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Jacobs Journal of Dentistry and Research

Research article

Is There a Correlation Between Maxilla and Femur Bone Loss in Osteopenic Rats?

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Received: 10-11-2017

Accepted: 10-31-2017

Published: 11-01-2017

Abstract

Objective: The aim of this study was to test the correlation between bone loss in the maxilla and femur in osteopenic rats.

Material and Methods: Fourteen female Wistar rats, 60 days old, weighing on average 200-220 grams: 1) control group (C) n = 7; 2) ovariectomized group (OVX) n = 7. Bilateral laparotomy was performed and induce osteopenia in the OVX group. In C group, laparotomy was performed, but ovaries were not removed. The femur and maxilla of the animals were removed, analyzed, and the bone metabolism by collagen evidenciation was evaluated for indirect determination of bone.

Results: There was moderate correlation in the alterations of collagen between the femur and maxilla (r = 0.64). There was also a statistically significant difference in the percentage of collagen between C and OVX groups for both the femur and maxilla ($p < 0.001$). The area occupied by collagen tissue was significantly greater in the maxilla in the C group compared to the OVX group ($p < 0.01$).

Conclusions: There was a correlation between bone loss in the maxilla and femur in ovariectomized-osteopenic rats, confirming that ovariectomy decreases bone formation for both sites evaluated, with greater impact on the femur.

Keywords: Histology; Ovariectomy; Osteoporosis; Maxilla, Bone

Introduction

Oral bone loss originating from osteoporosis is a great problem for dentists. Total or partial tooth loss is greater in patients suffering from systemic osteoporosis than in patients without this condition [1,2].

The quantity and quality of the maxillary and mandibular bone is important for treatment planning, especially in the areas that require bone quality for a successful treatment outcome such as in orthodontics, periodontics and implantology.

After the age of 50, mandible cortical bone porosity augments. The alveolar region is more affected than body mandible and it is most evident in women[3–5]. Age and estrogen deficiency affect alveolar crestal, thus leading to tooth loss[6–8].

Age is a significant factor which affects tooth movement. Young growing patients exhibit intense cellular activity, low bone density and large medullary spaces, a situation favoring bone movement. In contrast, adults have very dense bones with a thick cortical plate and low cellular activity, which explains why hyalinization and delayed cell proliferation (8 to 10 days) are frequent features in adults undergoing orthodontic treatment[9,10]. However, alveolar bone turnover and change in the alveolar bone resulting from orthodontic treatment do not seem to make tooth displacement any more difficult in healthy adults aged 50/60 than in subjects aged 20/25[9–11].

Alterations stemming from osteoporosis in the mandible and maxilla also affect stability[12,13] and promote an increase in the space of the periodontal ligament[14]. However, the mandible is loaded by forces applied to the teeth during mastication or biting and this factor seems to be more important than estrogen deficiency[15–17]. It has been reported that mechanical loading accelerates formation and suppresses bone resorption[18,19]. The mandibular bone that was under conditions in intermittent loading by occlusion, frequently stimulates bone formation in opposition to the bone resorption induced by estrogen deficiency[18,17].

Most experimental studies that assess bone loss in the mandible in ovariectomized animals are compared with the femur[20,21]. However, the proportion of trabecular/cortical bone of the femur is more akin to the maxilla than the mandible. The maxilla has a lower alveolar bone mineral density and trabecular bone ratio higher than that of cortical bone as compared to the mandible which has the inverse ratio. This is conceivable, because of rat skull morphology and arrangement of masticatory muscles, as the maxilla is part of the craniofacial complex of bones and can spread occlusal forces across the wide area of the temporalis muscle and the zygomatic arch during mastication. In contrast, the mandible must absorb these forces alone; as such, the bony requirements are likely different[22]. In orthodontic treatment there are some differences in tooth movement: the maxillary molars will move distally/mesially more easily than the mandibular molars[23].

Ovariectomy in rats causes a differential effect on the mandible, vertebra and femoral bone[24–26]. Some studies have demonstrated it as being more marked in the femur than in the mandible because estrogen deficiency affects the trabecular bone sooner than the cortical bone[14,27].

Thus, considering that a skeletal alteration associated with ovariectomy varies in the region observed, regarding the proportion of cortical and trabecular bone, estrogen deficiency affects a lot earlier the trabecular than the cortical bone and turnover of the maxilla is more like the turnover of the femur than that of the mandible; two null hypotheses were tested: (1) ovariectomy does not alter the degree of bone loss in the alveolar bone of the maxilla and (2) there is no correlation between bone mass in the femur and in the maxilla.

Material and Methods

The sample consisted of 14 female, Wistar rats (Rattus norvegicus), 60 days old, weighing on average 200-220 grams (Statement of Animal Rights of the State University of Maringá-UEM number 052/2006). The animals were divided into two groups: 1) control group (C) n = 7; 2) ovariectomized group (OVX) n = 7.

Model for induction of osteopenia

After sedating the animals with 0.1 ml / 100g Xylazine (Parke-Davis Ache, São Paulo, Brazil) and 0.1ml / 100g of Ketamine (Bayer S /A of Brazil, São Paulo, Brazil), bilateral laparotomy was performed to remove ovaries (ovariectomy), and induce osteopenia in the OVX group. In C group, laparotomy was performed, without removing the ovaries (sham-operated). Ninety days after ovariectomy, all animals were euthanized[28]. The left femur and maxilla were removed, dissected, and submitted to histological routine. Longitudinal semi-serial sections of 5μm were performed in relation to the longest axis of the bone. In the maxilla the sections were transverse (longitudinal) relative to the occlusal plane. All sections were stained with hematoxylin and eosin.

Quantitative analysis of area occupied by collagen

The literature report that bone densitometry, conventional X-ray and histological determination of the area occupied by collagen are valid methods to evaluate the loss of bone mass due to ovariectomy[29]. The collagenous tissue is precursor of bone tissue and that large amounts of collagenous tissue in a bone are indicative of bone neoformation. Therefore, in this study, we chose to use the technique of histological determination of the area occupied by collagen to evaluate the loss of bone mass resulting from ovariectomy.

Three histological images from semi-serial sections of the femur and maxilla using an optical microscope Olympus BX41 (Olympus, Tokyo, Japan), scanned with a high resolution camera Olympus 3 Q.Color (Tokyo, Japan) attached to the microscope with a 10x objective.

In maxilla, the collagen occupied area in selected region for quantification was first measured (Fig. 1A-D). Soon after, the total tissue collagen area collagen was quantified (Fig. 1E). This formula was used to make the proportion between cuts and improve results accuracy, because in the same animal, the selected cuts did not present equal total areas. Figure 2 presents the methodology used for analyzing the collagen occupied area in interradicular septal area. As the femur`s covered view the entire captured area, there was no need to define a specific part of the bone, as in figure 1A-B. Figure 2 illustrates the

sections of the femur for the group OVX (A-C) and the group C (DF).

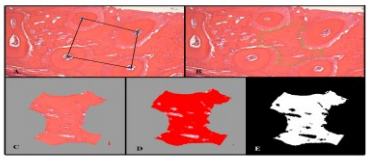

Figure 1. Technique to delimit the area occupied by collagen in the maxilla (magnification 10x);. (A) Demarcation of interradicular area taking as reference point the pulp center of each of the four roots (mesial and medium) of the first molar; (B) Demarcation of the alveolar bone in the region outlined in figure 1A; (C) Extraction of the alveolar bone in the region outlined in figure 1B; (D) All the pink area is stained collagen of red by the program Image Pro-plus ™; (E) The blank area is the area occupied by fully differentiated collagen from other tissues to quantification.

In the maxilla, we evaluated the region from the bone septum, composed of trabecular bone, between the mean and the mesial roots (Figure 1) from the first molar[6,30]. The proximal diaphyseal region in the femur, also composed of trabecular bone, was evaluated. The area occupied by collagen was analyzed with image analysis software Image-Pro Plus® (Version 4.5, Media Cybernetics, Silver Spring, MD). This software can automatically calculate the area in square micrometers (µm2) of stained proteins[31]. The results were expressed as means (x) standard deviation (± sd) of the area (mm^2) occupied by the collagen.

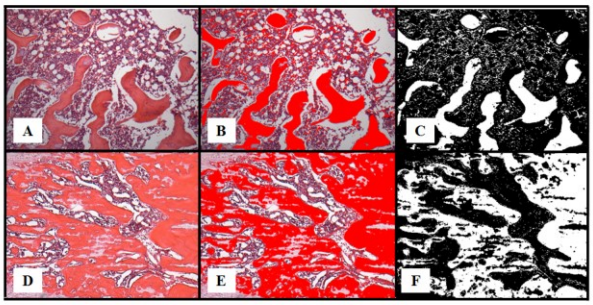

Figure 2. Technique to delimit the area occupied by collagen in the femur (magnification 10x); (A and D) region of the proximal shaft of the left femur of ovariectomized group (A) and control (D); (B and E) All the pink area is stained collagen of red by the program Image Pro-plus ™; (C and F) The white area is the area occupied by fully differentiated collagen from other tissues to quantification.

Statistical Analysis

For sample calculation we used the G Power Test with power of 0.97. The results were analyzed using the Biostat 5.0 program (Biostatistician Institute of Science and Technology, Pará, Brazil). The Pearson Correlation Test (r) was used to assess the correlation between bone loss in the maxilla and femur. The Pearson correlation coefficient indicated that there was a positive moderate correlation between the femur and the maxilla (r = 0.64). Anova test was used to compare the percentage averages between groups, and for intra-group evaluations we used Test-t. Results were expressed as mean (x) and standard deviation (sd) and the level of significance was 5%.

Results

There was a statistically significant difference in the percentage of collagen in the maxilla group C (96.28 ± 1.96) and OVX (80.70 ± 10.84) (Figure 3).

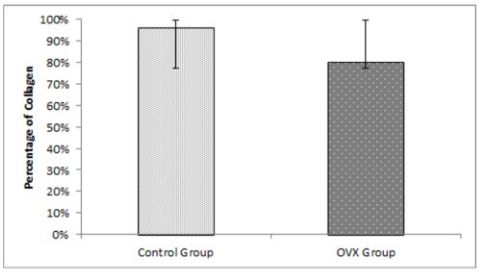

Figure 3. Percentage of area occupied by collagen at maxilla control group and ovariectomized (OVX): p = 0.003.

Comparing the femur with the maxilla in the OVX and C groups, it was observed that both showed statistically significant differences (p <0.01) (Table 1). In addition, the Pearson correlation test indicated that there was moderate correlation of changes in collagen between the femur and maxilla in the OVX group (r = 0.64).

Table 1: Percentages comparison of the area occupied by collagen at maxilla control group and ovariectomized (OVX): mean (x), standard deviation (SD) and t test (p).

	Maxilla	Femur	
	x (SD)	x (SD)	p
Control Group	96.28% (1.96)	46.21% (7.09)	0.014
OVX Group	80.70% (10.84)	22.13% (5.99)	0.001*

* p<0.01

Table 2: Pearson correlation test (r = 0.64) showing moderate correlation of bone loss between the maxilla and the femur of OVX group.

	Maxilla	Femur	
	x (SD)	x (SD)	r
OVX Group	80.70% (10.84)	22.13% (5.99)	0.64

Discussion

Ovariectomy causes turnover imbalance in long bones, providing for a short term, rapid increase in bone resorption, and a small increase in bone formation in the long term[29]. The experimental model of ovariectomy allows for the study of different treatment options used in the recovery of bone mass[32-34].

Many studies used the femur, tibia and vertebrae to evaluate the effects of osteoporosis in rats[29,35-41]. However, few studies evaluate the effects of osteoporosis on the maxillar bone, and the majority of these made analysis on the mandible and not the maxilla[20,21,30,42].

As in all connective tissues, the basic constituents of the bone are an extensive matrix and diverse cell types that function in bone synthesis and continuous remodelling. The bone matrix consists of an organic component (synthesized by osteoblasts) reinforced by inorganic calcium salt deposits. The collagen fibres comprise about 90% of the total protein in the bone and are oriented in a preferential direction, conferring the structure of the lamellar bone. The remaining 10% of the organic matrix is composed of proteoglycans and cell attachment proteins[43]. Histological analysis of the area occupied by collagen provides data on the amount of bone neoformation. Therefore, the area occupied by collagen is directly proportional to the amount of bone formation and indirectly proportional to bone loss. Thus, if a particular bone contains a large area of collagen, it also means that there is a large amount of neoformation in the bone. On the other hand, if a bone shows a small amount of collagen, this bone will be in the process of bone loss.

Bone turnover in the adult maxilla and mandible were approximately three- and sixfold higher (19–37%/yr) than in the femoral site (6.4%/yr), respectively. This data shows that maxillary bone turnover is more like the femur bone turnover than that of the mandible, justifying our analysis in the maxilla and not in the mandible[44].

There is a positive correlation between systemic osteoporosis caused by estrogen deficiency and low mineral density in the mandible[30]. However, there is little data in the literature correlating the amount of bone loss in the maxilla and femur. In the present study, we observed a correlation between bone mass reduction in the maxilla and femur ($r = 0.64$), indicating that there was osteopenia induced by ovariectomy in both the maxilla and femur. Study of women in pre-menopause stage, maxillary alveolar bone density had a moderate correlation with bone density of the femoral neck ($r = 0.43$)[45]. Our study supports this correlation ($r = 0.64$).

Knowledge of the relationship between sex steroids and bone metabolism helps the dentist to advise the patients about the risks of systemic and oral bone loss[21]. This deficiency results in osteoporotic changes in trabecular bone in the interradicular septum[30] as well as the periodontal ligament[21]. Other study found that the region of the alveolar bone has significantly lower mineral bone density than the mandibular cortical bone, both in OVX rats and control rats[42]. These findings indicate that bone remodeling increased by estrogen deficiency probably produces a high percentage of immature bone, in other words, a high percentage of collagen (bone neo formation), resulting in increased variability of mineralization. This data corroborates our study, because we observed a large amount of collagen tissue in the interadicular septum region of the evaluated maxillary bone (OVX group = 96.28% and C group = 80.70%).

An experimental study in adults rats showed that mandibular bone was less affected by ovariectomy and / or isocaloric protein undernutrition when comparing this site with proximal tibia, suggesting that mechanical loading of alveolar process during mastication may protect the alveolar bone from the detrimental effects observed in other skeletal sites[16]. The alteration of food consistency in young growing rats induced a lower mandibular alveolar bone, mineral density and decreased trabecular bone volume and thickness caused by a reduction of masticatory functional and mechanical demands[46]. Masticatory forces applied on the teeth are directly transferred to the alveolar bone through the periodontal ligament, resulting in stimulation of bone remodeling. However, the normal occlusal forces serve as a protective mechanism against the mandibular osteopenia in ovariectomized rats[20]. In the present study, the maxillary occlusal forces remained constant in groups C and OVX, and even then, ovariectomy impacted significantly in less bone formation in the maxilla OVX group ($p = 0.003$). Moreover, we found that in both groups, C and OVX, bone formation was significantly greater in the maxilla (C = 96.28%; OVX = 80.70%) than femur (C group = 46.21%; OVX group = 22.13%) ($p = 0.014$; $p = 0.001$). The data could agree with other study that report the normal occlusal forces protect against osteopenia in ovariectomized rats[20]. However, we must not forget that the femur also receives a large amount of load.

All the data induces the dentist to enhance the knowledge about the variation of maxilla bone density during menopause stage in order to improve dental procedures such as orthodontic tooth movement, periodontal treatment and implant placement. These procedures require a bone response for a successful treatment outcome.

We conclude in this study that (1) bone loss in osteopenic rats occurs both in the femur and in the maxilla, confirming that ovariectomy decreases bone formation in both of the sites evaluated, with greater impact on the femur and (2) there is a correlation between bone loss in the maxilla and in the femur of ovarietomized rats.

Aknowledgments

We would like to thank the Histology Laboratory at Universidade Estadual de Maringá (UEM) for all the help during experimental period.

References

1. Darcey J, Horner K, Walsh T, Southern H, Marjanovic EJ et al. Tooth loss and osteoporosis: to assess the association between osteoporosis status and tooth number. BDJ. 2013, 214(4): E10–E10.

2. Fu X, Chen J, Wu D, Du Z, Lei Q et al. Effects of Ovariectomy on Rat Mandibular Cortical Bone: A Study Using Raman Spectroscopy and Multivariate Analysis. Anal Chem. 2012, 84(7): 3318–3323.

3. Cao T, Shirota T, Ohno K, Michi KI. Mineralized bone loss in partially edentulous trabeculae of ovariectomized rabbit mandibles. J Periodontal Res. 2004, 39(1): 37–41.

4.Wright NC, Looker AC, Saag KG, Curtis JR, Delzell ES et al. The Recent Prevalence of Osteoporosis and Low Bone Mass in the United States Based on Bone Mineral Density at the Femoral Neck or Lumbar Spine. J Bone Miner Res. 2014, 29(11): 2520–2526.

5. Odén A, McCloskey EV, Johansson H, Kanis JA. Assessing the Impact of Osteoporosis on the Burden of Hip Fractures. Calcif Tissue Int. 2013, 92(1): 42–49.

6.Dai QG, Zhang P, Wu YQ, Ma XH, Pang J et al. Ovariectomy induces osteoporosis in the maxillary alveolar bone: an in vivo micro-CT and histomorphometric analysis in rats. Oral Dis. 2014, 20(5): 514–520.

7. Sanfilippo F, Bianchi AE. Osteoporosis: the effect on maxillary bone resorption and therapeutic possibilities by means of implant prostheses--a literature review and clinical considerations. Int J Periodontics Restorative Dent. 2003, 23(5): 447–457.

8. von Wowern N. General and oral aspects of osteoporosis: a review. Clin Oral Investig. 2001, 5(2): 71–82.

9. Dudic A, Giannopoulou C, Kiliaridis S. Factors related to the rate of orthodontically induced tooth movement. Am J Orthod Dentofacial Orthop. 2013, 143(5): 616–621.

10. Sastre J, Le Gall M. Tooth movement – clinical implications. Int Orthod. 2010, 8(2): 105–123.

11. Reitan K. Clinical and histologic observations on tooth movement during and after orthodontic treatment. Am J Orthod. 1967, 53(10): 721–745.

12. Jiang G, Matsumoto H, Fujii A. Mandible bone loss in osteoporosis rats. J Bone Miner Metab. 2003, 21(6): 388–395.

13. Birkenfeld L, Yemini M, Kase NG, Birkenfeld A. Menopause-related oral alveolar bone resorption: a review of relatively unexplored consequences of estrogen deficiency. Menopause. 1999, 6(2): 129–133.

14. Teófilo JM, Azevedo ACB, Petenusci SO, Mazaro R, Lamano-Carvalho TL. Comparison between two experimental protocols to promote osteoporosis in the maxilla and proximal tibia of female rats. Pesqui Odontol Bras. 2003, 17(4): 302–306.

15. Elsubeihi ES, Heersche JN. Comparison of the effect of ovariectomy on bone mass in dentate and edentulous mandibles of adult rats. Eur J Prosthodont Restor Dent. 2009, 17(1): 9–21.

16. Mavropoulos A, Rizzoli R, Ammann P. Different Responsiveness of Alveolar and Tibial Bone to Bone Loss Stimuli. J Bone Miner Res. 2007, 22(3): 403–410.

17. Tanaka M, Ejiri S, Nakajima M, Kohno S, Ozawa H. Changes of cancellous bone mass in rat mandibular condyle following ovariectomy. Bone. 1999, 25(3): 339–347.

18. Tanaka E, Sano R, Kawai N, Langenbach GEJ, Brugman P et al. Effect of food consistency on the degree of mineralization in the rat mandible. Ann Biomed Eng. 2007, 35(9): 1617–1621.

19. Sakata T, Sakai A, Tsurukami H, Okimoto N, Okazaki Y et al. Trabecular Bone Turnover and Bone Marrow Cell Development in Tail-Suspended Mice. J Bone Miner Res. 1999, 14(9): 1596–1604.

20. Patullo IM, Takayama L, Patullo R, Jorgetti V, Pereira RM. Influence of ovariectomy and masticatory hypofunction on mandibular bone remodeling. Oral Dis. 2009, 15(8): 580–586.

21. Armada L, Nogueira C, Neves U, Santos Souza P, Detogne J et al. Mandible analysis in sex steroid-deficient rats. Oral Dis. 2006, 12(2): 181–186.

22. Cox PG, Rayfield EJ, Fagan MJ, Herrel A, Pataky TC et al. Functional Evolution of the Feeding System in Rodents. Goswami A. PLoS ONE. 2012, 7(4): e36299.

23. Devlin H, Horner K, Ledgerton D. A comparison of maxillary and mandibular bone mineral densities. J Prosthet Dent. 1998, 79(3): 323–327.

24. Reginster JY, Burlet N. Osteoporosis: A still increasing prev-

alence. Bone. 2006, 38(2): 4–9.

25. Yamashiro T, Takano-Yamamoto T. Differential responses of mandibular condyle and femur to oestrogen deficiency in young rats. Arch Oral Biol. 1998, 43(3): 191–195.

26. Li X, Nishimura I. Altered bone remodeling pattern of the residual ridge in ovariectomized rats. J Prosthet Dent. 1994, 72(3): 324–330.

27. Johnson RB, Gilbert JA, Cooper RC, Parsell DE, Stewart BA et al. Effect of Estrogen Deficiency on Skeletal and Alveolar Bone Density in Sheep. J Periodontol. 2002, 73(4): 383–391.

28. Thompson DD, Simmons HA, Pirie CM, Ke HZ. FDA Guidelines and animal models for osteoporosis. Bone. 1995, 17(4): 125s–133s.

29. Hernandes L, Ramos AL, Micheletti KR, Santi AP, Cuoghi OA et al. Densitometry, radiography, and histological assessment of collagen as methods to evaluate femoral bones in an experimental model of osteoporosis. Osteoporos Int. 2012, 23(2): 467–473.

30. Tanaka M, Ejiri S, Toyooka E, Kohno S, Ozawa H. Effects of ovariectomy on trabecular structures of rat alveolar bone. J Periodontal Res. 2002, 37(2): 161–165.

31. Cuttle L, Nataatmadja M, Fraser JF, Kempf M, Kimble RM et al. Collagen in the scarless fetal skin wound: Detection with Picrosirius-polarization. Wound Repair Regen. 2005, 13(2): 198–204.

32. Kavuncu V, Sahin S, Baydas G, Ilhan N, Ozercan I et al. A Comparison of Estrogen and Two Different Doses of Calcitonin in Ovariectomized Rats. Yonsei Med J. 2003, 44(3): 508-516.

33. Hornby SB, Evans GP, Hornby SL, Pataki A, Glatt M et al. Long-Term Zoledronic Acid Treatment Increases Bone Structure and Mechanical Strength of Long Bones of Ovariectomized Adult Rats. Calcif Tissue Int. 2003, 72(4): 519–527.

34. Turner RT, Aloia RC, Segel LD, Hannon KS, Bell NH. Chronic Alcohol Treatment Results in Disturbed Vitamin D Metabolism and Skeletal Abnormalities in Rats. Alcohol Clin Exp Res. 1988, 12(1): 159–162.

35. Sato M, Zeng GQ, Turner CH. Biosynthetic Human Parathyroid Hormone (1–34) Effects on Bone Quality in Aged Ovariectomized Rats. Endocrinology. 1997, 138(10): 4330–4337.

36. Ma YL, Bryant HU, Zeng Q, Schmidt A, Hoover J et al. New Bone Formation with Teriparatide [Human Parathyroid Hormone-(1–34)] Is Not Retarded by Long-Term Pretreatment with Alendronate, Estrogen, or Raloxifene in Ovariectomized Rats. Endocrinology. 2003, 144(5): 2008–2015.

37. Kasukawa Y, Miyakoshi N, Itoi E, Tsuchida T, Tamura Y et al. Effects of h-PTH on cancellous bone mass, connectivity, and bone strength in ovariectomized rats with and without sciatic-neurectomy. J Orthop Res. 2004, 22(3): 457–464.

38. Kurth AHA, Eberhardt C, Müller S, Steinacker M, Schwarz M et al. The bisphosphonate ibandronate improves implant integration in osteopenic ovariectomized rats. Bone. 2005, 37(2): 204–210.

39. Ma YL, Bryant HU, Zeng Q, Schmidt A, Jee WSS et al. Raloxifene and teriparatide (hPTH 1-34) have complementary effects on the osteopenic skeleton of ovariectomized rats. J Bone Miner Metab. 2005, 23 Suppl: 62–68.

40. Zhang Y, Lai WP, Leung PC, Wu CF, Wong MS. Short- to Mid-Term Effects of Ovariectomy on Bone Turnover, Bone Mass and Bone Strength in Rats. Biol Pharm Bull. 2007, 30(5): 898–903.

41. Du Z, Steck R, Doan N, Woodruff MA, Ivanovski S et al. Estrogen Deficiency-Associated Bone Loss in the Maxilla: A Methodology to Quantify the Changes in the Maxillary Intra-radicular Alveolar Bone in an Ovariectomized Rat Osteoporosis Model. Tissue Eng Part C Methods. 2015, 21(5): 458–466.

42. Libouban H, Blouin S, Moreau MF, Baslé MF, Audran M et al. Effects of risedronate in a rat model of osteopenia due to orchidectomy and disuse: Densitometric, histomorphometric and microtomographic studies. Micron. 2008, 39(7): 998–1007.

43. Ames MS, Hong S, Lee HR, Fields HW, Johnston WM et al. Estrogen deficiency increases variability of tissue mineral density of alveolar bone surrounding teeth. Arch Oral Biol. 2010, 55(8): 599–605.

44. Titorencu I, Pruna V, Jinga VV, Simionescu M. Osteoblast ontogeny and implications for bone pathology: an overview. Cell Tissue Res. 2014, 355(1): 23–33.

45. Huja SS, Beck FM. Bone Remodeling in Maxilla, Mandible, and Femur of Young Dogs. Anat Rec Adv Integr Anat Evol Biol. 2008, 291(1): 1–5.

46. Scheibel PC, Ramos AL, Iwaki LCV. Is there correlation between alveolar and systemic bone density? Dental Press J Orthod. 2013, 18(5): 78–83.