Estrogen deficiency reduces the expression of estrogen receptor-beta in Wistar rats' periodontal tissues

Mossad M. Al-Sherbini, BDS, PhD, Mohammad S. Al-Zahrani, BDS, PhD, Zienab A. Alrefaie, MD, Hanan A. Amin, MD, Khalid H. Zawawi, BDS, DSc.

ABSTRACT

الأهداف: لتقييم تأثير استئصال المبيض على ظهور مستقبلات هرمون الاستروجين بيتا في الأنسجة اللثوية والعظم السنخي.

الطريقة: أجريت هذه الدراسة بجامعة الملك عبد العزيز على عينة من الفئران خلال الفترة من مارس 2012م وحتى أكتوبر 2012م وقد تم تقسيم 30 من إناث الفئران، والتي تبلغ من العمر 12 أسبوعاً إلى مجموعتين (في كل مجموعة منهما 15 فأراً): المجموعة التجريبية و التي تم فيها استئصال المبيض، والمجموعة الضابطة والتي تم فيها إجراء الجراحة بدون استئصال المبيض. ثم تم قياس مستويات هرمون الاستروجين والبروجستيرون باستخدام تقنيات اليزا. كما تم قياس مقدار ظهور مستقبلات هرمون الاستروجين بيتا في الأربطة اللثوية والعظم السنخي وفي عظام الساق.

النتائج: انخفضت بشكل ملحوظ مستويات هرمونات الاستروجين والبروجسترون في فئران المجموعة التجريبية مقارنة بالفئران في المجموعة الضابطة. كما انخفضت بشكل ملحوظ سماكة ونسبة الترابيق في عظام الساق والعظام السنخية في فئران المجموعة التجريبية مقارنة بالفئران في المجموعة الضابطة. كما وجد أن ألياف الأربطة اللثوية كانت منظمة تنظيما جيدا وموجهة بشكل مناسب في المجموعة الضابطة بينما كانت غير منظمة وغير موجهة بشكل مناسب في المجموعة التجريبية. وقد انخفض بشكل كبير مقدار ظهور مستقبلات هرمون الاستروجين بيتا في الأربطة اللثوية والعظم السنخي و في عظام الساق في المجموعة التجريبية مقارنة بالمجموعة الضابطة.

خاتمة: أدى نقص هرمون الاستروجين إلى انخفاض كبير في مقدار ظهور مستقبلات هرمون الاستروجين بيتا في كل من الساق والأنسجة اللثوية.

Objectives: To assess the effect of ovariectomy on the expression of estrogen receptor-beta (ER-ß) in periodontal ligament and alveolar bone.

Methods: This animal study was conducted at King Fahad Research Center, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia from March to October 2012. Thirty 12-week-old female Wistar rats were divided into 2 groups (15 each): ovariectomized (OVX) and sham-operated. Levels of estrogen and progesterone in the sera were measured using the enzyme linked immunosorbent assay (ELISA). To detect the expression of ER-ß, immunostaining was performed on the tibia, alveolar bone, and periodontal ligament specimens followed by quantitative histomorphometric analysis.

Results: Estrogen (p=0.001) and progesterone (p=0.007) levels were significantly decreased in the OVX rats compared to their controls. Histologically, the thickness and area percentage of the tibia and alveolar bone trabeculae were significantly reduced in OVX rats compared to the controls (p=0.001). The periodontal ligament fibers in the control group exhibited well-organized and appropriately oriented fibers, while in the OVX group they appeared disrupted with loss of orientation. The ER-ß expression in the OVX rats was significantly decreased in the periodontal tissues (p=0.005) and tibia (p=0.008).

Conclusions: Estrogen deficiency resulted in a significant decrease in the expression of ER-ß in both tibia and periodontal tissues.

Saudi Med J 2014; Vol. 35 (3): 242-247

From the Departments of Oral Basic and Clinical Sciences (Al-Sherbini, Al-Zahrani), and Preventive Dental Science (Zawawi), Faculty of Dentistry, and the Departments of Physiology (Alrefaie), and Anatomy (Amin), Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia.

Received 2nd October 2013. Accepted 20th January 2014.

Address correspondence and reprint request to: Dr. Khalid H. Zawawi, Department of Preventive Dental Science, Faculty of Dentistry, PO Box 80209, King Abdulaziz University, Jeddah 21589, Kingdom of Saudi Arabia. Tel. +966 (12) 6402000 Ext. 20388. Fax. +966 (12) 6403316. E-mail: kzawawi@kau.edu.sa

There is a growing interest in the association between osteoporosis and craniofacial bone loss, periodontitis and tooth loss. It was suggested that certain systemic conditions could predispose individuals to osteoporosis and periodontal tissue destruction.[1] A recent review found several similarities and correlations between osteoporosis and periodontal disease.[2] Osteoporosis and periodontitis are bone-resorptive and non-symptomatic diseases, where symptoms develop late in the disease process. Some of their common risk factors include age, smoking, and certain systemic diseases, and both diseases appear to have a hereditary component. The endocrine hormones play a fundamental function in the stability of the periodontium by regulating reproduction, growth and development, preservation of cellular activity, and energy production, utilization, and storage.[3-6] Estrogen and progesterone play a crucial role in several vital activities, as well as having significant biological actions that can affect the oral cavity.[3,7-9] For example, estrogen affects gingival epithelium keratinization and cellular proliferation and differentiation, whereas microvasculature permeability and collagen production is affected by progesterone.[7,10-12] An increased concentration of sex hormones in the serum, saliva, crevicular fluid, and gingival tissues has been reported during pregnancy, and observed throughout womens' menstrual cycle. These hormonal changes can cause biological changes in tissues of the periodontium, and may impact periodontal health.[13] Regulation of most cellular processes by hormones occurs through its interaction with receptors. When estrogen is rapidly transported across the cell membrane by simple diffusion, it binds to its receptor; this causes an activation of the receptor as a conformational change of the hormone-receptor complex, revealing a binding site that causes gene activation.[14] Both estrogen and progesterone receptors have been demonstrated in fibroblasts of the gingiva, periosteum, and periodontal ligament.[3,15] Estrogen acts as a ligand for estrogen receptors-alpha (ER-α) and estrogen receptors-beta (ER-ß), which show a tissue specific distribution.[16] The ER-α and ER-ß are not only expressed in typical organs for estrogen, such as the ovary, uterus, and breast, but are also commonly expressed on other tissues, such as the periodontal tissues.[17,18] Estrogen deficiency accelerates the bone remodeling process leading to higher levels of bone resorption than formation.[19] Estrogen affects bone metabolism and regulates the function of several cell types, including those of the periodontal ligaments. It has been suggested that estrogen could play a central role in the pathogenesis of periodontal disease.[12] Estrogen receptors are suggested to play a role in estrogen-induced effects on the differentiation of osteoblasts, proliferation of periodontal ligament cells, and expression of key molecules that contribute to the structural integrity and function of the periodontal tissues.[20] The objectives of this study were to assess the effect of ovariectomy on the expression of ER-ß in the periodontal ligament, and to study alveolar bone changes using immunohistochemical and histomorphometrical analyses. The null hypothesis was that there is no difference in the expression of ER-ß in the tibia, alveolar bone, and periodontal ligament between ovariectomized rats (OVX), and sham-operated controls.

Disclosure. This study was funded by the Deanship of Scientific Research (DSR), King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia (Grant No. 486/165-1432).

Methods.

Animals. All procedures were performed at King Fahad Research Center, King Abdulaziz University, Jeddah, Saudi Arabia from March to October 2012, according to the animal care guidelines. The study was reviewed and approved by the Research Ethics Committee, Faculty of Dentistry, King Abdulaziz University. Thirty, 12-week-old, female Wistar rats weighing between 200-250 g were divided into 2 groups with equal number, and randomly assigned to either the OVX, or the sham-operated group. Both groups did not receive medications during the study period (16 weeks). All rats were fed normal chow, and allowed free access to water with light/dark cycles of 12/12 hours at constant temperature and humidity.

Surgical procedure. The rats were either bilaterally OVX, or sham-operated (control) using the dorsal approach.[21] Briefly, under aseptic conditions, anesthesia (100 mg/kg ketamine, intramuscular) was administered. The dorsal aspect of the lumbar region was shaved, and a small one to 2 cm midline incision was made in the skin, approximately halfway between the midline of the back (the hump), and the base of the tail. Both ovaries in the OVX rats were dissected, ligated, and removed. The same surgical procedure was performed on the sham-operated group, except for the removal of the ovaries.

Blood sample collection and measurement of serum estrogen and progesterone. By the end of the sixteenth week, blood samples were collected through retro-orbital route, left to coagulate at room temperature, and centrifuged at 3000 rate per minute (rpm) for

30 minutes. The clear supernatant sera was separated and stored at -2°C. The levels of both estrogen and progesterone in the sera were then measured using the enzyme linked immunosorbent assay (ELISA) system kit according to the manufacturers' instructions (EIAab, Wuhan, China for estrogen assay, and ALPCO, Salem, NH, USA for the progesterone assay). The ELISA for estrogen level was performed as follows: samples were placed in a 96-well microplate coated with the primary antibodies and incubated for 2 hours at 37°C. Liquids were then removed from each well without washing, and a detection reagent-A was added, and the plate was incubated for one hour at 37°C. The plate was then washed 3 times and detection reagent-B was added, and the plate was incubated for another one hour at 37°C. The plate was washed 5 times, and a substrate solution was added, and the plate was incubated at 37°C. After 30 minutes, a stop solution was added. The ELISA for progesterone assay was performed as follows: samples were added to each well, an incubation buffer was added followed by enzyme conjugate. The plate was incubated for one hour at room temperature. The plate was then washed 4 times and the substrate solution was added, and the plate was incubated for another 30 minutes in the dark. A stop solution was added, and the absorbance was then measured.

Sample collection and preparation. After 16 weeks, 6 rats were randomly selected from each group and were euthanized with diethyl ether. Marked atrophy of the uterine horns, and no evidence of ovarian tissue indicated the success of ovariectomy procedure. The tibia and mandible were harvested by carefully detaching the soft tissues, and then they were fixed in formalin for 2 days and decalcified in 10% buffered ethylenediaminetetraacetic acid (EDTA) for one week, and embedded in paraffin. Slides from the paraffin blocks of the tibia and periodontal tissues at the interradicular septum of the second molar were prepared for Hematoxylin and Eosin stain (H&E stain) and immunohistochemical staining.

Immunohistochemical staining. Immunostaining for ER-ß was performed on the slides from the paraffin blocks. This was carried out using a primary anti-serum to ERs according to the manufacturer instructions (DAKO Corp, Denmark) followed by biotinylated horse anti-mouse anti-serum, avidin-biotin complex and 3,3'-diaminobenzidine (DAB) in chromogen solution (DAKO Corp. Denmark). To confirm the reliability of the technique, a control slide was used, in which the primary antibody was omitted.

Quantitative histomorphometric analysis. The thickness of bone trabeculae and trabecular numbers per square millimeter was measured and calculated in the tibial bones. Similarly, at the site of the interradicular septum of the second molar, changes in the thickness of the alveolar bone and area percentage, as well as the periodontal ligament width and orientation were assessed. An image analyzing system (Leica Q 500 MCO Analyzer, Germany) was used for histomorphometric analysis. Quantitative measurements for ER-ß were performed on the immunostained slides by selecting 5 fields from each slide that best reflected the overall immunostaining. Brown cytoplasmic and nuclear DAB chromogen stains of ER-ß were considered a positive reaction. The mean area percentage of ER-ß expression in the trabecular bone of the tibia and in the alveolar bone and periodontal ligament were analyzed, and automatically measured using the image analyzer.

Statistical analysis. Data set is presented as means ± standard deviations. Comparison of data between both groups was performed using the independent Student's t-test. Statistical significance was considered at $p<0.05$.

Results. There was no difference in the weight of the rats between the groups at baseline. Sixteen weeks after the surgical procedure, a significant increase in the weight of OVX rats compared to the sham-operated controls was observed ($p=0.001$). There was a significant decrease in the serum level of both estrogen ($p=0.001$) and progesterone ($p=0.007$) hormones in the OVX rats compared to their sham-operated controls. The estrogen/progesterone ratio in the OVX rats was higher compared to sham-operated rats ($p=0.004$) (**Table 1**). Histologically, the trabeculation of the alveolar bone at the interradicular septum of the second molar in the OVX rats appeared less than the control rats. Similarly, in the proximal epiphyseal area of the tibia, the trabeculation was also reduced. Both the alveolar

Table 1 - Body weights and calculated mean weight changes and serum levels of estrogen, progesterone, and estrogen/progesterone ratio in the ovariectomy group (OVX) rats after 4 months compared with the sham-operated control rats.

Variables	Sham-operated rats	OVX rats	*P*-value
Initial weight (gm)	211 ± 4.56	218 ± 13.84	0.083
Final weight (gm)	236 ± 16.46	321 ± 25.30	0.001
Final - initial weight (gm)	36.36 ± 17.38	103 ± 22.27	0.001
Estrogen (pmol/L)	143 ± 66.35	49.61 ± 19.21	0.001
Progesterone (nmol/L)	52.26 ± 6.25	6.97 ± 4.16	0.007
Estrogen/progesterone ratio	4.82 ± 2.82	9.01 ± 4.35	0.004

Data are expressed as mean ± standard deviation

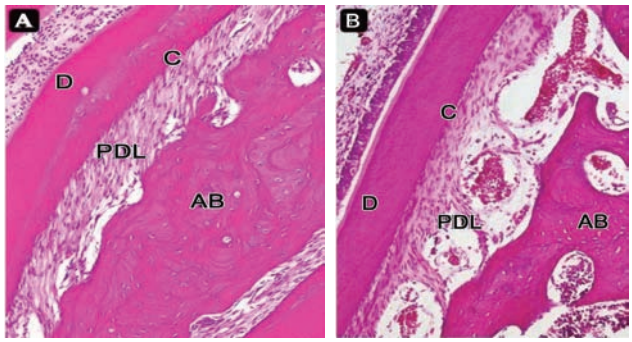

Figure 1 - Histological sections of sham-operated rats (**A**) and ovariectomized rats (**B**) showing the periodontal ligament (PDL), dentin (D), cementum (C), and alveolar bone (AB).

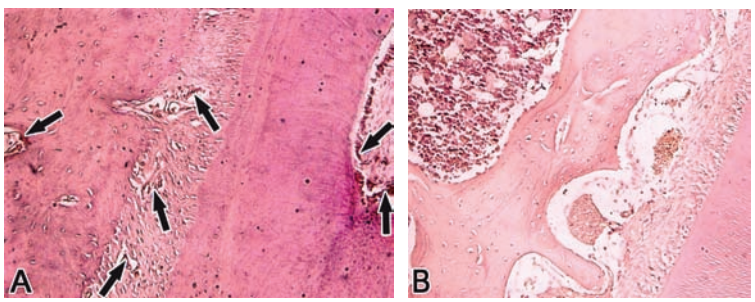

Figure 2 - Estrogen receptor (ER)-ß immunoexpression in the periodontal tissue: **A**) sham-operated rats, showing strong ER-ß expression (arrows); and **B**) ovariectomized rats showing very weak immunostaining, (original magnification × 200).

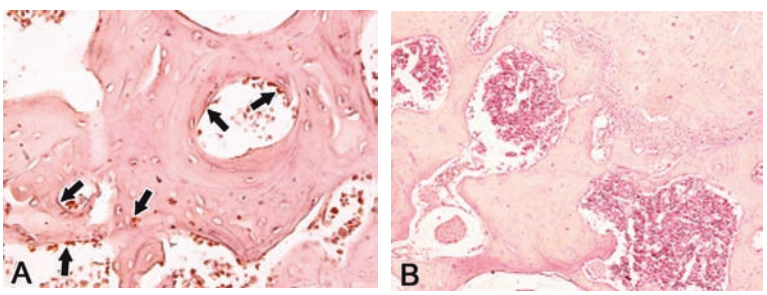

Figure 3 - Estrogen receptor (ER)-ß immunoexpression in the tibia: **A**) sham-operated rats, showing strong ER-ß expression (arrows); and **B**) ovariectomized rats showing very weak immunostaining with large marrow spaces, (original magnification ×200).

bone and tibia presented a larger area of bone marrow in the OVX rats when compared with the control group. The control group exhibited well-organized and appropriately oriented periodontal ligament fibers (PDL) while in the OVX group, the PDL space appeared wider and disrupted with loss of orientation (**Figure 1**). These findings were confirmed statistically by higher mean thickness and trabecular area percentages of tibia and alveolar bone in the sham-operated compared with the OVX rats. Furthermore, there was a significant decrease in the mean weight of tibia and alveolar bone of the OVX group compared with the control as shown in **Table 2**. The immunoexpression of ER-ß in the OVX rats was weak in the periodontal tissue (**Figure 2**) and tibia (**Figure 3**). The mean percent area of ER-ß immunoexpression was significantly lower in the OVX group when compared with the sham-operated group (**Table 3**).

Discussion. This study was conducted to evaluate the effect of estrogen depletion on the expression of ER-ß in the periodontal tissues and tibia. The results demonstrated a decrease in the expression of the ER-ß in the periodontal ligament and alveolar bone of ovariectomized rats compared with sham-operated controls. This substantiates the important role of estrogen hormones in the maintenance of the periodontal structures. Few studies have investigated the expression

Table 2 - The weight and trabecular bone thickness and area percentage of the tibia and mandible.

Variables	Sham-operated rats	Ovariectomy group rats	*P*-value
Tibia			
Weight	0.73 ± 0.06	0.68 ± 0.05	0.001
Trabeculae thickness	85.78 ± 10.68	47.09 ± 5.14	0.001
Area percentage	42.01 ± 5.30	25.09 ± 3.80	0.001
Mandible			
Weight	0.66 ± 0.07	0.58 ± 0.03	0.035
Trabeculae thickness	89.02 ± 11.96	64.74 ± 14.19	0.001
Area percentage	83.50 ± 7.70	60.45 ± 9.20	0.001
Data are expressed as mean ± standard deviation			

Table 3 - The percentage of areas expressing estrogen receptor (ER)-ß in the ovariectomy group (OVX) and control rats.

Variables	Sham-operated rats	OVX rats	*P*-value
Trabecular bone of the tibia	7.39 ± 0.02	0.450 ± 0.01	0.008
Periodontal tissues	5.501 ± 0.01	0.36 ± 0.01	0.005
Data are expressed as mean ± standard deviation			

of ER at the molecular level using mesenchymal cells and periodontal ligament cells, while there are sparse data regarding the effect of altered estrogen levels on the expression of ER in periodontal tissues,[12] which was addressed in the current study.

The mechanism by which estrogen depletion decreases the expression of ER-ß is not fully understood. A possible explanation is that the expression of ER-ß is dependent on the continuous intra- and extra-cellular presence of estrogen. Estrogen regulates the transcription of ER-ß intracellularly. This could be caused by the ability of estrogen to affect its own receptor concentrations through increasing target tissue responsiveness to itself.[14] Female sex hormones act via specific receptors. Thus far, 2 estrogen receptors have been identified and existed in 2 isoforms; ER-α and ER-ß.[12] Regulation of most cellular process by hormones occur through its interaction with intracellular receptors. It has been shown that periodontal tissues contain receptors for androgens, estrogens, and progesterone.[15] These receptors have been identified in the basal and spinous layers of the epithelium and connective tissue and are suggested as targets to manifest hormonal effects. Mamalis et al[20] identified the major receptor for estrogen in the periodontium and examined how estrogen through its major receptors can regulate proliferation, osteoblastic differentiation, collagen synthesis, and periostin gene expression in periodontal ligament cells. They concluded that estrogen receptors play key roles in estrogen-induced effects on proliferation of periodontal ligament cells, differentiation of osteoblasts, and expression of important molecules for the functional and structural integrity of the periodontal tissues.

The current study demonstrated that estrogen deficiency in ovariectomized rats caused a significant decrease in alveolar bone mass. A significant reduction in trabecular number and thickness was also observed. Both the alveolar bone and tibia presented a larger area of bone marrow in ovariectomized rats when compared with the sham group. Moreover, the current findings showed that the mean weight of mandible and tibia was significantly reduced in ovariectomized rats. These findings are in line with earlier studies.[22,23] It has been documented that estrogen deficiency stimulates bone resorption by increasing the number and activity of osteoclast cells due to enhancement of their formation and reduction in their apoptosis.[24] Wattanaroonwong et al[25] documented that estrogen inhibits osteoclast formation produced by periodontal ligament fibroblasts. For this reason, estrogen deficiency has been suggested as a risk factor for periodontal disease in postmenopausal women, and seemed to enhance the loss of alveolar bone volume resulting in the progression of periodontitis.[25-27] Al Habashneh et al[28] found that osteoporosis was significantly related to severity of alveolar bone loss and prevalence of periodontitis. Jabbar et al[29] concluded that periodontitis is more common among women affected with osteoporosis, and is correlated with lower vitamin D and higher concentration of receptor activator of nuclear factor kappa-B ligand (RANKL) and osteoprotegerin (OPG).

The current study has limitations. This is an animal study, and thus, the results might not be generalized to human subjects. Furthermore, the expression of ER-ß was measured using a semi-quantitative method. Another limitation is that only ER-ß was assessed. Future studies to detect and quantify the expression of ER-ß and ER-α in the periodontal tissues using real-time polymerase chain reaction are warranted.

In conclusion, this study demonstrated that ER-ß is expressed in the periodontal and bone cells. Estrogen deficiency resulted in a significant decrease in the expression of ER-ß in both tibia and periodontal tissues, and also caused osteoporotic changes. Continued research is very important to understand the mechanism and relationship between osteoporosis and periodontitis. It is necessary to conduct further research to characterize the expression of ER-ß using ovariectomized animals with periodontitis.

Acknowledgment. *The authors gratefully acknowledge the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia for their technical and financial support.*

References

1. Lai YL. Osteoporosis and periodontal disease. ***J Chin Med Assoc*** 2004; 67: 387-388.
2. Wactawski-Wende J. Periodontal diseases and osteoporosis: association and mechanisms. ***Ann Periodontol*** 2001; 6: 197-208.
3. Guncu GN, Tozum TF, Caglayan F. Effects of endogenous sex hormones on the periodontium--review of literature. ***Aust Dent J*** 2005; 50: 138-145.
4. Armitage GC. Development of a classification system for periodontal diseases and conditions. ***Northwest Dent*** 2000; 79: 31-35.
5. Bole C, Wactawski-Wende J, Hovey KM, Genco RJ, Hausmann E. Clinical and community risk models of incident tooth loss in postmenopausal women from the Buffalo Osteo Perio Study. ***Community Dent Oral Epidemiol*** 2010; 38: 487-497.
6. Mealey BL, Moritz AJ. Hormonal influences: effects of diabetes mellitus and endogenous female sex steroid hormones on the periodontium. ***Periodontol 2000*** 2003; 32: 59-81.
7. Mascarenhas P, Gapski R, Al-Shammari K, Wang HL. Influence of sex hormones on the periodontium. ***J Clin Periodontol*** 2003; 30: 671-681.
8. McCauley LK, Tozum TF, Kozloff KM, Koh-Paige AJ, Chen C, Demashkieh M, et al. Transgenic models of metabolic bone disease: impact of estrogen receptor deficiency on skeletal metabolism. ***Connect Tissue Res*** 2003; 44 Suppl 1: 250-263.
9. McCauley LK, Tozum TF, Rosol TJ. Estrogen receptors in skeletal metabolism: lessons from genetically modified models of receptor function. ***Crit Rev Eukaryot Gene Expr*** 2002; 12: 89-100.
10. Gordon CM, LeBoff MS, Glowacki J. Adrenal and gonadal steroids inhibit IL-6 secretion by human marrow cells. ***Cytokine*** 2001; 16: 178-186.
11. Soory M. Hormonal factors in periodontal disease. ***Dent Update*** 2000; 27: 380-383.
12. Zhang B, Li Y, Zhou Q, Ding Y. Estrogen deficiency leads to impaired osteogenic differentiation of periodontal ligament stem cells in rats. ***Tohoku J Exp Med*** 2011; 223: 177-186.
13. Becerik S, Ozcaka O, Nalbantsoy A, Atilla G, Celec P, Behuliak M, et al. Effects of menstrual cycle on periodontal health and gingival crevicular fluid markers. ***J Periodontol*** 2010; 81: 673-681.
14. Fritz MA, Speroff L. Clinical Gynecologic Endocrinology and Infertility. 8th ed. Philadelphia (PA): Wolters Kluwer Health/ Lippincott Williams & Wilkins; 2011.
15. Kawahara K, Shimazu A. Expression and intracellular localization of progesterone receptors in cultured human gingival fibroblasts. ***J Periodontal Res*** 2003; 38: 242-246.
16. Nilsson S, Gustafsson JA. Estrogen receptors: therapies targeted to receptor subtypes. ***Clin Pharmacol Ther*** 2011; 89: 44-55.
17. Nilsson S, Makela S, Treuter E, Tujague M, Thomsen J, Andersson G, et al. Mechanisms of estrogen action. ***Physiol Rev*** 2001; 81: 1535-1565.
18. Xie YF, Shu R. [Expression of estrogen and progesterone receptors in the gingival tissues of female patients with moderate and advanced periodontitis]. ***Shanghai Kou Qiang Yi Xue*** 2003; 12: 366-369. Chinese
19. Robert PH. Remodeling and skeletal fragility. ***Osteoporos Int*** 2003; 14 Suppl 5: S12-S15.
20. Mamalis A, Markopoulou C, Lagou A, Vrotsos I. Oestrogen regulates proliferation, osteoblastic differentiation, collagen synthesis, and periostin gene expression in human periodontal ligament cells through oestrogen receptor beta. ***Arch Oral Biol*** 2011; 56: 446-455.
21. Giavaresi G, Borsari V, Fini M, Martini L, Tschon M, De Terlizzi F, et al. Different diagnostic techniques for the assessment of cortical bone on osteoporotic animals. ***Biomed Pharmacother*** 2004; 58: 494-499.
22. 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: 161-165.
23. Yang J, Farnell D, Devlin H, Horner K, Graham J. The effect of ovariectomy on mandibular cortical thickness in the rat. ***J Dent*** 2005; 33: 123-129.
24. Riggs BL. The mechanisms of estrogen regulation of bone resorption. ***J Clin Invest*** 2000; 106: 1203-1204.
25. Wattanaroonwong N, Schoenmaker T, de Vries TJ, Everts V. Oestrogen inhibits osteoclast formation induced by periodontal ligament fibroblasts. ***Arch Oral Biol*** 2011; 56: 212-219.
26. Haas AN, Rosing CK, Oppermann RV, Albandar JM, Susin C. Association among menopause, hormone replacement therapy, and periodontal attachment loss in southern Brazilian women. ***J Periodontol*** 2009; 80: 1380-1387.
27. Lerner UH. Inflammation-induced bone remodeling in periodontal disease and the influence of post-menopausal osteoporosis. ***J Dent Res*** 2006; 85: 596-607.
28. Al Habashneh R, Alchalabi H, Khader YS, Hazza'a AM, Odat Z, Johnson GK. Association between periodontal disease and osteoporosis in postmenopausal women in Jordan. ***J Periodontol*** 2010; 81: 1613-1621.
29. Jabbar S, Drury J, Fordham J, Datta HK, Francis RM, Tuck SP. Plasma vitamin D and cytokines in periodontal disease and postmenopausal osteoporosis. ***J Periodontal Res*** 2011; 46: 97-104.