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Review

Effects of osteoporosis on alveolar bone repair after tooth extraction: A systematic review of preclinical studies

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ABSTRACT

Objective: This systematic review aimed to address whether the alveolar socket repair after a tooth extraction is impacted by an osteoporotic phenotype and propose methodological observations.
Design: A search strategy in MEDLINE/PubMed, EMBASE, Web of Science, and Scopus databases was performed. Quality assessment was carried out through the SYRCLE Risk of Bias tool.
Results: Out of the 1147 potentially relevant records, 25 met the inclusion criteria. Most of the studies were performed in rats, and ovariectomy (OVX) was the most frequent osteoporosis induction method. Histomorphometry, micro-computed tomography (microCT), and immunohistochemistry were the main bone repair evaluation methods. Most of the included studies (88 %) presented negative impacts of osteoporosis on the alveolar socket repair. Only three studies (12 %) showed no statistical differences among groups. Overall, most of the quality assessment categories presented a high percentage of unclear risk of bias due to insufficient information in the studies.
Conclusions: The results indicated that an osteoporotic phenotype seems to impair alveolar socket repair after tooth extraction. However, there is still a lack of information and standardization. Therefore, further studies should consider the proposed methodological aspects regarding animal characteristics, OVX associated with a low calcium diet, waiting 8 weeks to osteoporosis induction, maxillary molars as the best option for tooth extraction, confirming and reporting OVX and osteoporosis success, and an appropriate method of repair analysis.

1. Introduction

Osteoporosis is a systemic skeletal disease characterized by lower bone mass and bone microstructure deterioration (Kanis et al., 2019). It was estimated that 27 million people were affected by this disease up to 2010 only in the European Union (Hernlund et al., 2013). Besides, the actual current scenario may be at least 10 times higher since most cases still go underdiagnosed, and these estimates are expected to worsen with the aging population (Hernlund et al., 2013; Hiligsmann et al., 2019; Nuti et al., 2019; Pietschmann et al., 2009).

The pathophysiology of osteoporosis is characterized by a high osteoclastic activity, which prevails over the osteoblastic one (Pietschmann et al., 2009; Rachner et al., 2011). Menopause is the leading risk factor of osteoporosis, characterized by decreased ovarian function, consequently causing an estrogen deficiency. Estrogen is a hormone with variable functions in bone homeostasis, such as stimulating osteoblastic function and differentiation; inhibiting osteoclastic differentiation and activity by cellular effects or inhibiting the production of RANKL by mesenchymal stem cells, osteoblasts, and osteocytes; and regulation of cytokines related to the stimulus of bone resorption (Curtis et al., 2016; Eastell et al., 2016; Kerschan-Schindl et al., 2018; Pietschmann et al., 2015). Considering the estrogen function and its effects, a lack of this hormone may lead to an unbalance in bone turnover, ultimately favoring its resorption (Kerschan-Schindl et al., 2018).

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Even though menopause is the most frequent determinant for osteoporosis, there are many other potentially associated risk factors, namely advanced age, genetics, smoking, and alcohol consumption (Curtis et al., 2016). These other potential risk factors for osteoporosis also present similar pathogenic mechanisms related to a consequent lack of estrogen (Kerschan-Schindl et al., 2018).

The consequent bone deterioration caused by osteoporosis significantly increases the susceptibility to bone fractures, potentially impairing the process of bone repair (Cheung et al., 2016; Kanis et al., 2019; Nikolaou et al., 2009). However, whether osteoporosis actually impairs osseous healing is still a matter of debate with no consensus (Féron & Mauprivez, 2016; Gorter et al., 2020; Hak, 2018). The current literature of clinical studies evaluating bone healing is scarce due to the difficulty of conducting research with osteoporotic patients and not being affected by other variables such as aging, endocrine disorders, and medications (Hak, 2018). On the other hand, the literature currently supposes that osteoporotic healing is similar to the normal bone yet delayed (Féron & Mauprivez, 2016). Apart from that, there are some studies that held that the osteoporosis aggravates the chances of implants loss, functional outcomes issues, and non-unions during the fracture healing processes (Barrios et al., 1993; Büyükkurt et al., 2012; Fitzpatrick et al., 2012)

To reduce the risk of bone fractures, different medications are prescribed for osteoporotic patients (Hegde et al., 2016; Kanis et al., 2019; Simpson et al., 2020). In jawbones, a focus on the effect of osteoporosis medications on the jaws-related osteonecrosis events has been extensively studied (Beth-Tasdogan et al., 2017; Escobedo et al., 2020; Khan et al., 2015; Nicolatou-Galitis et al., 2019). On the other hand, the impact of osteoporosis without the effect of medications is still unclear and needs to be further addressed since a significant number of aged patients' lives with an osteoporotic phenotype and may be underdiagnosed or even failing to take the medications regularly (Hiligsmann et al., 2019; Martin et al., 2020).

Tooth extraction is one of the most common procedures in alveolar bones, and tooth loss is one of the most critical oral health indicators (de Weijden et al., 2009; Haworth et al., 2018; Susin et al., 2005). Even though its rates have been dropping over the last years, 158 million people are still affected by severe tooth loss (Kassebaum et al., 2014). Epidemiological studies reveal a strong positive correlation between age determinant and missing teeth (Haworth et al., 2018; Susin et al., 2005). In parallel, osteoporosis is also strongly correlated to age (Johnell & Kanis, 2006; Kerschan-Schindl et al., 2018; Khosla et al., 2018; Pietschmann et al., 2009). In this context, the relationship between osteoporosis and tooth extraction should be further verified. Some preclinical studies have already addressed conclusions about this issue, indicating that an osteoporotic-phenotype delay alveolar bone repair after tooth extraction (Arioka et al., 2019; Chen et al., 2018; de Oliveira Puttini et al., 2019; Liu et al., 2019; Luvizuto et al., 2010a, 2010b, 2011; Miranda et al., 2020; Pereira et al., 2007; Shimizu et al., 2000; Teófilo et al., 2004). Although the process of extraction socket healing is widely comprehended (de Weijden et al., 2009; Marmary et al., 1986), no review has been conducted to verify whether this process occurs differently in osteoporotic bones.

To the best of our knowledge, there are no clinical studies that evaluate the bone repair of a socket post tooth extraction in osteoporotic patients without medication. The OVX procedure has been widely used to induce osteoporosis in experimental animal studies (Iwaniec & Turner, 2013; Thompson et al., 1995; Yousefzadeh et al., 2020). Other procedures to induce osteoporosis in animals have also been described (Iwaniec & Turner, 2013).

Considering that osteoporosis is a prevalent disease, and tooth extraction is a common surgical procedure for aged individuals, the comprehension of the socket repair process in these cases is of crucial clinical relevance. Taking the pathophysiology of osteoporosis into account, we hypothesize that an osteoporotic condition may impair the process of alveolar socket repair. In this sense, the current systematic review aimed to address whether alveolar socket repair after a tooth extraction is impacted by an osteoporotic phenotype and propose methodological aspects for future preclinical studies.

2. Methods

2.1. Protocol registration and focused question

This review was registered at the National Institute for Health Research, International Prospective Register of Systematic Reviews (http://www.crd.york.ac.uk/PROSPERO#CRD42019150539). According to Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines (Moher et al., 2009), the following specific question was framed for this systematic review: "Does osteoporosis impair alveolar socket repair after tooth extraction?"

2.2. Search strategy

According to the Populations, Interventions, Comparison, Outcomes, and Study Design (PICOS) principle, the search strategy was constructed. Individual search strategies were designed for the following electronic databases: MEDLINE/PubMed, EMBASE, Web of Science, and Scopus. The electronic databases were searched to identify relevant studies published up to and including August 2020. The searched publications were only considered in the English language, with no restrictions on publication year. The search strategy contained a combination of controlled predefined Medical Subject Heading (MeSH) terms and free terms using the Boolean operators (*i.e.*, OR, AND), always adapted to the syntax rules of each bibliographic database. Supplementary Table 1 details the search strategies performed in the electronic databases. Additionally, it was also conducted a manual search of bibliographies and reference lists of the included studies to locate any potential unidentified study.

2.3. Eligibility criteria

2.3.1. Inclusion criteria

The present systematic review was based on original preclinical research studies considering animal models with osteoporotic-like bony phenotype. The animals should be submitted to tooth extraction procedures and evaluation of alveolar bone repair. The studies inclusion criteria were: (1) populations: extraction socket; (2) intervention: osteoporosis induction; (3) comparisons: no intervention; (4) primary outcomes: quantitative data on the newly formed bone; or secondary outcomes: qualitative data on the newly formed bone.

2.3.2. Exclusion criteria

Review papers, letters to the editor, monographs, conference papers, book chapters, unpublished data, and studies published in a language other than English were excluded. *Ex vivo* and *in vitro* studies were also disregarded. Original animal research studies were excluded when: (1) the animals presented other systemic diseases or comorbidities; (2) the study did not show a non-osteoporotic control group; and (3) the study compared different treatments for osteoporosis; (4) the outcomes involved alveolar ridge preservation.

Table 1
Reasons for exclusion after full-text reading.

Reason for exclusion	n (studies)
No osteoporosis induction	13
No alveolar socket repair analysis	16
No healthy/sham/control group	9
Not an experimental study	9
Group OVX subjected to other treatments/interventions	3
Full-text article not found;	1
n=	51

2.4. Study selection and data extraction

Titles and abstracts of all studies were reviewed. It was first selected the studies that met the inclusion criteria based on their titles and abstracts. Studies that failed the inclusion criteria were excluded. The finally included papers were thoroughly read. Two reviewers (F.M.S.; B. B.S.) conducted the process of research independently and in duplicate. The two reviewers discussed with a third one (M.D.D) when there was a disagreement. The required information from the eligible studies was collected by one of the reviewers (B.B.S.). For each study, the following data were extracted, using a standardized data collection form: (1) publication details (first author and year); (2) animal model; (3) samples characteristics (groups/environmental characteristics/sex/age); (4) tooth extraction characteristics; (5) osteoporosis induction method; (6) method of repair analysis; (7) primary outcomes on the newly formed bone; and/or secondary outcomes on the newly formed bone. All the data extracted were inserted in a database on the EndNote software (Thompson Reuters, New York, NY, USA).

2.5. Risk of bias assessment

The assessment for possible existing bias at the selected studies was performed using *the Systematic Review Centre for Laboratory Animal Experimentation Risk of Bias (*SYRCLE RoB Tool). This instrument was specifically developed for the risk of bias in animal research and based on the Cochrane Collaboration (Hooijmans et al., 2014). The SYRCLE RoB Tool consists of 10 entries, classified as the sort of bias: selection bias, performance bias, detection bias, attrition bias, reporting bias, and others. For "others" section, 3 entries were added to adapt this tool to the present study. The following 3 new entries were considered to be essential for bias assessment in the field of osteoporosis and alveolar socket repair: (1) Reporting an evaluation method for the success of OVX; (2) Adequate time between OVX and tooth extraction; (3) Evaluating and reporting osteoporosis confirmation. For each entry, the studies received a score of "high risk of bias" (when the study does not meet one or more criteria for that entry); "unclear risk of bias" (when the study does not present the necessary data for this entry or partly meet one or more criteria), or "low risk of bias" (when all the requirements were met for this entry).

2.6. Data synthesis and statistical analysis

Due to methodological heterogeneity, a quantitative analysis of the obtained results was not feasible. Consequently, the results were qualitatively analyzed and descriptively summarized. In order to increase the comprehension and better address the focused question of this review, the results will be separated into two parts: "primary outcomes" in which the results of the newly formed bone area or volume will be presented. In the second part, there will be the "secondary outcomes" in which the results from qualitative analysis of the newly formed bone obtained by other methods will be described. Data were tabulated and processed using Microsoft Excel®.

3. Results

3.1. Study selection

A total of 1147 potentially relevant records were identified and further processed according to the PRISMA statement (Fig. 1) (Moher et al., 2009). After that, duplicate removal was performed, and there were 759 records to examine based on title and/or abstract. Then, 684 records were removed since they did not meet the eligibility criteria for this study. Seventy-five articles were selected to be full-text read. Subsequently, 51 articles were excluded according to the reasons described in Table 1. After a manual search of bibliographies, one study was further included. Overall, a total of 25 studies were finally included in the present review (Arioka et al., 2019; Bezerra et al., 2013; Chavarry et al., 2019; Chen et al., 2018; de Oliveira et al., 2019; de Oliveira Puttini

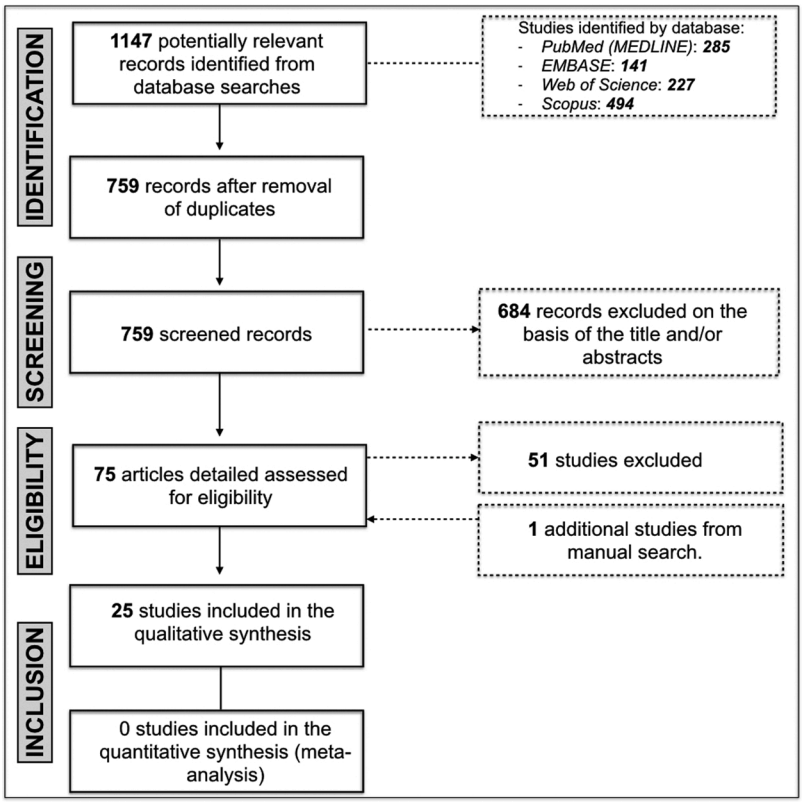

Fig. 1. PRISMA flow diagram for systematic search and studies selection strategy.

et al., 2019; Hsieh et al., 1995; Jee et al., 2010; Kim et al., 2015; Liu et al., 2014; Liu et al., 2019; Luvizuto et al., 2010a, 2010b, 2011; Machado et al., 2010; Miranda et al., 2020; Pereira et al., 2007; Prado et al., 2012; Ramalho-Ferreira et al., 2017; Shimizu et al., 1998, 2000; Shoji et al., 2008; Tanaka et al., 2001; Teófilo et al., 2004; Zecchin et al., 2005).

3.2. Study characteristics

The general descriptions of the included studies are summarized in Table 2. The 25 included articles were published between 1995 and 2020. The studies were conducted in different countries: the USA, Canada, Brazil, United Kingdom, Japan, China, and South Korea. The majority of the studies (56 %) were performed in Brazil. Considering the animal models, 22 (88 %) out of the 25 studies used rats, 2 (8 %) mice, and 1 (4 %) rabbits. From the 22 studies using rats, the majority of lineages were Wistar. From the available data, the age of the animals ranged from 4 weeks to 6 months (mean age: 99 ± 57days). The majority of the studies used female animals (92 %), and the maxillary molars (44 %) and the upper incisors (32 %) were the most prevalent teeth groups submitted to tooth extraction. Some studies presented additional experimental groups that were not of interest to the present review.

3.3. Osteoporosis induction methods

The period between the induction of osteoporosis and tooth extraction ranged from 0 to 60 days (33.7 ± 22.2 days). Among the studies that confirmed that the animals were osteoporotic, the mean period between osteoporosis induction until confirmation, and then tooth extraction, was 50.5 days (±18.1).

For the osteoporosis induction method, 19 studies (76 %) employed OVX, 2 (8 %) the orchiectomy (ORQ), and 3 (12 %) the OVX and low calcium and phosphate diet (Chavarry et al., 2019; Ramalho-Ferreira et al., 2017; Teófilo et al., 2004). Prado et al. (2012) was the only study that performed two different methods and compared them, an OVX group and an OVX with a low calcium and phosphate diet group.

Confirmation of OVX procedure was reported in 9 (36 %) studies by estrous cycle assessment, estradiol depletion, absence of ovaries, and atrophy of the uterine horns after autopsy observation. Additionally, confirmation of osteoporosis was reported in only 7 (28 %) of the studies by bone densitometry, microCT, histomorphometry of tibia, humerus, femur, and lumbar vertebrae. Only 3 (12 %) studies confirmed an osteoporotic phenotype in the jaw skeleton (Arioka et al., 2019; Chen et al., 2018; Liu et al., 2019).

3.4. Method of analysis for alveolar socket repair after tooth extraction

Several methods for bone repair analysis were employed in the included studies. Thirteen studies (52 %) used the histomorphometric analysis to assess the bone repair process. Nine studies (36 %) used immunohistochemistry through the following different antibodies: RANKL, OPG, osteocalcin, TRAP, alkaline phosphatase, Wnt, osteopontin, bone sialoprotein. The imaginological examination for bone repair analysis was used in 8 (32 %) of the included studies. The other types of analysis are described in Table 2.

The period of socket repair analysis ranged from 3–70 days. The most frequent analysis period was 7 days, which was performed in 13 (52 %) of the studies. After 7 days, the other most frequent analysis periods were 14 and 28 days, both used in 9 (36 %) of the studies. The different analysis periods performed in the studies are presented in Table 2. The analysis period's median was 21 days.

3.5. Overall results of the included studies

Twenty-two studies (88 %) presented negative effects of osteoporosis on alveolar socket repair, and only 3 (12 %) reported no differences in alveolar socket repair when comparing osteoporosis inducted and control groups. None of the studies in this review presented positive effects of osteoporosis on alveolar socket repair. Relevant topics regarding the primary outcomes (quantitative results of the newly formed bone) and secondary outcomes (qualitative results from the newly formed bone) of the negative effects will be presented in the following section.

3.6. Primary outcomes

The following studies conducted quantitative analysis of the newly formed bone which were considered to be primary outcomes of this review. Out of the 20 studies that performed this quantitative assessment, 80 % showed significantly less amount of neoformed bone tissue in OVX groups compared to control (evaluated through histomorphometry, microCT, scanning electron microscope or backscattered electron image). Table 4 highlights the amount of newly formed bone with absolute values provided by some studies with histomorphometric analysis.

In the study of Teófilo et al. (2004), a significant decrease in the bone volume fraction was observed by means of histometric volume fraction of bone trabeculae of the alveolar sockets of OVX rats at 3- and 9-weeks post-extraction (PE), associated with a larger volume fractions of connective tissue and/or coagulum remnants. Also, by histomorphometry, Pereira et al. (2007) demonstrated that the volume fraction of newly formed bone was significantly lower in the OVX group at 14, 21, and 28 days PE. Likewise, Luvizuto et al. (2010a, 2010b) evaluated neoformed bone area at 7, 14, 21, 28, and 42 days after tooth extraction also by means of histomorphometric analysis, and observed that OVX presented a significantly lower bone area comparing to the sham group at all these periods analyzed. The results obtained by Machado et al. (2010) also showed a decrease in the newly formed bone area in the middle and cervical thirds of the alveolar socket regions of rats in the OVX group compared with sham at 7 and 14 days PE. On the other hand, Prado et al. (2012) demonstrated that estrogen hormonal deficiency alone did not delay alveolar bone repair in OVX rats. However, the estrogen deficiency associated with a calcium-deficient diet led to a lower amount of alveolar trabecular volume at 7- and 45-days PE compared to control and estrogen-deficient + regular diet groups. At 8 days PE, Bezerra et al. (2013) showed a reduction of the new bone formation was observed in the OVX group alveolus, while the control group presented the highest newly formed bone percentage also by means of histometric analysis. Still, Liu et al. (2014) pointed out a decrease in the number of bone trabeculae in the OVX group comparing to control at 4 weeks PE. Another type of bone area quantification performed in pixels with Aniline blue staining was performed by Chen et al. (2018), and a significantly lower alveolar socket repair in the extraction socket was noticed at 7- and 21-days PE comparing to control. Still by means of histomorphometric analysis, Miranda et al. (2020) evaluated newly formed bone at 10, 20, and 30 days. But significant differences were perceived between OVX and control only at 30 days PE, being OVX with less newly formed bone.

Regarding microCT analysis, the study of Arioka et al. (2019) showed that bone volume was significantly lower at the OVX animals' extraction socket at 7 days PE. Likewise, Liu et al. (2019) demonstrated that extraction socket repair in OVX mice was delayed to 8 weeks PE while control healed at 4. Also, employing the bone volume analysis through microCT, the authors observed that, at the healed extraction socket, the control group presented a significantly smaller amount of bone volume fraction than OVX. Also employing microCT, De Oliveira Puttini et al. (2019) observed that the group with a osteoporosis induction presented a significantly lower amount of newly formed bone volume 60 days PE.

Employing Energy Dispersive X-Ray Microanalysis, the study of Tanaka et al. (2001) was not able to observe a difference in newly formed bone inside the alveolar socket neither in the quantities of calcium and phosphate between OVX and control groups. However, these authors showed a significant decrease in the vestibular bone surface of

Table 2
Description of the main characteristics from the included studies.

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
Hsieh et al. (1995)	Rats / NI	Females 35 days-old	Group 1: OVX + tooth extraction / 6 Group 2: tooth extraction + sham operation / 5 Group 3: sham operation + no tooth extraction / 5	OVX No waiting	Right maxillary molar	MS; MAR; Mean width of the alveolar bone10 days	MS: This dynamic measure represents the magnitude of active mineralization at the bone area analyzed at that specific time, indicated by the quantity of labeled surface, and did not show differences between OVX and sham in the present study MAR: This dynamic measure represents the rate of osteoblasts' work and also did not demonstrate differences among groups. Mean width of alveolar bone: there were no significant differences in the alveolar socket width from gingivobuccal to apical region among groups.
Shimizu et al. (1998)	Rats / Wistar	Females 6 months-old	Bilaterally OVX / 5 Non-OVX control: sham surgical procedure / 5	OVX 60 days	Right maxillary first molar	SEM: mean percentage of bone formative or resorptive areas from the surfaces around the extracted sockets 4, 7, 30, 60 days	SEM: New bone formation was increased in OVX in the early periods in comparison to sham but then decreased. At 60 days, bone formation was already static in sham. Bone resorption was stimulated and long-lasting in OVX rats comparing to sham.
Shimizu et al. (2000)	Rats / Wistar	Females 6 months-old	Bilaterally OVX / NI Non-OVX control: sham surgical procedure / NI	OVX 60 days	Maxillary first molars	BSE: mean area of newly formed maxillary bone at buccal side of extraction socket and mean percentage of newly formed alveolar bone of extracted alveolar socket 7, 30, 60 days	BSE: newly formed bone area was significantly greater in sham than in OVX rats at both 7 and 60 days. OVX significantly decreased bone formation throughout the healing processes both at the maxillary bone surface and within the extracted alveolar sockets. Bone resorption was stimulated and long-lasting in OVX.
Tanaka et al. (2001)	Rats / Wistar	Females 6 months-old	Bilaterally OVX / NI Non-OVX control: sham surgical procedure / NI	OVX 7 days	Maxillary first molars	SEM: mean percentage of bone formative or bone resorptive areas in bone surface observed from the occlusal side. BSE: mean area of newly formed bone at the buccal side of alveolar sockets; mean percentage of newly alveolar bone of extracted alveolar sockets. EDX: quantitative analysis of Ca and P weight percentage 7, 30, 60 days	SEM: No differences were observed regarding the mean percentage of the newly formed bone area on maxillary surfaces at 7 and 30 days. At 60 days, OVX presented the highest. BSE: Within the alveolar socket, no differences were observed in the mean percentage of newly formed bone between sham and OVX at 30 and 60 days. At the same periods, significantly less newly formed bone area on the buccal side of OVX but only at 60 days. EDX: microanalysis showed that on newly formed bone matrices of OVX and sham, Ca and P weight % and the ratio

(*continued on next page*)

Table 2 (*continued*)

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
							between Ca/P, suggesting that OVX neither increased nor decreased mineralization processes.
		Females	Sham / 30	OVX + low Ca and P diet	Upper right	Qualitative histological analysis;	HMM: A significant decrease was observed in the bone volume fraction filling the alveolar sockets of OVX rats, both 3 and 9 weeks after tooth extraction, in parallel with larger volume fractions of connective tissue and/or coagulum remnants.
Teófilo et al. (2004)	Rats / Wistar	50–60 days	OVX / 30	2 weeks	incisors	HMM: volume fraction of bone trabeculae, connective tissue, and coagulum remnants 1, 2, 3, 9 weeks	
		Females	Control (sham) / 33	OVX		Gene expression: semi-quantitative RT-PCR (MMP-2, MMP-9, Type I collagen, Type III collagen);	Gene expression: The absence of estrogen in OVX animals promoted a decrease of gelatinolytic activities of MMP-2 and MMP-9 in all time-periods and types I and III collagen mRNA levels.
Zecchin et al. (2005)	Rats / Wistar	4-weeks	OVX / 33	21 days	Second mandibular molars	Protein expression: western blots ((MMP-2, MMP-9, Type I collagen, Type III collagen). 3, 5, 7 days	
		Females	Control (sham) / 30	OVX		RA: Bone densitometry	RA: Densitometric x-rays showed asignificant reduction in OD readings in the OVX group, which means a decreased bone content in the absence of estrogen.
Pereira et al. (2007)	Rats / Wistar	4–6-weeks	OVX / 30	21 days	First mandibular molars	HMM: % epithelial coverage,volume fraction of fibroblasts, collagen, and newly formed bone 3, 5, 7, 14, 21, 28 days	HMM: During the first week, there were no differences between the studied groups in respect to bone formation. At 14, 21, and 28 days.Sham group presented significantly higher amounts of bone volume.
		Females	Control / 8	OVX		Colony assays;	Colony assays: Short-term estrogen depletion significantly increased the percentage of AP-positive CFU-F in both vehicle and Dex-treated cultures derived from mandibular bone, suggesting that the effect of OVX on osteoprogenitors in the regenerating mandible is either smaller or later occurring.
Shoji et al. (2008)	Rats /Sprague-Dawley	6-months	OVX / 8	14 days	Incisor of the right mandible	Gene expression: in situ hybridization (osteocalcin mRNA expression). 4-weeks	Gene expression: Osteocalcin mRNA present on labeled osteoblasts of the regenerating bone surface was not significantly different between control and OVX.
		Females	Sham-operated / 5	OVX		MicroCT: a qualitative analysis of new bone formation; RA: radiographic density	MicroCT: No clear difference in the healing process was observed.
Jee et al. (2010)	Rats /Sprague-Dawley	5-weeks	OVX + saline / 7	NI	Left maxillary first molars	2, 4, 6-weeks	RA: At 4-weeks post-extraction, there were significant differences in radiographic densities between the OVX saline and sham-operated, OVX rats presented low bone densities in the extraction

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Table 2 (*continued*)

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
							socket comparing to control rats.
		Females	Sham-operated / 40	OVX		HMM: neoformed bone area; IHC: RANKL and OPG	HMM: Animals in the sham group showed greater bone formation in the middle third in all periods of analysis, whereas OVX had lower bone formation. There was a significant difference between the quantity of bone formation in the sham group in comparison with the bone formation in the other groups (7 and 14-days).
Luvizuto et al. (2010a)	Rats / Wistar	70-days	OVX + oil / 40	At least 26 days (depending on the period of analysis)	Right maxillary incisor	7, 14, 21, 28, 42-days	
		Females	Sham-operated / 40	OVX		HMM: neoformed bone area and trabecular thickness;	HMM: Sham group showed a greater bone mass in the middle term in all periods analyzed. In OVX, the trabecular thickness was significantly thinner and quantitatively smaller than it was in the other experimental groups.
Luvizuto et al. (2010b)	Rats / Wistar				Right maxillary incisor		
		70-days	OVX + oil / 40	At least 26 days (depending on the period of analysis)		IHC: osteocalcin 7, 14, 21, 28, 42-days	IHC: OVX decreases the mineralization process and osteocalcin expression.
		Female	Sham OVX/NaCl / 20	OVX		HMM: Bone area.	HMM: A marked decrease in the newly formed bone area was observed in the middle and cervical thirds of rat alveolar regions in the OVX/NaCl compared to the sham OVX/NaCl group 7 and 14 days after extraction. The results obtained show that there was considerable delay in new bone formation in OVX rats.
Machado et al. (2010)	Rats / Wistar	4–6 weeks old	OVX/NaCl / 20	At least 30 days (not specified)	Right maxillary incisor	7 and 14 days	
		Females	Sham-operated / 40	OVX		IHC: OPG/RANKL, TRAP.	IHC: At 14 postoperative days, it was observed RANKL immunolabelling similar to the previous period of all groups. Sham showed similar OPG immunolabelling compared to the previous analyzed period, whilst OVX + oil showed a decrease in OPG immunolabelling. OVX + oil group showed intense TRAP immunolabelling, discrete for sham. At 21 postoperative days, the OVX + oil group showed a decreasing OPG immunolabelling. At 28 and 42 postoperative days, the OVX + oil group showed a decrease in the OPG immunolabelling, as well as a significant increase in RANKL immunolabelling.
Luvizuto et al. (2011)	Rats / Wistar	70-days	OVX + oil / 40	At least 26 days (depending on the period of analysis)	Right maxillary incisor	7, 14, 21, 28, 42-days	
Prado et al. (2012)	Rats / Wistar	Female	Sham / 21	OVX	Bilateral		

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Table 2 (*continued*)

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
Bezerra et al. (2013)	Rats / Wistar	3-month-old	OVX / 21 OVX + low calcium diet / 21	15 days	mandibular first molars	Qualitative histological analysis; HMM: trabecular volume and osteoid volume; Analysis of mast cells. 7, 21, and 45 days	HMM analysis: OVX only did not modify the trabecular and osteoid volume in all periods analyzed (7, 21- and 45-days PE). However, the OVX + low calcium diet demonstrated lower trabecular volume at 7- and 45-days PE. After all, extraction socket healing was impaired only by OVX with a low calcium diet. Analysis of mast cells: sham and OVX groups had similar counts of mast cells, but the OVX + low-calcium diet increased the presence of these cells. The presence of these cells may indicate an increased bone resorption activity.
		Female 90 days	Control / 15 OVX and non-ingestion of caffeine / 15	OVX 43 days	Bilateral maxillary molars	HMM: Bone healing (newly formed bone percentage calculated the number of intersections with new bone x 100/total number of intersections on the alveolus); Gene expression: quantitative RT-PCR (BMP-2, BMP-7, BSP, OPN). 8 days	HMM: Percentages of bone healing in the distobuccal root of the maxillary first molar were lower in the OVX than in the control group, showing reduced newly formed bone in the alveolus. Gene expression: No gene evaluated presented any significant differences among groups.
Liu et al. (2014)	Rats / Sprague-Dawley	Female 3-months	Control (sham)/ 20 OVX / 20	OVX 8 weeks	First left maxillary molar	HMM: new bone volume, trabeculae number, trabecular separation; Gene expression: quantitative RT-PCR (TGF-β1 and TNF-α); ELISA assay: TGF-β1 and TNF-α. 4 and 8 weeks	HMM: At 4 w, the OVX group presented a significantly lower trabecular number and significantly higher separation compared to the control group. Gene expression: TNF-α mRNA was significantly higher in the OVX group at both 4 and 8 weeks indicating higher osteoclastic activity. TGF-β1 mRNA was significantly lower in the OVX group at both 4 and 8 weeks indicating less bone formation activity. ELISA assay: TNF-α protein was significantly higher in the OVX group at both 4 and 8 weeks indicating higher osteoclastic activity. TGF-β1 protein was significantly lower in the OVX group at both 4 and 8 weeks indicating less bone formation activity.
Kim et al. (2015)	Rats / Sprague-Dawley	Female 16 weeks	OvC (Ovariectomy Control) ShC (Sham Control)	OVX 6 weeks	The lower left first to third molars	MicroCT: tissue volume, bone volume, bone mineral density, trabecular number, trabecular thickness, and trabecular separation;	All the OvC and sham control animals presented a normal healing course.

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Table 2 (*continued*)

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
						Qualitative histological analysis; HMM: tissue area, bone area, bone surface, and number of osteoclasts. Bone area/Total area, number osteoclasts/ bone area and number of osteoclasts/bone surface, and empty lacunae; Bone biomarkers: serum CTX, C-terminal crosslinked telopeptide of type I collagen and serum TRACP 5b, Tartrate-resistant acid phosphatase isoform 5b; 8 weeks	MicroCT: No differences between control and OvC in all parameters analyzed. HMM: No differences between control and OvC in all parameters analyzed. Bone biomarkers: No differences between control and OvC neither for CTX or TRACP 5b.
Ramalho-Ferreira et al. (2017)	Rats / Wistar	Female 3 months	Sham / 16 OVX / 16	OVX 38 days	Right upper incisor	HMM: blood clot area, connective tissue area, and bone formation area; IHC: OPG, TRAP, RANKL, and Osteocalcin. 14 and 42 days	HMM: Sham group showed the greatest responses regarding bone formation as well as a decrease in blood clot and connective tissue area, indicating that the alveolar bone healing process occurred normally However no differences were observed in terms of newly formed bone. area IHC: Sham presented higher osteocalcin expression than the OVX group. RANKL was higher in the OVX group. Alveolar bone healing dynamics were negatively affected in osteoporotic rats.
Chen et al. (2018)	Rats / Wistar and Charles River	Female 6-week-old (young) and 12-month-old (aged)	Group 1 (extraction performed at the time of OVX) / (30) Group 2 (extraction was performed 8 weeks following the OVX) / 30	OVX 8 weeks	Bilateral maxillary first molars	HMM: Newly formed bone area detected by aniline blue pixels; IHC: AP. 1, 7, and 21 days.	IHC: On day 7, AP indicated that there was new bone at the extraction sockets from the control group, and OVX presented significantly less. HMM: In 7 days, control presented significantly more newly formed bone than OVX. By day 21, there was significantly less bone tissue in OVX group sockets, and it was significantly less than in uninjured sites. As control had a similar bone area as the uninjured tissues surrounding, it was concluded that the extraction sockets of OVX rats were still in the process of healing, and control was already healed by 21 days.
De Oliveira et al. (2019)	Rats / Wistar	Male NI	SHAM / 18 ORQ NT (no treatment) / 18	ORQ 30 days	Right upper incisor	IHC: TRAP, AP, WNT, and Osteocalcin; MicroCT: bone volume fraction, trabecular thickness, trabecular	IHC: Wnt protein was more intensely expressed in sham than ORQ NT, revealing that bone formation was active,

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Table 2 (*continued*)

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
						separation, and trabecular number.	which represents normal processes of alveolar bone healing and a slower process for the ORQ NT group.
						14 and 42 days	MicroCT: Trabecular thickness, trabecular number, and separation between the trabeculae indicated that a better-quality bone was observed in animals of the sham group, while a worse-quality bone was observed in the ORQ NT group. However, no differences were found in terms of newly formed bone volume.
	Mice / BALB/c;	Female	Young / 9	OVX	Bilateral maxillary	MicroCT: bone volume/total volume;	MicroCT: At day 7, OVX alveolar sockets presented significantly less bone volume fraction compared to young control.
Arioka et al. (2019)	Axin2$^{CreERT2/+}$; R26R^{mTmG}/+	6 weeks (OVX) and 8 weeks (young)	OVX / 9	8 weeks	molars	Histological; IHC: WNT responsive cells. 0, 1, 3 (IHC) and 7 (MicroCT)	Histological: On day 7, histologic evaluation demonstrated that OVX extraction sockets were significantly slower to heal. IHC: At all time points examined, it remained a deficit of Wnt- responsive cells and their progenitors.
		Female	OVX + Pbo / 10	OVX	Upper and lower left first premolars (10 weeks analysis)	MicroCT: bone volume fraction, trabecular pattern factor, structure model index, trabecular number, trabecular thickness, trabecular separation, connectivity density, bone surface density, and degree of anisotropy; CBCT: fractal dimension, Type I collagen analysis by High-performance liquid chromatography 4 and 10 weeks	MicroCT: No differences were found among groups in all parameters analyzed.
Chavarry et al. (2019)	White rabbits / New Zealand (Oryctolagus cuniculus)	6 months	SHAM + Pbo / 6	1 month and 6 weeks	Upper and the lower right first premolars (4 weeks analysis)		CBCT: Significantly higher amounts of type 1 collagen were found in the control group and higher amounts of pentosidine, deoxypyridinoline, and pyridinoline combined in the OVX group.
		Female	Young / 4	OVX		MicroCT: Bone volume/total volume; Histology.	MicroCT and histology: In the young control group, extraction sites were healed by week 4. In the OVX, extraction sites took 8 weeks to heal. At the healed extraction site, the OVX group presented a significantly less percentage of bone volume.
			OVX / 4				
Liu et al. (2019)	Mice / Wild-type BALB/C and Axin2LacZ/+	1.5 months	Aged / 4	No waiting	Bilateral maxillary molars	4 and 8 weeks	
		Male	SHAM / 26	ORQ		PCR: RANKL, OPG, RANKL/OPG;	IHC: Higher ratio of RANKL/OPG was observed in the ORQ group.
Puttini et al. (2019)	Rats / Wistar	6 months	ORQ / 26	30 days	Right upper incisor	Histological; IHC: OPG, RANKL; MicroCT: total bone volume and porosity;	MicroCT: The worse trabecular pattern was observed in the ORQ group. Bone volume was significantly higher in sham group at the periods analyzed.

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Table 2 (*continued*)

Author, year	Animal species / Strain	Samples characteristics *Sex Age*	Experimental groups / Number of animals	Osteoporosis induction: method/ period	Tooth extraction characteristics	Method of healing analysis *Type Period of analysis*	Main outcomes
Miranda et al. (2020)	Rats / Wistar	Female HMM: No differences on the newly formed bone at 10 but at 20 days and 30 days post-extraction, the estrogen-deficient group presented lower bone healing than the others. The estrogen-deficient group presented larger spaces between the trabeculae formed at 20- and 30-days. IHC: OCN, OPN, BSP, RANKL, and OPG. IHC: The estrogen-sufficient group exhibited lower RANKL expression.	Estrogen-sufficient / 24 90 days	OVX Estrogen-deficient / 24	Maxillary left first molars	Confocal laser microscopy: MAR and fluorochrome area. 42 and 60 days 21 days 10, 20, and 30 days	Confocal laser microscopy: Loss of calcium deposition in bone from ORQ group. HMM: Bone healing (newly formed bone calculated by the [number of intersections presenting new bone × 100]/total number of intersections on the entire socket); Histochemistry: TRAP; Histochemistry: TRAP staining did not differ among groups at 10 and 20 days.

AP, alkaline phosphatase; **BMP-7,** bone morphogenetic protein; **BMP-9**, bone morphogenetic protein 9; **BSE**, backscattered electron image; **BSP**, bone sialoprotein; **BSP**, bone sialoprotein; **CBCT**, Cone-beam computed tomography; **CFU-F**, colony formation unit, fibroblast; **CFU-O**, colony formation unit, osteoprogenitors; **Dex**, dexamethasone; **EDX**, energy-dispersive X-Ray; **HMM**, histomorphometry; **IHC**, immunohistochemistry; **MAR**, mineral apposition rate; **MicroCT**, microtomography; **MS**, mineralizing surfaces; **NI**, not informed; **OCN**, osteocalcin; **OD**, optical densitometry; **OPG**, osteoprotegerin; **OPN**, osteopontin; **OPN**, osteopontin; **ORQ**, orchiectomy; **OVX**, ovariectomy; **Pbo**, placebo; **RA**, radiographic analysis. **RANKL**, receptor activator of nuclear factor kappa B ligand; **RT-PCR**, real-time polymerase chain reaction; **SEM**, scanning electron microscopy; **TBA**, trabecular bone area; **TGF-β1**, transforming growth factor beta 1; **TNF-α**, Tumor Necrosis Factor alfa; **TRAP**, tartrate-resistant acid phosphatase.

the OVX group, concluding that the acute estrogen deficiency induced by OVX stimulated bone resorption associated with a minor effect on bone formation.

Shimizu et al. (2000) demonstrated by quantitative backscattered electron image analysis that the newly formed bone area significantly was greater in sham-operated controls than OVX rats at 30 and 60 days, without differences at 7 days PE. Another study by Shimizu et al. (1998) showed that bone resorption was stimulated and long-lasting in OVX compared to the control group by means of scanning electron microscopy analysis around the alveolar socket.

3.7. Secondary outcomes

This section will focus on the qualitative outcomes of the newly formed bone within the alveolar socket obtained from the included studies.

Luvizuto et al. (2010a, 2010b, 2011) demonstrated that OVX was associated with an increase in osteoclastogenesis-related immunoexpressions compared to control groups. Similarly, in the study of Ramalho-Ferreira et al. (2017), the expression of RANKL was higher in the OVX group, and the sham group presented the highest osteocalcin immunoexpression. In another study by Luvizuto et al. (2010b), it was found that OVX decreased osteocalcin expression, indicating a delay in the alveolar socket repair process due to interference in the extracellular matrix turnover. On the other hand, de Oliveira et al. (2019) found no significant difference in the immunoexpression of osteocalcin, TRAP, and alkaline phosphatase between ORQ and sham groups. In this study, the only difference was found in Wnt immunolabelling, which was moderate and intense for the sham group, and mild and intense for the ORQ group at 14- and 42-days PE for both, respectively. The authors concluded that there is a delay in the chronology of socket repair in ORQ animals. De Oliveira Puttini et al. (2019) observed a significant difference in the RANKL/OPG ratio between sham and ORQ groups in all periods analyzed in the study. Moreover, ORQ alveolar bone repair presented a worse trabecular pattern with a more significant amount of intertrabecular connective tissue and a loss of bone calcium deposition. In the study of Chen et al. (2018), alkaline phosphatase activity was also lower in the OVX sockets at 7 days PE. In the study of Arioka et al. (2019), Ki67 and Runx2 markers were also significantly lower in the OVX group at 3 days PE.

Regarding the studies that performed gene expression analysis, the results from Liu et al. (2014) showed that TGF-β1 was significantly lower in the OVX group than the control. Simultaneously, TNF-α was significantly higher in OVX than in the control group. With similar analysis

methods but different genes, Bezerra et al. (2013) observed no differences among groups in the gene expression of BMP-2, BMP-7, BSP, and OPN. In the study of Zecchin et al. (2005), it was found that the absence of estrogen in OVX animals lacking estrogen presented a decrease of MMP-9 and types I and III collagen mRNA.

There were 2 studies that evaluated radiographic density analysis. Pereira et al. (2007) performed a densitometric analysis and verified that OVX had lower densitometric values than control at all periods PE analyzed (3, 5, 7-, 12-, 21-, and 28-days PE). Contrastingly, Jee et al. (2010) also evaluated radiographic density between OVX and control at 0-, 2-, 4- and 6-weeks PE buy observed that the OVX group presented a lower bone density comparing to control only at 4 weeks PE.

An analysis of type 1 collagen by Chavarry et al. (2019), showed significantly higher amounts in the control group and higher amounts of pentosidine, deoxypyridinoline, and pyridinoline in the OVX group, concluding that there was increased bone resorption in the OVX group.

3.8. Risk of bias assessment

Considering all the twelve entries evaluated through the adapted SYRCLE's RoB tool, the one with the highest risk of bias was "Evaluating and reporting osteoporosis confirmation," followed by "Reporting an evaluation method for the success of ovariectomy," both from "other source of bias category." In decrescent order, the following entries also presented some degree of bias: "Selective outcome reporting (reporting bias)," "Random housing (performance bias)," "Adequate time between ovariectomy and tooth extraction (other sources of bias), "Blinding (performance bias)," "Baseline characteristics (selection bias)" and "Incomplete outcome data (attrition bias)." The remaining entries, "Sequence generation (selection bias)," "Allocation concealment (selection bias)," "Random outcome assessment (detection bias)," and "Blinding (detection bias)" did not present any clear risk of bias. The results from the risk of bias assessment and authors' judgment for each included study are shown in Table 3 and Fig. 2.

4. Discussion

Osteoporosis is a systemic condition that causes major public health concerns because of its high prevalence, risk of bone fractures, and a consequent increase in hospitalizations (Eastell et al., 2016; Kanis et al., 2019). Additionally, this disease is a challenge to health professionals since only a small fraction of the population who should receive treatment is provided with it compared to the great majority of those undertreated (Hiligsmann et al., 2019; Kanis et al., 2019). Considering that, it is justified the necessity to enlighten the effect of osteoporosis on the alveolar bone repair after tooth extraction, which is one of the most common oral clinical procedures. To the best of our knowledge, no systematic review has been yet carried out on this subject. The analysis performed in the present systematic review demonstrated that an osteoporotic phenotype in the majority of the included experimental studies negatively impacted the alveolar socket repair.

Table 3
Risk of bias assessment.

	Low	Unclear	High
Sequence generation (selection bias)	0 %	100 %	0 %
Baseline characteristics (selection bias)	40 %	56 %	4 %
Allocation concealment (selection bias)	0 %	100 %	0 %
Random housing (performance bias)	12 %	60 %	28 %
Blinding (performance bias)	88 %	0%	12 %
Random outcome assessment (detection bias)	0 %	100 %	0 %
Blinding (detection bias)	44 %	56 %	0 %
Incomplete outcome data (attrition bias)	36 %	60 %	4 %
Selective outcome reporting (reporting bias)	68 %	0 %	32 %
Reporting an evaluation method for the success of ovariectomy (other)	36 %	0 %	64 %
Adequate time between ovariectomy and tooth extraction (other)	72 %	8 %	20 %
Evaluating and reporting the osteoporosis confirmation (other)	28 %	0 %	72 %

Adapted from the SYRCLE's risk of bias tool (Hooijmans et al., 2014).

In this context, animal models have been remarkably useful to osteoporosis studies. Among them, OVX has been the most common method to induce osteoporosis, being recommended by the US Food and Drug Administration (FDA) (Thompson et al., 1995). Accordingly, the present review also found that 76 % used OVX or even 88 %, including OVX associated with other methods. It is clearly not ethically feasible to perform a clinical study with osteoporotic patients and not treat them even though a great part of the osteoporotic population is undertreated (Hiligsmann et al., 2019; Nuti et al., 2019). For this reason, systematic reviews of animal studies are a valuable tool for studying osteoporosis, and several methods have already been proposed (Iwaniec & Turner, 2013; Thompson et al., 1995).

Regarding socket repair analysis, most of the included studies performed histomorphometric analysis. With this method, it is possible to measure the bone area, trabecular number, trabecular thickness, and other parameters to assess bone repair and bone quality. Through the histomorphometric analysis, the majority of the included studies demonstrated a significantly lower bone area in osteoporosis inducted animals (Bezerra et al., 2013; Luvizuto et al., 2010b, 2011; Miranda et al., 2020; Pereira et al., 2007; Shimizu et al., 2000; Teófilo et al., 2004) in the periods analyzed. Histomorphometry is a valuable approach to analyze socket healing as it evidences bone cellularity and metabolism. Besides, it is currently considered the gold standard for trabeculae and cortical bone crest analysis. Nevertheless, an important weakness related to histomorphometric analysis is that it only provides data on the two-dimensional stereological characteristic of bone, thus precluding the assessment of bone volume microstructure (Romão et al., 2015). On the other hand, microCT is a method of analysis that can perform reconstructions and provide high-definition three-dimensional information of the cortical and trabecular bone (Bouxsein et al., 2010). This socket healing method of analysis allowed 32 % of the studies to measure bone volume, trabecular number, trabecular thickness, and other parameters in the alveolar socket and compare the osteoporotic to the healthy bone. The studies that performed this type of analysis observed that the osteoporotic group presented a lower bone volume and a more inferior bone quality in the parameters analyzed (Arioka et al., 2019; de Oliveira Puttini et al., 2019; Jee et al., 2010; Liu et al., 2019). Immunohistochemistry is a useful technique to verify in which stage the process of bone healing is as it can identify specific proteins expressed in a tissue. Several proteins participate in bone healing, being OPG and RANKL likely the most important ones. The ratio between these two antibodies can point out the tendency to bone resorption (RANKL) or formation (OPG) (Hassumi et al., 2018). In this review, 36 % of the studies performed this method of analysis and observed that osteoporotic animals presented a higher proportion of osteoclastic markers and/or lower proportion of osteoblastic markers (Chen et al., 2018; de Oliveira et al., 2019; de Oliveira Puttini et al., 2019; Luvizuto et al., 2010a, 2010b, 2011; Miranda et al., 2020). Even though immunohistochemistry provides useful information regarding the stage of bone healing, a disadvantage of this method is that it is not possible to obtain data from the amount of neoformed bone tissue.

Conversely, three studies could not observe differences in alveolar socket repair between a healthy and an osteoporotic phenotype. A possible explanation is that there is still no consensus concerning the methodology in animal models, making it unfeasible to determine which aspect was different in these studies that provided them with adverse outcomes. Moreover, a systematic review of Calciolari et al. (Calciolari

Table 4
Ranges of newly formed bone in sham and OVX groups evaluated by histomorphometric analysis. Studies were included in this table only if the authors provided these values in the article.

Author; year	Method of analysis	Period of analysis													
		Early period (7-14 days)					Intermediate period (20-30 days)				Late period (42 to 60 days)				
		Amount of sham newly formed bone	Amount of OVX newly formed bone	Sham x OVX difference	Amount of sham newly formed bone	Amount of OVX newly formed bone	Sham x OVX difference	Amount of sham newly formed bone	Amount of OVX newly formed bone	Sham x OVX difference	Amount of sham newly formed bone	Amount of OVX newly formed bone	Sham x OVX difference	Amount of sham newly formed bone	Amount of OVX newly formed bone	Sham x OVX difference
Shimizu et al. (2000)	HMM	4.3 ± 1.3	4.9 ± 1.6	↑≈14%							43.7 ± 4.8	22.5 ± 3.5	↓ ≈49%	66.4 ± 5.1	56.6 ± 4.6	↓≈15%
		7 days	7 days	NS							30 days	30 days	P < 0.01	60 days	60 days	P < 0.05
Luvizuto et al. (2010a)	HMM	14.78 ± 1.2	6.28 ± 0.52	↓ ≈57,6%	41.17 ± 2.8	23.64 ± 1.6	↓ ≈43%	50.25 ± 1.2	29.5 ± 2.5	↓ ≈42%	59.26 ± 0.9	45.85 ± 2.62	↓≈ 23%	69.64 ± 3.1	49.81 ± 2.5	↓≈28,5%
		7 days	7 dias	P < 0.05	14 days	14 days	P < 0.05	21 days	21 days	P < 0.05	28 days	28 days	P < 0.05	42 days	42 days	P < 0.05
Bezerra et al. (2013)	HMM	32.6 +-3.8	5.9 +-1.8	↓≈82%												
		8 days	8 days	P < 0.05												
Miranda et al. (2020)	HMM	57.9 ± 6.3	52.7 ± 7.2	↓≈ 9 %				80.0 ± 6.8	65.1 ± 10.7	↓ ≈19 %	87.9 ± 6.7	75.8 ± 8.0	↓ ≈14 %			

HMM, histomorphometric analysis; **NS**, not significant.

et al., 2016) examined the relation of the skeletal mineral density and jawbone density in humans and concluded that it is still unclear to which extent osteoporosis affects the jawbone in humans due to the weak available evidence. Similarly, another study examined the effect of OVX in rats and observed that jawbones presented only a minor reduction in bone mineral density compared to other bones of the skeleton such as the tibia and femur (Liu et al., 2015). On the other hand, another study performed OVX in association with a low calcium diet and presented a significant reduction in bone mass and calcium content in the maxilla in comparison to the sham group (Teófilo et al., 2003). Even though the extent to which osteoporosis affects oral bones is quite controversial, this aspect added to the lack of methodological consensus may justify the absence of significant results in some of the studies in the present review.

Regarding the time points for socket healing analysis, the present review showed a wide range of 3–70 days. However, the most frequent time points were 7, 14, and 28 days. It is essential to highlight that the normal course of alveolar bone healing in rats is relatively well-established in the current literature. There are 3 main phases: early phase (1–5 days), bone formation phase (5–20 days), and bone remodeling phase (20–60 days) (Bodner et al., 1993). To evaluate neoformed bone tissue and compare healthy to the osteoporotic bone, we recommend performing healing analysis at a time point between 5–20 days, the bone formation phase. Additionally, according to previous studies, extraction socket healing is completed by 28 days in healthy rats (Hassumi et al., 2018; Luvizuto et al., 2010a, 2010b). On the other hand, it is also crucial to bear in mind that osteoporotic bone may present a slower healing process and, therefore, it may lengthen this period.

The variable methodologies used among the included studies were limiting factors for a solid and homogeneous analysis in the present review. Animals' age, extracted teeth, the period of socket repair analysis, methods for repair analysis, osteoporosis induction, the period after osteoporosis induction, and method of socket repair analysis are some examples of methodological aspects that considerably diverged between the studies. In the quality assessment, in most of the categories, a high amount of "unclear" risk of bias was attributed, highlighting the lack of information in experimental studies. Additionally, other crucial limitations were observed in most of the included studies, which were not confirming osteoporosis induction procedures and not reporting an osteoporosis establishment success. These categories represented the ones with the highest risk of bias. Considering the challenge that is to establish an osteoporotic phenotype in jawbones (Calciolari et al., 2016; Hara et al., 2001; Liu et al., 2015), performing the confirmation is fundamental and will increase the chances of the results being accurate and of showing whether osteoporosis actually produces an impact on tooth extraction healing. Once there was no pattern among studies, it becomes difficult to compare the outcomes, to draw a conclusion that may translate to direct clinical applicability. Similarly, a study evaluated the osteointegration of implants in osteoporotic conditions in animal models. Also, it verified that still no conclusions could be drawn regarding the placement of implants in jawbones based on the available evidence due to the small number of studies and overall low quality (Dereka et al., 2018).

Considering the detailed assessment performed on the studies and the lack of methodologic uniformity among them, the present review will suggest here a methodological proposal for future studies. The recommendations summarized after the article's conclusion may be applied for studies evaluating alveolar bone healing after tooth extraction. Some of them (items 1–7, for instance) may even be employed in other further studies that wish to develop an osteoporotic phenotype in jawbones to evaluate other primary outcomes such as alveolar ridge preservation. As observed in quality assessment, several studies failed to report baseline characteristics. A consolidated reporting of animal species, strain, sex, age, and weight in future studies is recommended. To better standardize future studies, it should be considered that rats are the most frequent animal model. Regarding animal age, the literature is

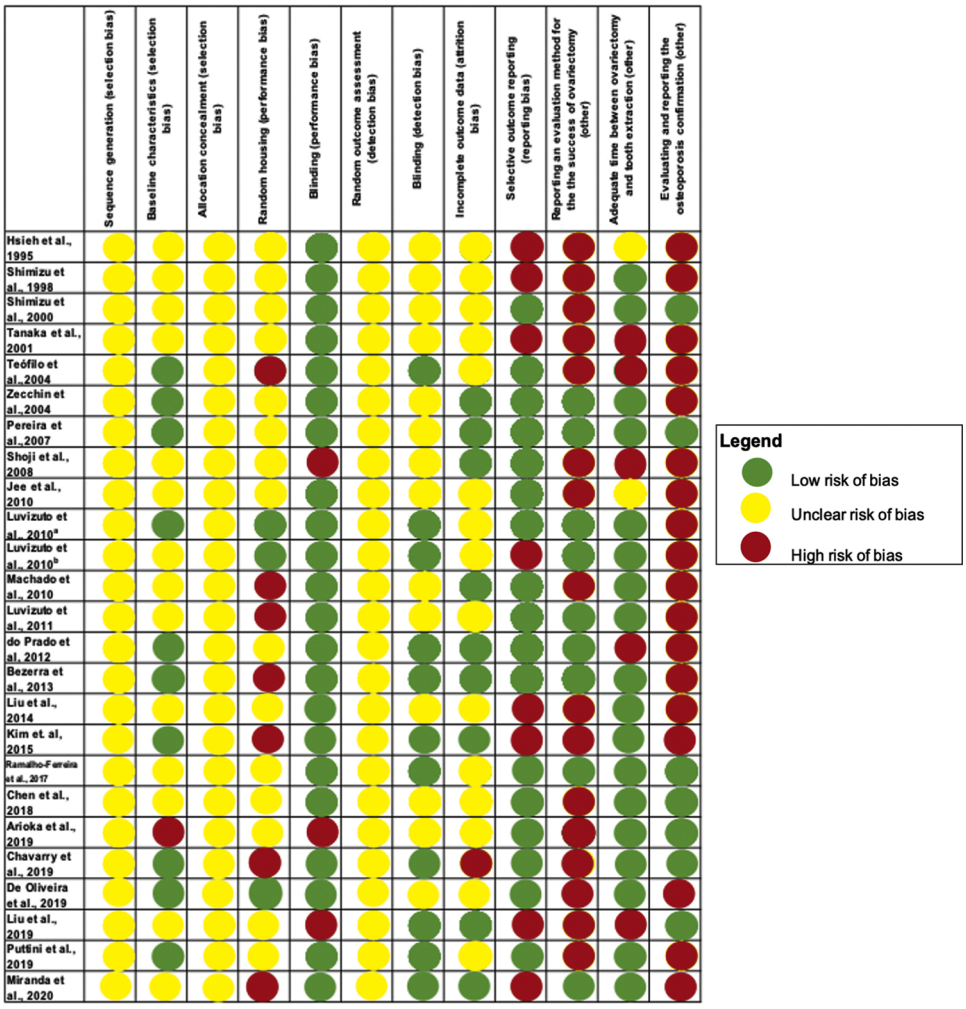

Fig. 2. Risk of bias assessment summary. Authors' judgment for each included stud.

controversial. Medical studies claim that studying postmenopausal osteoporosis rats should be around 8 months old (Iwaniec & Turner, 2013; Yousefzadeh et al., 2020). However, in the present review, the mean age was around 100 days (approximately 3 months and 10 days), and some studies in this review demonstrated an osteoporotic phenotype in much younger animals, even 6-weeks-old (Arioka et al., 2019; Chen et al., 2018; Liu et al., 2019). Conversely, such young animals are not recommended. According to Lee et al. (2019b) citing Kalu (1991), in young animals, lower bone mass in OVX animals may be confounded with the inhibited growth compared to the control group instead of the accelerated bone loss caused by OVX (Francisco et al., 2011; Iwaniec & Turner, 2013; Yousefzadeh et al., 2020). Considering that, for future studies, around 3 months of age, sexually mature models, at the time of the induction is recommended since the skeletally mature models (12 months old) may present comorbidities related to aging (Lee et al., 2019b). Concerning animal sex, female animals are suggested to perform OVX as an osteoporosis induction procedure since it is the most common method for osteoporosis induction, according to previous studies (Iwaniec & Turner, 2013; Lelovas et al., 2008; Yousefzadeh et al., 2020).

Although upper incisors and maxillary molars were the most frequent extracted teeth, maxillary molars are considered a better alternative since rat incisors have continuous growth, making them relatively different from the human tooth (Pereira et al., 2007). Additionally, a study of Hsu et al. (Hsu et al., 2016) observed that, even though osteoporosis had been confirmed in the femurs, OVX affected only the microarchitecture of trabecular bone in the mandible. Trabecular bone is primarily affected by osteoporosis (Osterhoff et al., 2016) and is more metabolically active than cortical bone (Kerschan--Schindl et al., 2018). Therefore, procedures may work better in the maxilla due to the more significant trabecular bone proportion compared to the mandible (Deguchi et al., 2008).

Previous studies have been demonstrating the challenge to develop an osteoporotic-phenotype in jawbones with OVX only (Hara et al., 2001; Prado et al., 2012; Ramalho-Ferreira et al., 2017; Teófilo et al., 2003). Considering that, some studies have investigated an association with other methods to OVX such removal of occlusal loading or low calcium diet to OVX and demonstrated that it accelerates the establishment an osteoporotic-phenotype in maxilla and mandible as well as in other bones of the skeleton (Ejiri et al., 2006; Elovic et al., 1995; Gao et al., 2014; Hara et al., 2001; Jiang et al., 2003; Lelovas et al., 2008; Prado et al., 2012; Ramalho-Ferreira et al., 2017; Teófilo et al., 2003, 2004; Zaffe et al., 1999). Therefore, even though this association of a low calcium diet to OVX was not the most employed method of osteoporosis induction in the present review, we still recommend employing this resource in future studies since it may increase the rates of success in developing an osteoporotic animal model in jawbones. Another important aspect to consider is the period of osteoporosis induction. The depletion of estradiol hormones is reached at least 21 days after OVX as previously reported (Marcondes et al., 2002). For this reason, assessing and reporting the animal estrous cycle is strongly recommended (Ceschin et al., 2004). Another approach to evaluate the success of OVX is to measure the plasmatic concentrations of estradiol (Luvizuto et al., 2010b). Even though the depletion is usually reached in 21 days

(Marcondes et al., 2002) and the median period was 30 days in this review, the currently available evidence consistently shows that a much longer period is necessary to induce osteoporosis in jawbones (Hsu et al., 2016; C. Lee et al., 2019a; X. L. Liu et al., 2015; Teófilo et al., 2003). A previous study by Lee et al. (2019a) demonstrated that a significant microarchitectural change in the mandible is observed 36 weeks after OVX. Another study associated OVX with a low calcium diet and observed that the combination of these two methods was capable of accelerating the bone loss in maxillary bone in rats, even in 5 weeks, some differences could be found comparing to OVX alone and sham group (Teófilo et al., 2003). Furthermore, in this review, a longer period for osteoporosis induction was observed in the studies that performed an evaluation and confirmed the osteoporotic phenotype in the animals' skeleton, approximately 50 days. According to the quality assessment in this review, these studies are less biased in terms of the osteoporotic establishment. Based on the scientific evidence above, we recommend 8 weeks to start the experimental procedures to guarantee osteoporosis induction success. In addition, reporting confirmation of the osteoporosis phenotype is also highly recommended. For this purpose, there are several methodological alternatives such as microCT (Arioka et al., 2019; Chen et al., 2018; Y. Liu et al., 2019), bone densitometry (Pereira et al., 2007; Zecchin et al., 2005), backscattered electron micrograph (Shimizu et al., 2000) and histometric analysis (Pereira et al., 2007) from the tibia (Pereira et al., 2007; Zecchin et al., 2005), femur (Arioka et al., 2019; Chen et al., 2018; Y. Liu et al., 2019), lumbar vertebrae (Y. Liu et al., 2019) or humerus (Shimizu et al., 2000).

Appropriate comprehension of the current state of knowledge of the impact of osteoporosis on socket healing is of extreme relevance. First, implant rehabilitation is usually performed in people over 50, the same age group of people with increased risk for osteoporosis (Kanis et al., 2019; Starr & Maksoud, 2006). Additionally, immediate implant placement still presents a considerable number of limitations, and several strict clinical criteria are required (Ragucci et al., 2020; Romão et al., 2015). Considering that implant placement immediately after a tooth extraction is not possible in all cases, an adequate bone repair process with a bone of good quality is necessary. This preclinical review demonstrated that it is likely that osteoporotic socket healing occurs slower than healthy ones and also may result in a smaller amount of bone after healed. On the other hand, further studies are still necessary to confirm these trends on the results due to current methodological heterogeneity and high risk of bias in crucial aspects. Supposing that the present review results' tendency remains in further research, in that case, new therapeutic strategies will demand investigations to improve bone healing in these situations and guarantee a safe implant placement for these patients.

According to the results observed in the present systematic review, it may be evidenced that an osteoporotic phenotype potentially impairs the alveolar socket repair after tooth extraction in animals. Linking the overall results to humans, we may assume that patients who are more likely to be affected by osteoporosis might present a slower alveolar socket repair or bone of lower quality after tooth extraction. However, limitations of experimental studies presented here require cautious interpretation since the quality assessment was overall unclear, methodologies were diversified, and poorly reported. Therefore, further studies should be conducted in this field and consider the methodological proposal to reduce the risk of bias and increase standardization among studies.

Summary of the methodological proposal for future studies:

1 Report animal species, strain, age, weight, and sex.
2 Female rats between 3 and 8 months of age.
3 Perform OVX associated with a low calcium diet to induce osteoporosis.
4 Wait at least 8 weeks after the osteoporosis induction to start experimental procedures.
5 Tooth extraction in maxillary molars.
6 Confirm OVX by evaluating levels of estradiol or animal estrous cycle.
7 Confirm the osteoporotic phenotype by microCT, bone densitometry, backscattered electron micrograph, or histometric analysis at the tibia, femur, lumbar vertebrae, or humerus bone.
8 Use histomorphometric, microCT, or immunohistochemical analysis of tooth extraction socket.
9 Consider the available evidence to choose a period of socket repair analysis to allow comparisons. The most frequent are 7, 14, and 28 days.

Author statement

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CRediT authorship contribution statement

Bruna Barcelos Só: Conceptualization, Methodology, Investigation, Validation, Writing - original draft. **Felipe Martins Silveira:** Methodology, Investigation, Validation, Writing - review & editing. **Gabriela Sauer Llantada:** Visualization, Writing - original draft, Writing - review & editing. **Luisa Comerlato Jardim:** Data curation, Resources, Formal analysis, Writing - review & editing. **Thiago Calcagnotto:** Visualization, Writing - review & editing. **Marco Antonio Trevizani Martins:** Visualization, Writing - review & editing. **Manoela Domingues Martins:** Project administration, Supervision.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.archoralbio.2021.105054.

References

Arioka, M., Zhang, X., Li, Z., Tulu, U. S., Liu, Y., Wang, L., Yuan, X., & Helms, J. A. (2019). Osteoporotic changes in the periodontium impair alveolar bone healing. *Journal of Dental Research, 98*(4), 450–458. https://doi.org/10.1177/0022034518818456

Barrios, C., Broström, L. A., Stark, A., & Walheim, G. (1993). Healing complications after internal fixation of trochanteric hip fractures: The prognostic value of osteoporosis. *Journal of Orthopaedic Trauma, 7*(5), 438–442. https://doi.org/10.1097/00005131-199310000-00006

Beth-Tasdogan, N. H., Mayer, B., Hussein, H., & Zolk, O. (2017). Interventions for managing medication-related osteonecrosis of the jaw. *The Cochrane Database of Systematic Reviews, 10*. https://doi.org/10.1002/14651858.CD012432.pub2. CD012432.

Bezerra, J. P., de Siqueira, A., Pires, A. G., Marques, M. R., Duarte, P. M., & Bastos, M. F. (2013). Effects of estrogen deficiency and/or caffeine intake on alveolar bone loss, density, and healing: A study in rats. *Journal of Periodontology, 84*(6), 839–849. https://doi.org/10.1902/jop.2012.120192

Bodner, L., Kaffe, I., Littner, M. M., & Cohen, J. (1993). Extraction site healing in rats. A radiologic densitometric study. *Oral Surgery, Oral Medicine, and Oral Pathology, 75* (3), 367–372. https://doi.org/10.1016/0030-4220(93)90153-u

Bouxsein, M. L., Boyd, S. K., Christiansen, B. A., Guldberg, R. E., Jepsen, K. J., & Müller, R. (2010). Guidelines for assessment of bone microstructure in rodents using micro-computed tomography. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research, 25*(7), 1468–1486. https://doi.org/10.1002/jbmr.141

Büyükkurt, C. D., Bülbül, M., Ayanoğlu, S., Esenyel, C. Z., Oztürk, K., & Gürbüz, H. (2012). The effects of osteoporosis on functional outcome in patients with distal radius fracture treated with plate osteosynthesis. *Acta Orthopaedica Et Traumatologica Turcica, 46*(2), 89–95. https://doi.org/10.3944/AOTT.2012.2440

Calciolari, E., Donos, N., Park, J.-C., Petrie, A., & Mardas, N. (2016). A systematic review on the correlation between skeletal and jawbone mineral density in osteoporotic subjects. *Clinical Oral Implants Research, 27*(4), 433–442. https://doi.org/10.1111/clr.12597

Ceschin, Á. P., de Biondo-Simões, M. L. P., Thomaz, B. A. C., & Totsugui, J. (2004). Avaliação hormonal indireta e estudo da preservação folicular em tecido ovariano autólogo transplantado para região inguinal em ratos. *Acta Cirurgica Brasileira, 19* (1), 27–30. https://doi.org/10.1590/S0102-86502004000100004

Chavarry, N. G. M., Perrone, D., Farias, M. L. F., Dos Santos, B. C., Domingos, A. C., Schanaider, A., & Feres-Filho, E. J. (2019). Alendronate improves bone density and type I collagen accumulation but increases the amount of pentosidine in the healing dental alveolus of ovariectomized rabbits. *Bone, 120*, 9–19. https://doi.org/10.1016/j.bone.2018.09.022

Chen, C.-H., Wang, L., Serdar Tulu, U., Arioka, M., Moghim, M. M., Salmon, B., Chen, C.-T., Hoffmann, W., Gilgenbach, J., Brunski, J. B., & Helms, J. A. (2018). An osteopenic/osteoporotic phenotype delays alveolar bone repair. *Bone, 112*, 212–219. https://doi.org/10.1016/j.bone.2018.04.019

Cheung, W. H., Miclau, T., Chow, S. K.-H., Yang, F. F., & Alt, V. (2016). Fracture healing in osteoporotic bone. *Injury, 47*, S21–S26. https://doi.org/10.1016/S0020-1383(16)47004-X

Curtis, E. M., Moon, R. J., Dennison, E. M., Harvey, N. C., & Cooper, C. (2016). Recent advances in the pathogenesis and treatment of osteoporosis. *Clinical Medicine (London, England), 16*(4), 360–364. https://doi.org/10.7861/clinmedicine.16-4-360

de Oliveira, D., de Oliveira Puttini, I., Silva Gomes-Ferreira, P. H., Palin, L. P., Matsumoto, M. A., & Okamoto, R. (2019). Effect of intermittent teriparatide (PTH 1-34) on the alveolar healing process in orchiectomized rats. *Clinical Oral Investigations, 23*(5), 2313–2322. https://doi.org/10.1007/s00784-018-2672-y

de Oliveira Puttini, I., Gomes-Ferreira, P. H., da, S., de Oliveira, D., Hassumi, J. S., Gonçalves, P. Z., & Okamoto, R. (2019). Teriparatide improves alveolar bone modelling after tooth extraction in orchiectomized rats. *Archives of Oral Biology, 102*, 147–154. https://doi.org/10.1016/j.archoralbio.2019.04.007

de Weijden, F. V., Dell'Acqua, F., & Slot, D. E. (2009). Alveolar bone dimensional changes of post-extraction sockets in humans: A systematic review. *Journal of Clinical Periodontology, 36*(12), 1048–1058. https://doi.org/10.1111/j.1600-051X.2009.01482.x

Deguchi, T., Takano-Yamamoto, T., Yabuuchi, T., Ando, R., Roberts, W. E., & Garetto, L. P. (2008). Histomorphometric evaluation of alveolar bone turnover between the maxilla and the mandible during experimental tooth movement in dogs. *American Journal of Orthodontics and Dentofacial Orthopedics: Official Publication of the American Association of Orthodontists, Its Constituent Societies, and the American Board of Orthodontics, 133*(6), 889–897. https://doi.org/10.1016/j.ajodo.2006.12.013

Dereka, X., Calciolari, E., Donos, N., & Mardas, N. (2018). Osseointegration in osteoporotic-like condition: A systematic review of preclinical studies. *Journal of Periodontal Research, 53*(6), 933–940. https://doi.org/10.1111/jre.12566

Eastell, R., O'Neill, T. W., Hofbauer, L. C., Langdahl, B., Reid, I. R., Gold, D. T., & Cummings, S. R. (2016). Postmenopausal osteoporosis. *Nature Reviews Disease Primers, 2*, 16069. https://doi.org/10.1038/nrdp.2016.69

Ejiri, S., Toyooka, E., Tanaka, M., Anwar, R. B., & Kohno, S. (2006). Histological and histomorphometrical changes in rat alveolar bone following antagonistic tooth extraction and/or ovariectomy. *Archives of Oral Biology, 51*(11), 941–950. https://doi.org/10.1016/j.archoralbio.2006.05.006

Elovic, R. P., Hipp, J. A., & Hayes, W. C. (1995). Maxillary molar extraction causes increased bone loss in the mandible of ovariectomized rats. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research, 10*(7), 1087–1093. https://doi.org/10.1002/jbmr.5650100713

Escobedo, M. F., Cobo, J. L., Junquera, S., Milla, J., Olay, S., & Junquera, L. M. (2020). Medication-related osteonecrosis of the jaw. Implant presence-triggered osteonecrosis: Case series and literature review. *Journal of Stomatology, Oral and Maxillofacial Surgery, 121*(1), 40–48. https://doi.org/10.1016/j.jormas.2019.04.012

Féron, J.-M., & Mauprivez, R. (2016). Fracture repair: General aspects and influence of osteoporosis and anti-osteoporosis treatment. *Injury, 47*(Suppl 1), S10–14. https://doi.org/10.1016/S0020-1383(16)30003-1

Fitzpatrick, S. K., Casemyr, N. E., Zurakowski, D., Day, C. S., & Rozental, T. D. (2012). The effect of osteoporosis on outcomes of operatively treated distal radius fractures. *The Journal of Hand Surgery, 37*(10), 2027–2034. https://doi.org/10.1016/j.jhsa.2012.06.025

Francisco, J. I., Yu, Y., Oliver, R. A., & Walsh, W. R. (2011). Relationship between age, skeletal site, and time post-ovariectomy on bone mineral and trabecular microarchitecture in rats. *Journal of Orthopaedic Research: Official Publication of the Orthopaedic Research Society, 29*(2), 189–196. https://doi.org/10.1002/jor.21217

Gao, X., Ma, W., Dong, H., Yong, Z., & Su, R. (2014). Establishing a rapid animal model of osteoporosis with ovariectomy plus low calcium diet in rats. *International Journal of Clinical and Experimental Pathology, 7*(8), 5123–5128.

Gorter, E. A., Gerretsen, B. M., Krijnen, P., Appelman-Dijkstra, N. M., & Schipper, I. B. (2020). Does osteoporosis affect the healing of subcapital humerus and distal radius fractures? *Journal of Orthopaedics, 22*, 237–241. https://doi.org/10.1016/j.jor.2020.05.004

Hak, D. J. (2018). The biology of fracture healing in osteoporosis and in the presence of anti-osteoporotic drugs. *Injury, 49*(8), 1461–1465. https://doi.org/10.1016/j.injury.2018.04.016

Hara, T., Sato, T., Oka, M., Mori, S., & Shirai, H. (2001). Effects of ovariectomy and/or dietary calcium deficiency on bone dynamics in the rat hard palate, mandible and proximal tibia. *Archives of Oral Biology, 46*(5), 443–451. https://doi.org/10.1016/S0003-9969(00)00135-7

Hassumi, J. S., Mulinari-Santos, G., da Fabris, A. L. S., Jacob, R. G. M., Gonçalves, A., Rossi, A. C., Freire, A. R., Faverani, L. P., Okamoto, R., Hassumi, J. S., Mulinari-Santos, G., da Fabris, A. L. S., Jacob, R. G. M., Gonçalves, A., Rossi, A. C., Freire, A. R., Faverani, L. P., & Okamoto, R. (2018). Alveolar bone healing in rats: Micro-CT, immunohistochemical and molecular analysis. *Journal of Applied Oral Science, 26*. https://doi.org/10.1590/1678-7757-2017-0326

Haworth, S., Shungin, D., Kwak, S. Y., Kim, H.-Y., West, N. X., Thomas, S. J., Franks, P. W., Timpson, N. J., Shin, M.-J., & Johansson, I. (2018). Tooth loss is a complex measure of oral disease: Determinants and methodological considerations. *Community Dentistry and Oral Epidemiology, 46*(6), 555–562. https://doi.org/10.1111/cdoe.12391

Hegde, V., Jo, J. E., Andreopoulou, P., & Lane, J. M. (2016). Effect of osteoporosis medications on fracture healing. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 27*(3), 861–871. https://doi.org/10.1007/s00198-015-3331-7

Hernlund, E., Svedbom, A., Ivergård, M., Compston, J., Cooper, C., Stenmark, J., McCloskey, E. V., Jönsson, B., & Kanis, J. A. (2013). Osteoporosis in the European Union: Medical management, epidemiology and economic burden. A report prepared in collaboration with the International Osteoporosis Foundation (IOF) and the European Federation of Pharmaceutical Industry Associations (EFPIA). *Archives of Osteoporosis, 8*, 136. https://doi.org/10.1007/s11657-013-0136-1

Hiligsmann, M., Cornelissen, D., Vrijens, B., Abrahamsen, B., Al-Daghri, N., Biver, E., Brandi, M. L., Bruyère, O., Burlet, N., Cooper, C., Cortet, B., Dennison, E., Diez-Perez, A., Gasparik, A., Grosso, A., Hadji, P., Halbout, P., Kanis, J. A., Kaufman, J. M., … Reginster, J. Y. (2019). Determinants, consequences and potential solutions to poor adherence to anti-osteoporosis treatment: Results of an expert group meeting organized by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO) and the International Osteoporosis Foundation (IOF). *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 30*(11), 2155–2165. https://doi.org/10.1007/s00198-019-05104-5

Hooijmans, C. R., Rovers, M. M., de Vries, R. B. M., Leenaars, M., Ritskes-Hoitinga, M., & Langendam, M. W. (2014). SYRCLE's risk of bias tool for animal studies. *BMC Medical Research Methodology, 14*, 43. https://doi.org/10.1186/1471-2288-14-43

Hsieh, Y. D., Devlin, H., & McCord, F. (1995). The effect of ovariectomy on the healing tooth socket of the rat. *Archives of Oral Biology, 40*(6), 529–531. https://doi.org/10.1016/0003-9969(94)00197-j

Hsu, P.-Y., Tsai, M.-T., Wang, S.-P., Chen, Y.-J., Wu, J., & Hsu, J.-T. (2016). Cortical bone morphological and trabecular bone microarchitectural changes in the mandible and femoral neck of ovariectomized rats. *PloS One, 11*(4), Article e0154367. https://doi.org/10.1371/journal.pone.0154367

Iwaniec, U. T., & Turner, R. T. (2013). Chapter 39—Animal models for osteoporosis. In R. Marcus, D. Feldman, D. W. Dempster, M. Luckey, & J. A. Cauley (Eds.), *Osteoporosis (Fourth edition)* (pp. 939–961). Academic Press. https://doi.org/10.1016/B978-0-12-415853-5.00039-X.

Jee, J.-H., Lee, W., & Lee, B. D. (2010). The influence of alendronate on the healing of extraction sockets of ovariectomized rats assessed by in vivo micro-computed tomography. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics, 110*(2), e47–53. https://doi.org/10.1016/j.tripleo.2010.03.025

Jiang, G., Matsumoto, H., & Fujii, A. (2003). Mandible bone loss in osteoporosis rats. *Journal of Bone and Mineral Metabolism, 21*(6), 388–395. https://doi.org/10.1007/s00774-003-0433-7

Johnell, O., & Kanis, J. A. (2006). An estimate of the worldwide prevalence and disability associated with osteoporotic fractures. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 17*(12), 1726–1733. https://doi.org/10.1007/s00198-006-0172-4

Kalu, D. N. (1991). The ovariectomized rat model of postmenopausal bone loss. *Bone and Mineral, 15*(3), 175–191. https://doi.org/10.1016/0169-6009(91)90124-i

Kanis, J. A., Cooper, C., Rizzoli, R., Reginster, J.-Y., & Scientific Advisory Board of the European Society for Clinical and Economic Aspects of Osteoporosis (ESCEO) and the Committees of Scientific Advisors and National Societies of the International Osteoporosis Foundation (IOF). (2019). European guidance for the diagnosis and management of osteoporosis in postmenopausal women. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 30*(1), 3–44. https://doi.org/10.1007/s00198-018-4704-5

Kassebaum, N. J., Bernabé, E., Dahiya, M., Bhandari, B., Murray, C. J. L., & Marcenes, W. (2014). Global burden of severe tooth loss: A systematic review and meta-analysis. *Journal of Dental Research, 93*(7 Suppl), 20S–28S. https://doi.org/10.1177/0022034514537828

Kerschan-Schindl, K., Valenzuela, E. N., & Pietschmann, P. (2018). Immunology of osteoporosis. In T. Fulop, C. Franceschi, K. Hirokawa, & G. Pawelec (Eds.), *Handbook*

of immunosenescence: Basic understanding and clinical implications (pp. 1–21). Springer International Publishing. https://doi.org/10.1007/978-3-319-64597-1_153-1.

Khan, A. A., Morrison, A., Hanley, D. A., Felsenberg, D., McCauley, L. K., O'Ryan, F., Reid, I. R., Ruggiero, S. L., Taguchi, A., Tetradis, S., Watts, N. B., Brandi, M. L., Peters, E., Guise, T., Eastell, R., Cheung, A. M., Morin, S. N., Masri, B., Cooper, C., … International Task Force on Osteonecrosis of the Jaw. (2015). Diagnosis and management of osteonecrosis of the jaw: A systematic review and international consensus. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research, 30*(1), 3–23. https://doi.org/10.1002/jbmr.2405

Khosla, S., Farr, J. N., & Kirkland, J. L. (2018). Inhibiting cellular senescence: A new therapeutic paradigm for age-related osteoporosis. *The Journal of Clinical Endocrinology and Metabolism, 103*(4), 1282–1290. https://doi.org/10.1210/jc.2017-02694

Kim, J.-W., Tatad, J. C. I., Landayan, M. E. A., Kim, S.-J., & Kim, M.-R. (2015). Animal model for medication-related osteonecrosis of the jaw with precedent metabolic bone disease. *Bone, 81*, 442–448. https://doi.org/10.1016/j.bone.2015.08.012

Lee, C., Lee, J.-H., Han, S.-S., Kim, Y. H., Choi, Y.-J., Jeon, K. J., & Jung, H. I. (2019a). Site-specific and time-course changes of postmenopausal osteoporosis in rat mandible: Comparative study with femur. *Scientific Reports, 9*(1), 1–9. https://doi.org/10.1038/s41598-019-50554-w

Lee, J.-H., Han, S.-S., Lee, C., Kim, Y. H., & Battulga, B. (2019b). Microarchitectural changes in the mandibles of ovariectomized rats: A systematic review and meta-analysis. *BMC Oral Health, 19*. https://doi.org/10.1186/s12903-019-0799-0

Lelovas, P. P., Xanthos, T. T., Thoma, S. E., Lyritis, G. P., & Dontas, I. A. (2008). The laboratory rat as an animal model for osteoporosis research. *Comparative Medicine, 58*(5), 424–430.

Liu, X. L., Li, C. L., Lu, W. W., Cai, W. X., & Zheng, L. W. (2015). Skeletal site-specific response to ovariectomy in a rat model: Change in bone density and microarchitecture. *Clinical Oral Implants Research, 26*(4), 392–398. https://doi.org/10.1111/clr.12360

Liu, X., Zhang, R., Zhou, Y., Yang, Y., Si, H., Li, X., & Liu, L. (2014). The effect of Astragalus extractive on alveolar bone rebuilding progress of tooth extracted socket of ovariectomied rats. *African Journal of Traditional, Complementary, and Alternative Medicines: AJTCAM, 11*(5), 91–98. https://doi.org/10.4314/ajtcam.v11i5.15

Liu, Y., Li, Z., Arioka, M., Wang, L., Bao, C., & Helms, J. A. (2019). WNT3A accelerates delayed alveolar bone repair in ovariectomized mice. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 30*(9), 1873–1885. https://doi.org/10.1007/s00198-019-05071-x

Luvizuto, E. R., Dias, S. S. M. D., Okamoto, T., Dornelles, R. C. M., & Okamoto, R. (2011). Raloxifene therapy inhibits osteoclastogenesis during the alveolar healing process in rats. *Archives of Oral Biology, 56*(10), 984–990. https://doi.org/10.1016/j.archoralbio.2011.03.015

Luvizuto, E. R., Dias, S. M. D., Queiroz, T. P., Okamoto, T., Garcia, I. R., Okamoto, R., & Dornelles, R. C. M. (2010a). Osteocalcin immunolabeling during the alveolar healing process in ovariectomized rats treated with estrogen or raloxifene. *Bone, 46*(4), 1021–1029. https://doi.org/10.1016/j.bone.2009.12.016

Luvizuto, E. R., Queiroz, T. P., Dias, S. M. D., Okamoto, T., Dornelles, R. C. M., Garcia, I. R., & Okamoto, R. (2010b). Histomorphometric analysis and immunolocalization of RANKL and OPG during the alveolar healing process in female ovariectomized rats treated with oestrogen or raloxifene. *Archives of Oral Biology, 55*(1), 52–59. https://doi.org/10.1016/j.archoralbio.2009.11.001

Machado, G. J. R., Dias, S. M. D., Bosco, A. F., Okamoto, T., Bedran de Castro, J. C., & Dornelles, R. C. M. (2010). Action of nicotine and ovariectomy on bone regeneration after tooth extraction in rats. *Journal of Oral and Maxillofacial Surgery: Official Journal of the American Association of Oral and Maxillofacial Surgeons, 68*(11), 2675–2681. https://doi.org/10.1016/j.joms.2010.04.018

Marcondes, F. K., Bianchi, F. J., & Tanno, A. P. (2002). Determination of the estrous cycle phases of rats: Some helpful considerations. *Brazilian Journal of Biology = Revista Brasleira de Biologia, 62*(4A), 609–614. https://doi.org/10.1590/s1519-69842002000400008

Marmary, Y., Brayer, L., Tzukert, A., & Feller, L. (1986). Alveolar bone repair following extraction of impacted mandibular third molars. *Oral Surgery, Oral Medicine, and Oral Pathology, 61*(4), 324–326. https://doi.org/10.1016/0030-4220(86)90409-3

Martin, J., Viprey, M., Castagne, B., Merle, B., Giroudon, C., Chapurlat, R., & Schott, A.-M. (2020). Interventions to improve osteoporosis care: A systematic review and meta-analysis. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 31*(3), 429–446. https://doi.org/10.1007/s00198-020-05308-0

Miranda, T. S., Napimoga, M. H., De Franco, L., Marins, L. M., de Malta, F. S., Pontes, L. A., Morelli, F. M., & Duarte, P. M. (2020). Strontium ranelate improves alveolar bone healing in estrogen-deficient rats. *Journal of Periodontology*. https://doi.org/10.1002/JPER.19-0561

Moher, D., Liberati, A., Tetzlaff, J., Altman, D. G., & PRISMA Group. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *PLoS Medicine, 6*(7), Article e1000097. https://doi.org/10.1371/journal.pmed.1000097

Nicolatou-Galitis, O., Schiødt, M., Mendes, R. A., Ripamonti, C., Hope, S., Drudge-Coates, L., Niepel, D., & Van den Wyngaert, T. (2019). Medication-related osteonecrosis of the jaw: Definition and best practice for prevention, diagnosis, and treatment. *Oral Surgery, Oral Medicine, Oral Pathology and Oral Radiology, 127*(2), 117–135. https://doi.org/10.1016/j.oooo.2018.09.008

Nikolaou, V. S., Efstathopoulos, N., Kontakis, G., Kanakaris, N. K., & Giannoudis, P. V. (2009). The influence of osteoporosis in femoral fracture healing time. *Injury, 40*(6), 663–668. https://doi.org/10.1016/j.injury.2008.10.035

Nuti, R., Brandi, M. L., Checchia, G., Di Munno, O., Dominguez, L., Falaschi, P., Fiore, C. E., Iolascon, G., Maggi, S., Michieli, R., Migliaccio, S., Minisola, S., Rossini, M., Sessa, G., Tarantino, U., Toselli, A., & Isaia, G. C. (2019). Guidelines for the management of osteoporosis and fragility fractures. *Internal and Emergency Medicine, 14*(1), 85–102. https://doi.org/10.1007/s11739-018-1874-2

Osterhoff, G., Morgan, E. F., Shefelbine, S. J., Karim, L., McNamara, L. M., & Augat, P. (2016). Bone mechanical properties and changes with osteoporosis. *Injury, 47*(Suppl 2), S11–S20. https://doi.org/10.1016/S0020-1383(16)47003-8

Pereira, M. C., Zecchin, K. G., Campagnoli, E. B., & Jorge, J. (2007). Ovariectomy delays alveolar wound healing after molar extractions in rats. *Journal of Oral and Maxillofacial Surgery: Official Journal of the American Association of Oral and Maxillofacial Surgeons, 65*(11), 2248–2253. https://doi.org/10.1016/j.joms.2006.11.040

Pietschmann, P., Mechtcheriakova, D., Meshcheryakova, A., Föger-Samwald, U., & Ellinger, I. (2015). Immunology of osteoporosis: A mini-review. *Gerontology, 62*. https://doi.org/10.1159/000431091

Pietschmann, P., Rauner, M., Sipos, W., & Kerschan-Schindl, K. (2009). Osteoporosis: An age-related and gender-specific disease–A mini-review. *Gerontology, 55*(1), 3–12. https://doi.org/10.1159/000166209

Prado, R. F. D., Silveira, V.Á. S., Rocha, R. F. D., Vasconcellos, L. M. R. D., & Carvalho, Y. R. (2012). Effects of experimental osteoporosis and low calcium intake on postextraction sockets of rats. *International Journal of Experimental Pathology, 93*(2), 139–147. https://doi.org/10.1111/j.1365-2613.2012.00809.x

Rachner, T. D., Khosla, S., & Hofbauer, L. C. (2011). Osteoporosis: Now and the future. *Lancet (London, England), 377*(9773), 1276–1287. https://doi.org/10.1016/S0140-6736(10)62349-5

Ragucci, G. M., Elnayef, B., Criado-Cámara, E., Del Amo, F. S.-L., & Hernández-Alfaro, F. (2020). Immediate implant placement in molar extraction sockets: A systematic review and meta-analysis. *International Journal of Implant Dentistry, 6*(1), 40. https://doi.org/10.1186/s40729-020-00235-5

Ramalho-Ferreira, G., Faverani, L. P., Momesso, G. A. C., Luvizuto, E. R., de Oliveira Puttini, I., & Okamoto, R. (2017). Effect of antiresorptive drugs in the alveolar bone healing. A histometric and immunohistochemical study in ovariectomized rats. *Clinical Oral Investigations, 21*(5), 1485–1494. https://doi.org/10.1007/s00784-016-1909-x

Romão, M. M. A., Marques, M. M., Cortes, A. R. G., Horliana, A. C. R. T., Moreira, M. S., & Lascala, C. A. (2015). Micro-computed tomography and histomorphometric analysis of human alveolar bone repair induced by laser phototherapy: A pilot study. *International Journal of Oral and Maxillofacial Surgery, 44*(12), 1521–1528. https://doi.org/10.1016/j.ijom.2015.08.989

Shimizu, M., Furuya, R., Kawawa, T., & Sasaki, T. (2000). Bone wound healing after maxillary molar extraction in ovariectomized aged rats: Quantitative backscattered electron image analysis. *The Anatomical Record, 259*(1), 76–85. https://doi.org/10.1002/(SICI)1097-0185(20000501)259:1<76::AID-AR9>3.0.CO;2-0

Shimizu, M., Sasaki, T., Ishihara, A., Furuya, R., & Kawawa, T. (1998). Bone wound healing after maxillary molar extraction in ovariectomized aged rats. *Journal of Electron Microscopy, 47*(5), 517–526. https://doi.org/10.1093/oxfordjournals.jmicro.a023623

Shoji, K., Basso, N., Elsubeihi, E. S., & Heersche, J. N. M. (2008). Short-term effect of ovariectomy on osteoprogenitors in the healing rat mandibular incisor extraction socket. *Osteoporosis International: A Journal Established as Result of Cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA, 19*(8), 1193–1201. https://doi.org/10.1007/s00198-007-0558-y

Simpson, E. L., Martyn-St James, M., Hamilton, J., Wong, R., Gittoes, N., Selby, P., & Davis, S. (2020). Clinical effectiveness of denosumab, raloxifene, romosozumab, and teriparatide for the prevention of osteoporotic fragility fractures: A systematic review and network meta-analysis. *Bone, 130*, Article 115081. https://doi.org/10.1016/j.bone.2019.115081

Starr, C. B., & Maksoud, M. A. (2006). Implant treatment in an urban general dentistry residency program: A 7-year retrospective study. *The Journal of Oral Implantology, 32*(3), 142–147. https://doi.org/10.1563/807.1

Susin, C., Oppermann, R. V., Haugejorden, O., & Albandar, J. M. (2005). Tooth loss and associated risk indicators in an adult urban population from south Brazil. *Acta Odontologica Scandinavica, 63*(2), 85–93. https://doi.org/10.1080/00016350510019694

Tanaka, S., Shimizu, M., Debari, K., Furuya, R., Kawawa, T., & Sasaki, T. (2001). Acute effects of ovariectomy on wound healing of alveolar bone after maxillary molar extraction in aged rats. *The Anatomical Record, 262*(2), 203–212. https://doi.org/10.1002/1097-0185(20010201)262:2<203::AID-AR1030>3.0.CO;2-#

Teófilo, J. M., Azevedo, A. C. B., Petenusci, S. O., Mazaro, R., & Lamano-Carvalho, T. L. (2003). Comparison between two experimental protocols to promote osteoporosis in the maxilla and proximal tibia of female rats. *Pesquisa Odontológica Brasileira, 17*(4), 302–306. https://doi.org/10.1590/S1517-74912003000400002

Teófilo, J. M., Brentegani, L. G., & Lamano-Carvalho, T. L. (2004). Bone healing in osteoporotic female rats following intra-alveolar grafting of bioactive glass. *Archives of Oral Biology, 49*(9), 755–762. https://doi.org/10.1016/j.archoralbio.2004.02.013

Thompson, D. D., Simmons, H. A., Pirie, C. M., & Ke, H. Z. (1995). FDA Guidelines and animal models for osteoporosis. *Bone, 17*(4 Suppl), 125S–133S. https://doi.org/10.1016/8756-3282(95)00285-l

Yousefzadeh, N., Kashfi, K., Jeddi, S., & Ghasemi, A. (2020). Ovariectomized rat model of osteoporosis: A practical guide. *EXCLI Journal, 19*, 89–107. https://doi.org/10.17179/excli2019-1990

Zaffe, D., Paganelli, C., & Cocchi, D. (1999). Induction and pharmacological treatment of oral osteopenia in rats. *Minerva Stomatologica, 48*(3), 45–62.

Zecchin, K. G., Pereira, M. C., Coletta, R. D., Graner, E., & Jorge, J. (2005). Ovariectomy reduces the gelatinolytic activity and expression of matrix metalloproteinases and collagen in rat molar extraction wounds. *Calcified Tissue International, 76*(2), 136–145. https://doi.org/10.1007/s00223-004-0013-4