (iii) Name and address of department, hospital or institution to which work should be attributed; (iv) Running title (no more than 30 letters and spaces); (v) No more than six keywords (in alphabetical order); (vi) Name, full postal address, telephone, fax number and e-mail address of author responsible for correspondence.

Abstract for Original Scientific Articles should be no more than 250 words giving details of what was done using the following structure:

• **Aim**: Give a clear statement of the main aim of the study and the main hypothesis tested, if any.

• **Methodology**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and statistical tests.

• **Results**: Give the main results of the study, including the outcome of any statistical analysis.

• **Conclusions**: State the primary conclusions of the study and their implications. Suggest areas for further research, if appropriate.

Abstract for Review Articles should be non-structured of no more than 250 words giving details of what was done including the literature search strategy.

Abstract for Mini Review Articles should be non-structured of no more than 250 words, including a clear research question, details of the literature search strategy and clear conclusions.

Abstract for Case Reports should be no more than 250 words using the following structure:

• **Aim**: Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Summary**: Describe the methods adopted including, as appropriate, the design of the study, the setting, entry requirements for subjects, use of materials, outcome measures and analysis if any.

• **Key learning points**: Provide up to 5 short, bullet-pointed statements to highlight the key messages of the report. All points must be fully justified by material presented in the report.

Abstract for Clinical Articles should be no more than 250 words using the following structure:

• **Aim:** Give a clear statement of the main aim of the report and the clinical problem which is addressed.

• **Methodology:** Describe the methods adopted.

• **Results:** Give the main results of the study.

• **Conclusions:** State the primary conclusions of the study.

Main Text of Original Scientific Article should include Introduction, Materials and Methods, Results, Discussion and Conclusion

Introduction: should be focused, outlining the historical or logical origins of the study and gaps in knowledge. Exhaustive literature reviews are not appropriate. It should close with the explicit statement of the specific aims of the investigation, or hypothesis to be tested.

Material and Methods: must contain sufficient detail such that, in combination with the references cited, all clinical trials and experiments reported can be fully reproduced.

(i) Clinical Trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A CONSORT checklist and flow diagram (as a Figure) should also be included in the submission material.

(ii) Experimental Subjects: experimentation involving human subjects will only be published if such research has been conducted in full accordance with ethical principles, including the World Medical Association Declaration of Helsinki (version 2008) and the additional requirements, if any, of the country where the research has been carried out. Manuscripts must

be accompanied by a statement that the experiments were undertaken with the understanding and written consent of each subject and according to the above mentioned principles. A statement regarding the fact that the study has been independently reviewed and approved by an ethical board should also be included. Editors reserve the right to reject papers if there are doubts as to whether appropriate procedures have been used.

When experimental animals are used the methods section must clearly indicate that adequate measures were taken to minimize pain or discomfort. Experiments should be carried out in accordance with the Guidelines laid down by the National Institute of Health (NIH) in the USA regarding the care and use of animals for experimental procedures or with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and in accordance with local laws and regulations.

All studies using human or animal subjects should include an explicit statement in the Material and Methods section identifying the review and ethics committee approval for each study, if applicable. Editors reserve the right to reject papers if there is doubt as to whether appropriate procedures have been used.

(iii) Suppliers: Suppliers of materials should be named and their location (Company, town/city, state, country) included.

Results: should present the observations with minimal reference to earlier literature or to possible interpretations. Data should not be duplicated in Tables and Figures.

Discussion: may usefully start with a brief summary of the major findings, but repetition of parts of the abstract or of the results section should be avoided. The Discussion section should progress with a review of the methodology before discussing the results in light of previous work in the field. The Discussion should end with a brief conclusion and a comment on the potential clinical relevance of the findings. Statements and interpretation of the data should be appropriately supported by original references.

Conclusion: should contain a summary of the findings.

Main Text of Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should be focused to place the subject matter in context and to justify the need for the review. The Review section should be divided into logical sub-sections in order to improve readability and enhance understanding. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The use of tabulated and illustrative material is encouraged. The Conclusion section should reach clear conclusions and/or recommendations on the basis of the evidence presented.

Main Text of Mini Review Articles should be divided into Introduction, Review and Conclusions. The Introduction section should briefly introduce the subject matter and justify the need and timeliness of the literature review. The Review section should be divided into logical sub-sections to enhance readability and understanding and may be supported by up to 5 tables and figures. Search strategies must be described and the use of state-of-the-art evidence-based systematic approaches is expected. The Conclusions section should present clear statements/recommendations and suggestions for further work. The manuscript, including references and figure legends should not normally exceed 4000 words.

Main Text of Clinical Reports and Clinical Articles should be divided into Introduction, Report, Discussion and Conclusion,. They should be well illustrated with clinical images,

radiographs, diagrams and, where appropriate, supporting tables and graphs. However, all illustrations must be of the highest quality

Acknowledgements: *International Endodontic Journal* requires that all sources of institutional, private and corporate financial support for the work within the manuscript must be fully acknowledged, and any potential conflicts of interest noted. Grant or contribution numbers may be acknowledged, and principal grant holders should be listed. Acknowledgments should be brief and should not include thanks to anonymous referees and editors. See also above under Ethical Guidelines.

5.3. References

It is the policy of the Journal to encourage reference to the original papers rather than to literature reviews. Authors should therefore keep citations of reviews to the absolute minimum.

We recommend the use of a tool such as EndNote or Reference Manager for reference management and formatting. The EndNote reference style can be obtained upon request to the editorial office (iejeditor@cardiff.ac.uk). Reference Manager reference styles can be searched for here: www.refman.com/support/rmstyles.asp

In the text: single or double authors should be acknowledged together with the year of publication, e.g. (Pitt Ford & Roberts 1990). If more than two authors the first author followed by *et al.* is sufficient, e.g. (Tobias *et al.* 1991). If more than 1 paper is cited the references should be in year order and separated by "," e.g. (Pitt Ford & Roberts 1990, Tobias *et al*. 1991).

Reference list: All references should be brought together at the end of the paper in alphabetical order and should be in the following form.

(i) Names and initials of up to six authors. When there are seven or more, list the first three and add *et al.*

(ii)Year of publication in parentheses

(iii) Full title of paper followed by a full stop (.)

(iv) Title of journal in full (in italics)

(v) Volume number (bold) followed by a comma (,)

(vi) First and last pages

Examples of correct forms of reference follow:

Standard journal article

Bergenholtz G, Nagaoka S, Jontell M (1991) Class II antigen-expressing cells in experimentally induced pulpitis. *International Endodontic Journal* **24**, 8-14.

Corporate author

British Endodontic Society (1983) Guidelines for root canal treatment. *International Endodontic Journal* **16**, 192-5.

Journal supplement

Frumin AM, Nussbaum J, Esposito M (1979) Functional asplenia: demonstration of splenic activity by bone marrow scan (Abstract). *Blood* **54** (Suppl. 1), 26a.

Books and other monographs

Personal author(s)

Gutmann J, Harrison JW (1991) *Surgical Endodontics*, 1st edn Boston, MA, USA: Blackwell Scientific Publications.

Chapter in a book

Wesselink P (1990) Conventional root-canal therapy III: root filling. In: Harty FJ, ed. *Endodontics in Clinical Practice*, 3rd edn; pp. 186-223. London, UK: Butterworth.

Published proceedings paper

DuPont B (1974) Bone marrow transplantation in severe combined immunodeficiency with an unrelated MLC compatible donor. In: White HJ, Smith R, eds. Proceedings of the Third Annual Meeting of the International Society for Experimental Rematology; pp. 44-46. Houston, TX, USA: International Society for Experimental Hematology.

Agency publication

Ranofsky AL (1978) Surgical Operations in Short-Stay Hospitals: United States-1975. DHEW publication no. (PHS) 78-1785 (Vital and Health Statistics; Series 13; no. 34.) Hyattsville, MD, USA: National Centre for Health Statistics.8

Dissertation or thesis

Saunders EM (1988) In vitro and in vivo investigations into root-canal obturation using thermally softened gutta-percha techniques (PhD Thesis). Dundee, UK: University of Dundee.

URLs

Full reference details must be given along with the URL, i.e. authorship, year, title of document/report and URL. If this information is not available, the reference should be removed and only the web address cited in the text.

Smith A (1999) Select committee report into social care in the community [WWW document]. URL http://www.dhss.gov.uk/reports/report015285.html [accessed on 7 November 2003]

5.4. Tables, Figures and Figure Legends

Tables: Tables should be double-spaced with no vertical rulings, with a single bold ruling beneath the column titles. Units of measurements must be included in the column title.

Figures: All figures should be planned to fit within either 1 column width (8.0 cm), 1.5 column widths (13.0 cm) or 2 column widths (17.0 cm), and must be suitable for photocopy reproduction from the printed version of the manuscript. Lettering on figures should be in a clear, sans serif typeface (e.g. Helvetica); if possible, the same typeface should be used for all figures in a paper. After reduction for publication, upper-case text and numbers should be at least 1.5-2.0 mm high (10 point Helvetica). After reduction, symbols should be at least 2.0-3.0 mm high (10 point). All half-tone photographs should be submitted at final reproduction size. In general, multi-part figures should be arranged as they would appear in the final version. Reduction to the scale that will be used on the page is not necessary, but any special requirements (such as the separation distance of stereo pairs) should be clearly specified.

Unnecessary figures and parts (panels) of figures should be avoided: data presented in small tables or histograms, for instance, can generally be stated briefly in the text instead. Figures should not contain more than one panel unless the parts are logically connected; each panel of a multipart figure should be sized so that the whole figure can be reduced by the same amount and reproduced on the printed page at the smallest size at which essential details are visible.

Figures should be on a white background, and should avoid excessive boxing, unnecessary colour, shading and/or decorative effects (e.g. 3-dimensional skyscraper histograms) and highly pixelated computer drawings. The vertical axis of histograms should not be truncated

to exaggerate small differences. The line spacing should be wide enough to remain clear on reduction to the minimum acceptable printed size.

Figures divided into parts should be labelled with a lower-case, boldface, roman letter, a, b, and so on, in the same typesize as used elsewhere in the figure. Lettering in figures should be in lower-case type, with the first letter capitalized. Units should have a single space between the number and the unit, and follow SI nomenclature or the nomenclature common to a particular field. Thousands should be separated by a thin space (1 000). Unusual units or abbreviations should be spelled out in full or defined in the legend. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. In general, visual cues (on the figures themselves) are preferred to verbal explanations in the legend (e.g. broken line, open red triangles etc.)

Figure legends: Figure legends should begin with a brief title for the whole figure and continue with a short description of each panel and the symbols used; they should not contain any details of methods.

Permissions: If all or part of previously published illustrations are to be used, permission must be obtained from the copyright holder concerned. This is the responsibilty of the authors before submission.

Preparation of Electronic Figures for Publication: Although low quality images are adequate for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit EPS (lineart) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented programmes. Scans (TIFF only) should have a resolution of 300 dpi (halftone) or 600 to 1200 dpi (line drawings) in relation to the reproduction size (see below). EPS files should be saved with fonts embedded (and with a TIFF preview if possible). For scanned images, the scanning resolution (at final image size) should be as follows to ensure good reproduction: lineart: >600 dpi; half-tones (including gel photographs): >300 dpi; figures containing both halftone and line images: >600 dpi.

Further information can be obtained at Wiley Blackwell's guidelines for figures: http:/authorservices.wiley.com/bauthor/illustration.asp.

Check your electronic artwork before submitting it: http://authorservices.wiley.com/bauthor/eachecklist.asp.

5.5. Supporting Information

Publication in electronic formats has created opportunities for adding details or whole sections in the electronic version only. Authors need to work closely with the editors in developing or using such new publication formats.

Supporting information, such as data sets or additional figures or tables, that will not be published in the print edition of the journal, but which will be viewable via the online edition, can be submitted. It should be clearly stated at the time of submission that the supporting information is intended to be made available through the online edition. If the size or format of the supporting information is such that it cannot be accommodated on the journal's website, the author agrees to make the supporting information available free of charge on a permanent Web site, to which links will be set up from the journal's website. The author must advise Wiley Blackwell if the URL of the website where the supporting information is located

changes. The content of the supporting information must not be altered after the paper has been accepted for publication.

The availability of supporting information should be indicated in the main manuscript by a paragraph, to appear after the References, headed 'Supporting Information' and providing titles of figures, tables, etc. In order to protect reviewer anonymity, material posted on the authors Web site cannot be reviewed. The supporting information is an integral part of the article and will be reviewed accordingly.

Preparation of Supporting Information: Although provision of content through the web in any format is straightforward, supporting information is best provided either in web-ready form or in a form that can be conveniently converted into one of the standard web publishing formats:

• Simple word-processing files (.doc or .rtf) for text.

• PDF for more complex, layout-dependent text or page-based material. Acrobat files can be distilled from Postscript by the Publisher, if necessary.

• GIF or JPEG for still graphics. Graphics supplied as EPS or TIFF are also acceptable.

• MPEG or AVI for moving graphics.

Subsequent requests for changes are generally unacceptable, as for printed papers. A charge may be levied for this service.

Video Imaging: For the on-line version of the Journal the submission of illustrative video is encouraged. Authors proposing the use such media should consult with the Editor during manuscript preparation.

6. AFTER ACCEPTANCE

Upon acceptance of a paper for publication, the manuscript will be forwarded to the Production Editor who is responsible for the production of the journal.

6.1. Figures

Hard copies of all figures and tables are required when the manuscript is ready for publication. These will be requested by the Editor when required. Each Figure copy should be marked on the reverse with the figure number and the corresponding author's name.

6.2 Proof Corrections

The corresponding author will receive an email alert containing a link to a web site. A working email address must therefore be provided for the corresponding author. The proof can be downloaded as a PDF (portable document format) file from this site. Acrobat Reader will be required in order to read this file. This software can be downloaded (free of charge) from the following Web site: www.adobe.com/products/acrobat/readstep2.html. This will enable the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof. Hard copy proofs will be posted if no e-mail address is available; in your absence, please arrange for a colleague to access your e-mail to retrieve the proofs. Proofs must be returned to the Production Editor within three days of receipt. As changes to proofs are costly, we ask that you only correct typesetting errors. Excessive changes made by the author in the proofs, excluding typesetting errors, will be charged separately. Other than in exceptional circumstances, all illustrations are retained by the publisher. Please note that the author is responsible for all statements made in his work, including changes made by the copy editor.

6.3 Early Online Publication Prior to Print

International Endodontic Journal is covered by Wiley Blackwell's Early View service. Early View articles are complete full-text articles published online in advance of their publication in a printed issue. Early View articles are complete and final. They have been fully reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after online publication. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the traditional way. They are therefore given a Digital Object Identifier (DOI), which allows the article to be cited and tracked before it is allocated to an issue. After print publication, the DOI remains valid and can continue to be used to cite and access the article.

6.4 Online Production Tracking

Online production tracking is available for your article through Blackwell's Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The author will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript. Visit http://authorservices.wiley.com/bauthor/ for more details on online production tracking and for a wealth of resources including FAQs and tips on article preparation, submission and more.

6.5 Author Material Archive Policy

Please note that unless specifically requested, Wiley Blackwell will dispose of all hardcopy or electronic material submitted two months after publication. If you require the return of any material submitted, please inform the editorial office or production editor as soon as possible.

6.6 Offprints

Free access to the final PDF offprint of your article will be available via Author Services only. Please therefore sign up for Author Services if you would like to access your article PDF offprint and enjoy the many other benefits the service offers.

Additional paper offprints may be ordered online. Please click on the following link, fill in the necessary details and ensure that you type information in all of the required fields: Offprint Cosprinters. If you have queries about offprints please email offprint@cosprinters.com

The corresponding author will be sent complimentary copies of the issue in which the paper is published (one copy per author).

6.7 Author Services

For more substantial information on the services provided for authors, please see Wiley Blackwell Author Services

6.8 Note to NIH Grantees: Pursuant to NIH mandate, Wiley Blackwell will post the accepted version of contributions authored by NIH grant-holders to PubMed Central upon acceptance. This accepted version will be made publicly available 12 months after publication. For further information, see www.wiley.com/go/nihmandate

7 Guidelines for reporting of DNA microarray data

The *International Endodontic Journal* gives authors notice that, with effect from 1st January 2011, submission to the *International Endodontic Journal* requires the reporting of

microarray data to conform to the MIAME guidelines. After this date, submissions will be assessed according to MIAME standards. The complete current guidelines are available athttp://www.mged.org/Workgroups/MIAME/miame_2.0.html. Also, manuscripts will be published only after the complete data has been submitted into the public repositories, such as GEO (http://www.ncbi.nlm.nih.gov/geo/) or ArrayExpress (http://www.ebi.ac.uk/microarray/submissions_overview.html), in MIAME compliant format, with the data accession number (the identification number of the data set in the database) quoted in the manuscript. Both databases are committed to keeping the data private until the associated manuscript is published, if requested.

Prospective authors are also encouraged to search for previously published microarray data with relevance to their own data, and to report whether such data exists. Furthermore, they are encouraged to use the previously published data for qualitative and/or quantitative comparison with their own data, whenever suitable. To fully acknowledge the original work, an appropriate reference should be given not only to the database in question, but also to the original article in which the data was first published. This open approach will increase the availability and use of these large-scale data sets and improve the reporting and interpretation of the findings, and in increasing the comprehensive understanding of the physiology and pathology of endodontically related tissues and diseases, result eventually in better patient care.

ANEXO B – NORMAS PARA PUBLICAÇÃO DE ARTIGO CIENTÍFICO NO PERIÓDICO *JOURNAL OF ENDODONTICS*.

Guidelines for Publishing Papers in the JOE

Writing an effective article is a challenging assignment. The following guidelines are provided to assist authors in submitting manuscripts.

The *JOE* publishes original and review articles related to the scientific and applied aspects of endodontics. Moreover, the *JOE* has a diverse readership that includes full-time clinicians, full-time academicians, residents, students and scientists. Effective communication with this diverse readership requires careful attention to writing style.

1. **General Points on Composition**

a. Authors are strongly encouraged to analyze their final draft with both software (*e.g.*, spelling and grammar programs) and colleagues who have expertise in English grammar. References listed at the end of this section provide a more extensive review of rules of English grammar and guidelines for writing a scientific article. Always remember that clarity is the most important feature of scientific writing. Scientific articles must be clear and precise in their content and concise in their delivery since their purpose is to inform the reader. The Editor reserves the right to edit all manuscripts or to reject those manuscripts that lack clarity or precision, or have unacceptable grammar or syntax. The following list represents common errors in manuscripts submitted to the *JOE*:
b. The paragraph is the ideal unit of organization. Paragraphs typically start with an introductory sentence that is followed by sentences that describe additional detail or examples. The last sentence of the paragraph provides conclusions and forms a transition to the next paragraph. Common problems include one-sentence paragraphs, sentences that do not develop the theme of the paragraph (see also section "c" below), or sentences with little to no transition within a paragraph.
c. Keep to the point. The subject of the sentence should support the subject of the paragraph. For example, the introduction of authors' names in a sentence changes the subject and lengthens the text. In a paragraph on sodium hypochlorite, the sentence, "In 1983, Langeland et al., reported that sodium hypochlorite acts as a lubricating factor during instrumentation and helps to flush debris from the root canals" can be edited to: "Sodium hypochlorite acts as a lubricant during instrumentation and as a vehicle for flushing the generated debris (Langeland et al., 1983)." In this example, the paragraph's subject is sodium hypochlorite and sentences should focus on this subject.
d. Sentences are stronger when written in the active voice, *i.e.*, the subject performs the action. Passive sentences are identified by the use of passive verbs such as "was," "were," "could," etc. For example: "Dexamethasone was found in this study to be a factor that was associated with reduced inflammation," can be edited to: "Our results demonstrated that dexamethasone reduced inflammation." Sentences written in a direct and active voice are generally more powerful and shorter than sentences written in the passive voice.

e. Reduce verbiage. Short sentences are easier to understand. The inclusion of unnecessary words is often associated with the use of a passive voice, a lack of focus or run-on sentences. This is not to imply that all sentences need be short or even the same length. Indeed, variation in sentence structure and length often helps to maintain reader interest. However, make all words count. A more formal way of stating this point is that the use of subordinate clauses adds variety and information when constructing a paragraph. (This section was written deliberately with sentences of varying length to illustrate this point.)
f. Use parallel construction to express related ideas. For example, the sentence, "Formerly, endodontics was taught by hand instrumentation, while now rotary instrumentation is the common method," can be edited to "Formerly, endodontics was taught using hand instrumentation; now it is commonly taught using rotary instrumentation." The use of parallel construction in sentences simply means that similar ideas are expressed in similar ways, and this helps the reader recognize that the ideas are related.
g. Keep modifying phrases close to the word that they modify. This is a common problem in complex sentences that may confuse the reader. For example, the statement, "Accordingly, when conclusions are drawn from the results of this study, caution must be used," can be edited to "Caution must be used when conclusions are drawn from the results of this study."
h. To summarize these points, effective sentences are clear and precise, and often are short, simple and focused on one key point that supports the paragraph's theme.
i. Authors should be aware that the *JOE* uses iThenticate, plagiarism detection software, to assure originality and integrity of material published in the *Journal*. The use of copied sentences, even when present within quotation marks, is highly discouraged. Instead, the information of the original research should be expressed by new manuscript author's own words, and a proper citation given at the end of the sentence. Plagiarism will not be tolerated and manuscripts will be rejected, or papers withdrawn after publication based on unethical actions by the authors. In addition, authors may be sanctioned for future publication.

2. **Organization of Original Research Manuscripts**
Please Note: *All abstracts should be organized into sections that start with a one-word title (in bold),* i.e.*, Introduction, Methods, Results, Conclusions, etc., and should not exceed more than 250 words in length.*

a. **Title Page:** The title should describe the major emphasis of the paper. It should be as short as possible without loss of clarity. Remember that the title is your advertising billboard—it represents your major opportunity to solicit readers to spend the time to read your paper. It is best not to use abbreviations in the title since this may lead to imprecise coding by electronic citation programs such as PubMed (*e.g.*, use "sodium hypochlorite" rather than NaOCl). The author list must conform to published standards on authorship (see authorship criteria in the Uniform Requirements for Manuscripts Submitted to Biomedical Journals at *www.icmje.org*). The manuscript title, name and address (including email) of one author designated as the corresponding author. This author will be responsible for editing proofs and ordering reprints when applicable. The contribution of each author should also be highlighted in the cover letter.

b. **Abstract:** The abstract should concisely describe the purpose of the study, the hypothesis, methods, major findings and conclusions. The abstract should describe the new contributions made by this study. The word limitations (250 words) and the wide distribution of the abstract (*e.g.*, PubMed) make this section challenging to write clearly. This section often is written last by many authors since they can draw on the rest of the manuscript. Write the abstract in past tense since the study has been completed. Three to ten keywords should be listed below the abstract.
c. **Introduction:** The introduction should briefly review the pertinent literature in order to identify the gap in knowledge that the study is intended to address and the limitations of previous studies in the area. The purpose of the study, the tested hypothesis and its scope should be clearly described. Authors should realize that this section of the paper is their primary opportunity to establish communication with the diverse readership of the *JOE*. Readers who are not expert in the topic of the manuscript are likely to skip the paper if the introduction fails to succinctly summarize the gap in knowledge that the study addresses. It is important to note that many successful manuscripts require no more than a few paragraphs to accomplish these goals. Therefore, authors should refrain from performing extensive review or the literature, and discussing the results of the study in this section.
d. **Materials and Methods:** The objective of the materials and methods section is to permit other investigators to repeat your experiments. The four components to this section are the detailed description of the materials used and their components, the experimental design, the procedures employed, and the statistical tests used to analyze the results. The vast majority of manuscripts should cite prior studies using similar methods and succinctly describe the essential aspects used in the present study. Thus, the reader should still be able to understand the method used in the experimental approach and concentration of the main reagents (*e.g.*, antibodies, drugs, etc.) even when citing a previously published method. The inclusion of a "methods figure" will be rejected unless the procedure is novel and requires an illustration for comprehension. If the method is novel, then the authors should carefully describe the method and include validation experiments. If the study utilized a **commercial product**, the manuscript must state that they either followed manufacturer's protocol *or* specify any changes made to the protocol. If the study used an ***in vitro*** **model** to simulate a clinical outcome, the authors must describe experiments made to validate the model, or previous literature that proved the clinical relevance of the model. Studies on **humans** must conform to the Helsinki Declaration of 1975 and state that the institutional IRB/equivalent committee(s) approved the protocol and that informed consent was obtained after the risks and benefits of participation were described to the subjects or patients recruited. Studies involving **animals** must state that the institutional animal care and use committee approved the protocol. The statistical analysis section should describe which tests were used to analyze which dependent measures; p-values should be specified. Additional details may include randomization scheme, stratification (if any), power analysis as a basis for sample size computation, drop-outs from clinical trials, the effects of important confounding variables, and bivariate versus multivariate analysis.
e. **Results:** Only experimental results are appropriate in this section (*i.e.*, neither methods, discussion, nor conclusions should be in this section). Include only those data that are critical for the study, as defined by the aim(s). Do not include all available data without justification; any repetitive findings will be rejected from publication. All Figures, Charts

and Tables should be described in their order of numbering with a brief description of the major findings. Author may consider the use of supplemental figures, tables or video clips that will be published online. Supplemental material is often used to provide additional information or control experiments that support the results section (*e.g.*, microarray data).

f. **Figures:** There are two general types of figures. The first type of figures includes photographs, radiographs or micrographs. Include only essential figures, and even if essential, the use of composite figures containing several panels of photographs is encouraged. For example, most photo-, radio- or micrographs take up one column-width, or about 185 mm wide X 185 mm tall. If instead, you construct a two columns-width figure (*i.e.*, about 175 mm wide X 125 mm high when published in the *JOE*), you would be able to place about 12 panels of photomicrographs (or radiographs, etc.) as an array of four columns across and three rows down (with each panel about 40 X 40 mm). This will require some editing to emphasize the most important feature of each photomicrograph, but it greatly increases the total number of illustrations that you can present in your paper. Remember that each panel must be clearly identified with a letter (*e.g.*, "A," "B," etc.), in order for the reader to understand each individual panel. Several nice examples of composite figures are seen in recent articles by Jeger et al (J Endod 2012;38:884–888); Olivieri et al., (J Endod 2012;38:1007 1011); Tsai et al (J Endod 2012;38:965–970). Please note that color figures may be published at no cost to the authors and authors are encouraged to use color to enhance the value of the illustration. Please note that a multipanel, composite figure only counts as one figure when considering the total number of figures in a manuscript (see section 3, below, for maximum number of allowable figures).

   The second type of figures are graphs (*i.e.*, line drawings including bar graphs) that plot a dependent measure (on the Y axis) as a function of an independent measure (usually plotted on the X axis). Examples include a graph depicting pain scores over time, etc. Graphs should be used when the overall trend of the results are more important than the exact numerical values of the results. For example, a graph is a convenient way of reporting that an ibuprofen-treated group reported less pain than a placebo group over the first 24 hours, but was the same as the placebo group for the next 96 hours. In this case, the trend of the results is the primary finding; the actual pain scores are not as critical as the relative differences between the NSAID and placebo groups.

g. **Tables:** Tables are appropriate when it is critical to present exact numerical values. However, not all results need be placed in either a table or figure. For example, the following table may not be necessary:

% NaOCl	**N/Group**	**% Inhibition of Growth**
0.001	5	0
0.003	5	0
0.01	5	0
0.03	5	0
0.1	5	100
0.3	5	100
1	5	100
3	5	100

h. Instead, the results could simply state that there was no inhibition of growth from 0.001-0.03% NaOCl, and a 100% inhibition of growth from 0.03-3% NaOCl (N=5/group). Similarly, if the results are not significant, then it is probably not necessary to include the results in either a table or as a figure. These and many other suggestions on figure and table construction are described in additional detail in Day (1998).
i. **Discussion:** This section should be used to interpret and explain the results. Both the strengths and weaknesses of the observations should be discussed. How do these findings compare to the published literature? What are the clinical implications? Although this last section might be tentative given the nature of a particular study, the authors should realize that even preliminary clinical implications might have value for the clinical readership. Ideally, a review of the potential clinical significance is the last section of the discussion. What are the major conclusions of the study? How does the data support these conclusions
j. **Acknowledgments:** All authors must affirm that they have no financial affiliation (*e.g.*, employment, direct payment, stock holdings, retainers, consultantships, patent licensing arrangements or honoraria), or involvement with any commercial organization with direct financial interest in the subject or materials discussed in this manuscript, nor have any such arrangements existed in the past three years. Any other potential conflict of interest should be disclosed. Any author for whom this statement is not true must append a paragraph to the manuscript that fully discloses any financial or other interest that poses a conflict. Likewise the sources and correct attributions of all other grants, contracts or donations that funded the study must be disclosed
k. **References:** The reference style follows Index Medicus and can be easily learned from reading past issues of the *JOE*. The *JOE*uses the Vancouver reference style, which can be found in most citation management software products. Citations are placed in parentheses at the end of a sentence or at the end of a clause that requires a literature citation. Do not use superscript for references. Original reports are limited to 35 references. There are no limits in the number of references for review articles.

3. **Manuscripts Category Classifications and Requirements**

Manuscripts submitted to the *JOE* must fall into one of the following categories. The abstracts for all these categories would have a maximum word count of 250 words:

A. CONSORT Randomized Clinical Trial-Manuscripts in this category must strictly adhere to the Consolidated Standards of Reporting Trials-CONSORT- minimum guidelines for the publication of randomized clinical trials. These guidelines can be found at *www.consort-statement.org/*. These manuscripts have a limit of 3,500 words, [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.
B. Review Article-Manuscripts in this category are either narrative articles, or systematic reviews/meta-analyses. Case report/Clinical Technique articles even when followed by extensive review of the literature will should be categorized as “Case Report/Clinical Technique”. These manuscripts have a limit of 3,500 words, [including abstract, introduction, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

C. Clinical Research (*e.g.*, prospective or retrospective studies on patients or patient records, or research on biopsies, excluding the use of human teeth for technique studies). These manuscripts have a limit of 3,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures and 4 tables*.

D. Basic Research Biology (animal or culture studies on biological research on physiology, development, stem cell differentiation, inflammation or pathology). Manuscripts that have a primary focus on biology should be submitted in this category while manuscripts that have a primary focus on materials should be submitted in the Basic Research Technology category. For example, a study on cytotoxicity of a material should be submitted in the Basic Research Technology category, even if it was performed in animals with histological analyses. These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or 4 tables*.

E. Basic Research Technology (Manuscripts submitted in this category focus primarily on research related to techniques and materials used, or with potential clinical use, in endodontics). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 3 figures and tables *.

F. Case Report/Clinical Technique (*e.g.*, report of an unusual clinical case or the use of cutting-edge technology in a clinical case). These manuscripts have a limit of 2,500 words [including abstract, introduction, materials and methods, results, discussion and acknowledgments; excluding figure legends and references]. In addition, there is a limit of a total of 4 figures or tables*.

* Figures, if submitted as multipanel figures must not exceed 1 page length. Manuscripts submitted with more than the allowed number of figures or tables will require approval of the *JOE* Editor or associate editors. If you are not sure whether your manuscript falls within one of the categories above, or would like to request preapproval for submission of additional figures please contact the Editor by email at *jendodontics@uthscsa.edu*.

Importantly, adhering to the general writing methods described in these guidelines (and in the resources listed below) will help to reduce the size of the manuscript while maintaining its focus and significance. Authors are encouraged to focus on only the essential aspects of the study and to avoid inclusion of extraneous text and figures. The Editor may reject manuscripts that exceed these limitations.

Available Resources:

Strunk W, White EB. The Elements of Style. Allyn & Bacon, 4th ed, 2000, ISBN 020530902X.

Day R. How to Write and Publish a Scientific Paper. Oryx Press, 5th ed. 1998. ISBN 1-57356-164-9.

Woods G. English Grammar for Dummies. Hungry Minds:NY, 2001 (an entertaining review of grammar).

Alley M. The Craft of Scientific Writing. Springer, 3rd edition 1996 SBN 0-387-94766-3.

Alley M. The Craft of Editing. Springer, 2000 SBN 0-387-98964-1.

ANEXO C – CARTA DE APROVAÇÃO DO PROTOCOLO DE PESQUISA NA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA PONTIFÍCIA UNIVERSIDADE CATÓLICA DO RIO GRANDE DO SUL (CEUA - PUCRS).

Pontifícia Universidade Católica do Rio Grande do Sul
PRÓ-REITORIA DE PESQUISA, INOVAÇÃO E DESENVOLVIMENTO
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Ofício 31/2015 - CEUA

Porto Alegre, 28 de maio de 2015.

Prezado Sr(a). Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou seu Protocolo de Pesquisa, registro CEUA 15/00436, intitulado **"Efeito da medicação intracanal à base de hidróxido de cálcio e da reposição com estrogênio sobre lesões periapicais em ratas com osteoporose induzida".**

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está **autorizada** a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Nº de Animais	Espécie	Duração do Projeto
80 animais	Rattus novergicus	02/2015 – 12/2016

Atenciosamente,

Prof. Dr. João Batista Blessmann Weber
Coordenador da CEUA/PUCRS

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