Original Research Article

Effects of ovariectomy on the structure of the periodontium and root resorption during orthodontic tooth movement in rats

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ABSTRACT

Aims: the objective of this study was to evaluate the effects of ovariectomy on the histological structure of periodontal tissues and root resorption during orthodontic movement in rats.

Study design: Experimental research.

Methodology:A total of 24 rats (Wistar) were divided into four experimental groups: Group 1 – control (CTL), composed of 6 rats that were not subjected to any experimental procedure; Group 2, composed of 6 rats that were subjected to induced tooth movement (ITM) for 7 days; Group 3, composed of 6 rats that underwent ovariectomy (OVX); Group 4, composed of 6 rats that were subjected to OVX +ITM for a period of 7 days. At the end of the experimental period, the animals were euthanized, and the jaws were removed, fixed in 10% formalin, decalcified, embedded in paraplast, cut to 5 µm and stained with hematoxylin and eosin. Morphological and morphometric analyses of the periodontal tissues, histological evaluation of the root surface and quantification of tooth movement were performed.

Results: The animals in the OVX + ITM group had a higher rate of tooth movement than the animals in the ITM group and greater root resorption than all experimental groups.

Conclusion: We conclude that induced tooth movement associated with ovariectomy induces a higher rate of tooth movement and a higher incidence of root resorption.

Keywords:*induced tooth movement, ovariectomy, female rats.*

1. INTRODUCTION

During orthodontic treatment, teeth move individually and at different rates, and the amount of dental movement caused by bone remodeling is influenced by drug use or systemic factors [1]. One of the most common systemic factors is osteoporosis, which is related to menopause or ovariectomy and results in pathological bone loss [2].

Estrogens are important regulators of bone metabolism [3]. Estrogen deficiency results in loss of jawbone and increased bone remodeling induced by mechanical load [4,5,6]. Low estrogen concentration is associated with unbalanced production of tumor necrosis factor alpha (TNF-α), nuclear factor activating receptor beta (κB) (RANKL) and interleukin-6 (IL-6) in periodontal tissue [6, 7,8, 9]. On the other hand, estrogen replacement prevents the release of these molecules [6,9]. Most estrogen actions are mediated by estrogen receptor α (ERα) and beta (ERβ); in bones, this action is partly via ERα, which is found in osteoblasts and osteoclasts [10,11, 12]. It is known that the activation of ERα in osteoblasts stimulates the production of osteoprotegerin (OPG) and IL-6, increasing bone mineral density [12].

Osteoporosis induced by estrogen and progesterone deficiencies in the postmenopausal period results in bone loss and increased bone turnover. Estrogens and androgens have traditionally been known to regulate bone turnover in women and men. Osteoporosis followed by the withdrawal of estrogen and progesterone affects the alveolar bone, promotes periodontal diseases and is the main cause of tooth loss. In orthodontic treatments, the increase in bone turnover induced by the lack of these hormones results in the progression of tooth movement in an unstable pattern [13].

Root resorption is one of the main sequelae studied in relation to orthodontic movement. This event may occur by the activation of clastic cells that reabsorb mineralized tissues by a mechanism similar to that of bone resorption, leading to loss of the dental element in extreme cases [14, 15, 16].

These observations suggest that periodontal tissues are targets of the actions of estrogens and that these hormones are important factors in the pathogenesis that affect the periodontium. The objective of this study was to evaluate the effects of ovariectomy on the

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histological structure of periodontal tissues and root resorption during orthodontic movement in rats.

2. METHODOLOGY

Twenty-four female Wistar rats (50 days old, weighing approximately 150 g) were acquired from central bioterium of the State University of West Paraná, UNIOESTE, Cascavel-PR, Brazil. The animals were adapted and kept in the sectorial bioterium of Center for Biological and Health Sciences – CCBS, UNIOESTE, in collective polyethylene cages (43x30x15), which were housed individually or in pairs under controlled temperature conditions (22º and 25º C), relative humidity close to 55% and a photoperiod of 12 hours (light period 7:00-19:00 h). The animals received food and water ad libitum. The experimental procedures were in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were analyzed by the Committee on Ethics in the Use of Animals (CEUA) of UNIOESTE.

2.1 Experimental groups

The animals were randomly divided into four experimental groups. Group 1, the control (CLT) group was composed of 6 rats that were not subjected to any experimental procedure. Group 2 was composed of 6 rats that were subjected to induced tooth movement (ITM). Group 3 was composed of 6 rats that underwent ovariectomy (OVX). Group 4 was composed of 6 rats that were subjected to OVX and ITM.

2.2 Sedation of animals

The experimental procedures (ovariectomy and installation of the MDI device) were performed under general anesthesia by applying ketamine hydrochloride-based anesthetic (DOPALEN, Sespo Indústria e Comércio, Paulínia-SP) at a dose of 75 mg/kg and xylazine hydrochloride-based muscle relaxant (ANASEDAN, SespoIndústria e Comércio, Paulínia-SP) at a dosage of 15 mg/kg, both intraperitoneally.

2.3 Ovariectomy

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At 60 days of age, the animals in Groups 3 and 4 underwent bilateral ovariectomy. They fasted for 12 to 16 hours during the night before the procedure. On the following morning, five minutes before onset, a single dose of antibiotic prophylaxis with intramuscular ceftriaxone (50 mg/kg) (EMS, Brazil) and analgesia with subcutaneous sodium dipyrone (50 mg/kg) (EMS, Brazil) was administered.

After anesthesia, the animals were placed in the surgical plane in the prone position, and the lateral abdominal region was shaved followed by antisepsis with iodized alcohol. The skin and muscles were incised longitudinally in the midline near the renal region below the last rib, and the ovary was identified and exposed. Hemostasis was performed by ligating the upper part of the uterus with No. 4 Ethicon – Johnson & Johnson silk thread and excision of the ovary along with the surrounding fat, the fallopian tube and a small portion of the uterus. At the end of the surgical procedure, the layers were sutured with absorbable #4 catgut thread, and the skin was sutured with #4 silk thread. The animals received postoperative analgesics (sodium dipyrone,50 mg/kg) intramuscularly every 12/12 h for 4 days (Figure 1).

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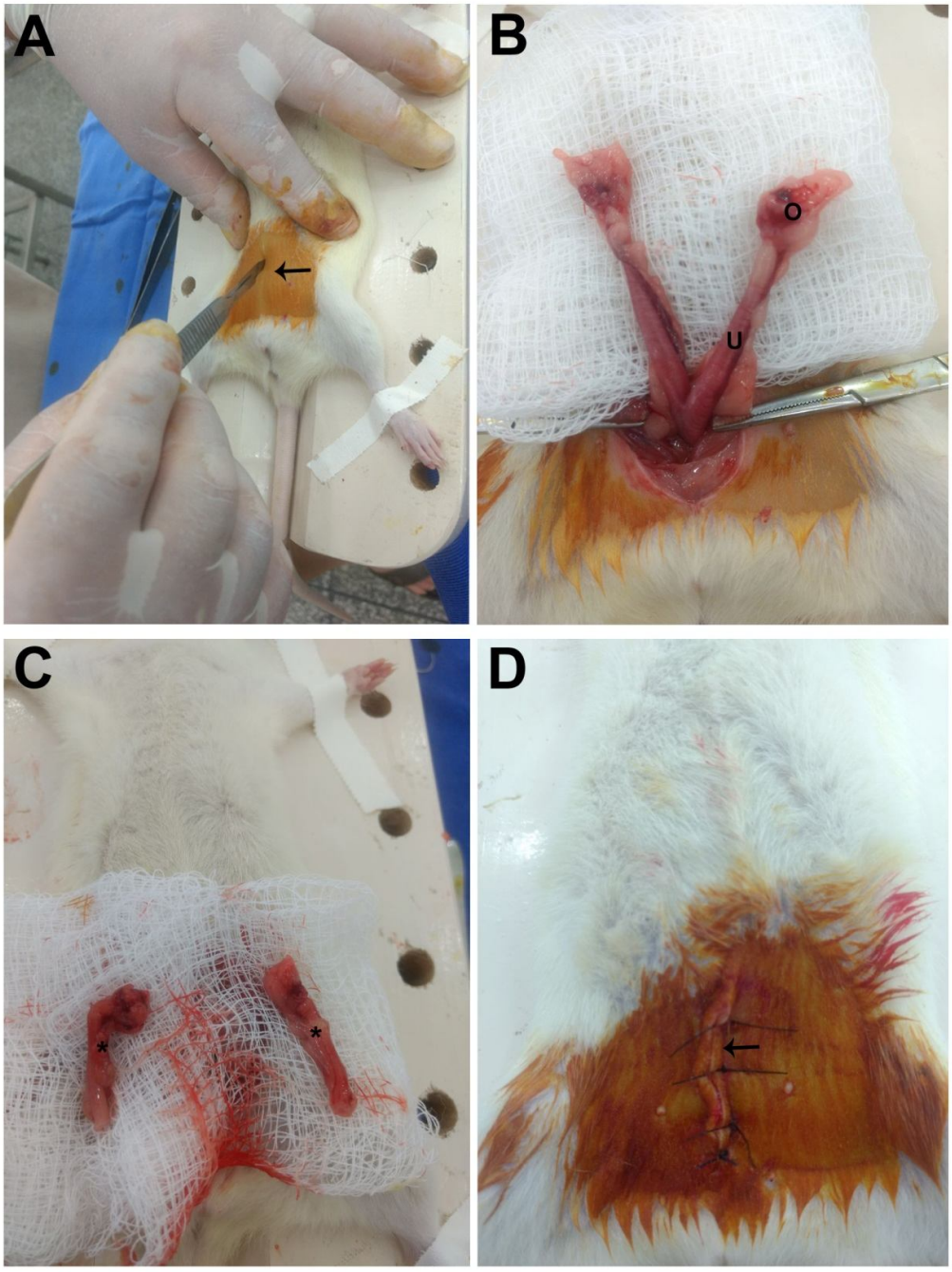

Figure 1. Photographs of the sequence of ovariectomy surgery (OVX). A. Median incision in the abdominopelvic region (arrow); B. Exposure of the uterine horn (U), uterine tube and ovaries (O); C. Excision of the ovary together with the surrounding fat, fallopian tubes and a small portion of the uterus (*); D. Sutured abdominal region (arrow).

2.4 Installation of induced tooth movement (ITM) device

At 90 days, the ITM device was installed in the animals of Groups 2 and 4. The device used in this study was similar to that proposed by Heller & Nanda[17], and the total period of ITM was 7 days. This modified device consisted of a closed-section nickel-titanium spring (Sentalloy®, GAC, NY, USA) with a force magnitude of 50 cN. The magnitude of the spring force was previously verified using a Zeusan tensiometer (Zeusan Exporting Ltd. Campinas, São Paulo, Brazil). In addition, two segments of ligature wire, with a thickness of 0.25 mm (Morelli, Sorocaba, SP, Brazil), were connected to each end of the spring, one surrounding the maxillary right first molar and the other segment surrounding the maxillary right central incisor of the animal. For the stability of the ligature wire on the buccal surface of the incisor, a slot was made in the cervical region and fixed with light-curing composite resin (Filtek™ Z350XT, 3M Company, St. Paul, MN, USA) to avoid displacement of the wire (Figure 2).

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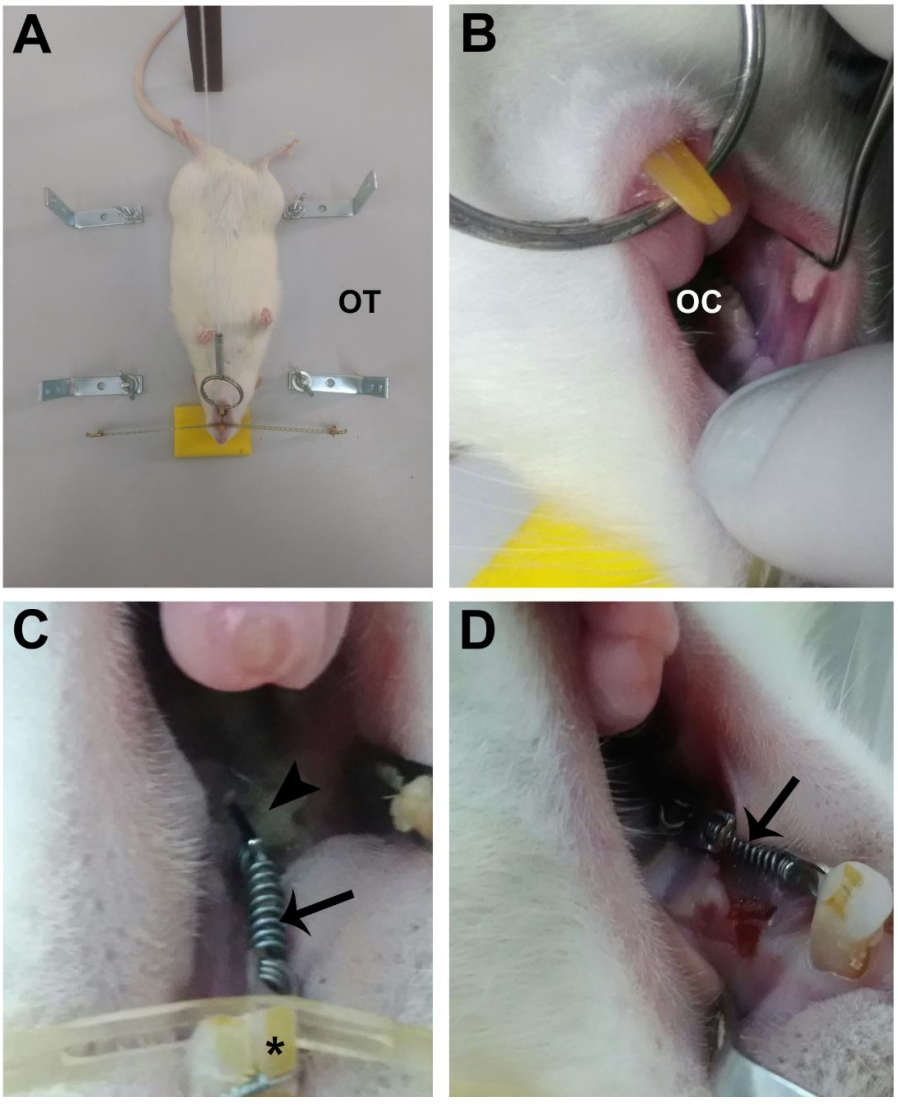

Figure 2.

Photographs of the sequence of the ITM device installation procedure. A. Positioning the animal in dorsal decubitus on the operating table (OT); B. Animal with an open oral cavity (OC); C and D. Nickel-titanium spring (arrow) with the ends connected to two segments of ligature wire, one surrounding the upper right first molar (arrowhead) and the other segment the upper right incisor (*) of the animal.

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2.5 Euthanasia and collection of biological material

At the end of 97 days, all animals were weighed and sacrificed in a CO_2 chamber with subsequent decapitation. The right hemimaxillae were removed and fixed in 10% buffered formalin for 24 hours, washed in running water for 48 hours and subsequently decalcified in decalcifying acid solution (Allkimia®) for 19 hours, and stored in 70% alcohol.

2.6 Quantitative analysis of tooth movement

Immediately after euthanasia, the amount of tooth movement was obtained as the difference between the distances from the mesial surface of the maxillary first molar to the distal surface of the maxillary third molar on the moved right side and the unmoved left side [18] The measurements were obtained using a digital caliper (Mitutoyo, São Paulo, Brazil) with the aid of an ocular magnifying glass (Intex, Brazil).

2.7 Laboratory processing

After decalcification, the pieces were dehydrated in an increasing series of alcohols, cleared in xylene and embedded in Paraplast. For the histological analyses, serial sections were performed in the longitudinal plane of the mesiobuccal and distobuccal roots of the maxillary right first molar, from mesial to distal, with 5-µm thickness, using a manual rotating microtome (Olympus 4060) equipped with a steel razor. The sections obtained were deparaffinized with xylene, hydrated with distilled water and subjected to staining with hematoxylin-eosin (HE) for analysis.

A light microscope (Olympus BX61) was used for histological analysis. An Olympus DP71 digital camera with DP Controller 3.2.1.276 software was used to obtain the photomicrographs at 200x and 400x magnification.

2.8 Descriptive analysis of histological slides

The specific areas of the descriptive analysis were 1) periodontal ligament of the mesiobuccal and distobuccal roots on the mesial and distal surfaces, cervical, middle and apical thirds; 2) periodontium of the furcation region; 3) mesial bone crest; 4) interradicular septum; and 5) interdental septum between the maxillary right first molar and the maxillary right second molar.

The histopathological events investigated were external root resorption, areas of hyalinization, acute inflammatory infiltrate, chronic inflammatory infiltrate, presence of multinucleated giant cells, presence of vascular changes, and organization of the periodontal ligament. Each event was evaluated as follows: absence, occasional presence, moderate presence and intense presence [19].

2.9 Histomorphometric analysis of external root resorption

For the quantitative analysis of external root resorption, the mesial surface of the distal root in its cervical and middle thirds was considered because this is the region most affected by compression of the periodontal ligament both in trauma from extrusive dislocation and in induced tooth movement. The photomicrographs at 400X magnification were analyzed using Image-Pro Plus 6.0 software (Media Cybernetics, Rockville, MD – USA), where the total area of each resorption was quantified in square micrometers (μm^2).

Each measurement was performed three times to obtain the mean of each value. When the root region presented more than one area of root resorption, the areas were added together to obtain the total area of resorption per area/animal.

2.10 Statistical Analysis

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For data analysis, Student's t-test or analysis of variance (ANOVA) with Tukey's posttest was used, according to the characteristics of each variable. Differences were considered statistically significant at *P*= .05. Statistical analyses and graphs were performed using SigmaPlot version 11.0 (Systat Software Inc., San Jose, CA, USA).

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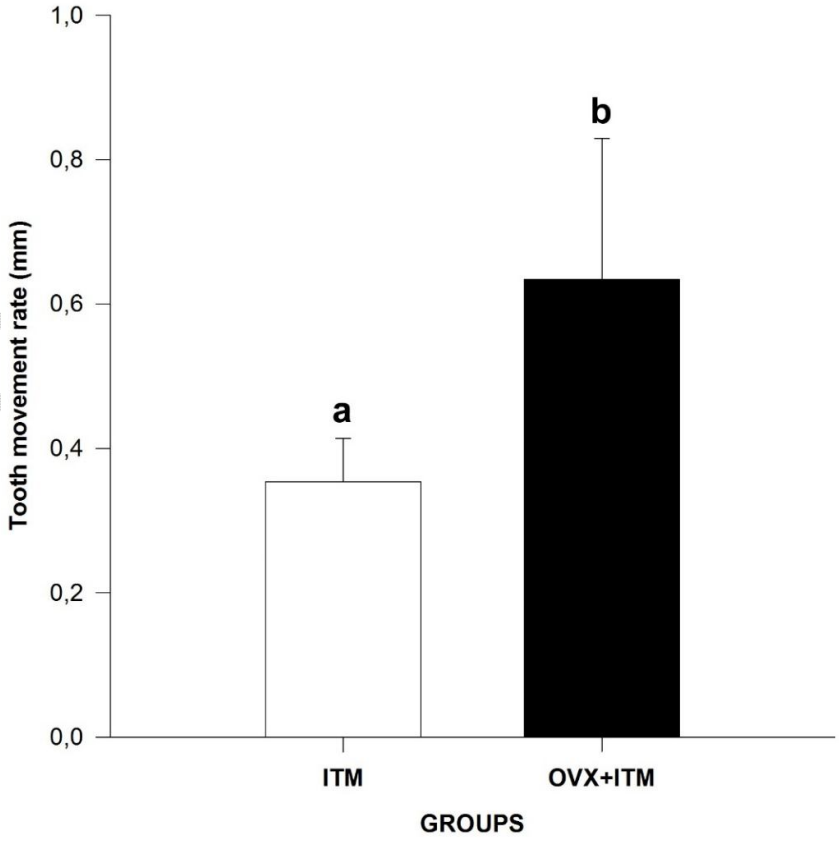
3. RESULTS

3.1Tooth movement rate

The animals in the OVX+ITM group had a significantly higher tooth movement rate (0.63 mm) than the animals in the ITM group (0.35 mm) (*P*=.05) (Graph 1).



Graph 1. Tooth movement rate in the experimental groups subjected to induced tooth movement (ITM). Data are expressed as the mean ± standard deviation. N= 6 animals/group. Student's t-test. Different letters (^{a,b}) indicate significant differences between groups (*P* =.05).

3.2 Descriptive analysis of histological slides

Group 1 - Control (CTL): The periodontal ligament (PL) was normal and rich in fibroblasts and collagen fibers. The surfaces of the roots were continuous throughout in most animals, with some occasions of root resorption. The interradicular septum and mesial bone crest were normal.

Group 2 - Induced Tooth Movement (ITM): The PL was found to be moderately disorganized near the interradicular septum region, with the presence of intense vascular changes. Root resorption was moderately present in the distobuccal root, mesial and distal surfaces, in the cervical and middle thirds, in the mesiobuccal root in the distal cervical and middle thirds, and in the periodontium region of the furcation.

Group 3 - Ovariectomy (OVX): The PL was slightly disorganized. Root surfaces exhibited resorption in the middle and cervical thirds of the mesial surface of the mesiobuccal root, in the furcation region, and in the cervical and middle thirds of the distal and mesial surfaces of the distobuccal root. The interradicular septum and mesial bone crest were normal.

Group 4 –Ovariectomy + movement (OVX + ITM): The PL was moderately disorganized in the periodontium of the furcation region. The interradicular septum showed moderate vascular changes. Presence of moderate root resorption in the mesiobuccal and distobuccal roots in all analyzed areas and in the periodontium of the furcation region.

3.3 Histomorphometric analysis of root resorption

The animals in the ITM group exhibited significantly greater areas of root resorption than the animals in the CTL and OVX groups (*P*<0.001). The OVX group had a larger root

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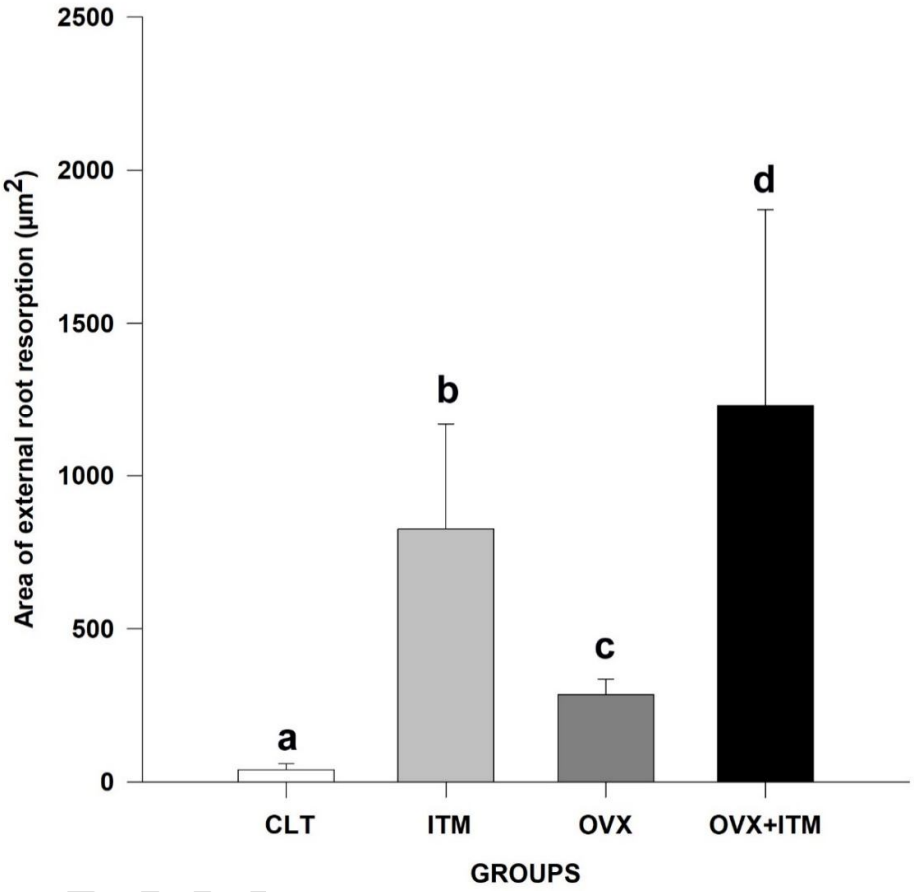
resorption area than the CTL group and a smaller root resorption area than the ITM and OVX + ITM groups (*P*<0.001). The OVX + ITM group had the highest amounts of root resorption areas when compared to all experimental groups (*P*<0.001) (Graph 2).



Graph 2. Area of external root resorption in the different experimental groups. Values are expressed as the mean ± standard deviation. N= 6 animals/group. Analysis of variance (one-way ANOVA) with Tukey's posttest. Different letters (^{a,b,c,d}) indicate significant differences between groups (*P*<0.001).

4. DISCUSSION

Orthodontic tooth movement is the result of remodeling of the periodontal ligament and alveolar bone, which consists of an interaction between bone resorption by osteoclasts and bone formation by osteoblasts.

Osteoporosis is one of the main diseases characterized by low bone mass and is often associated with postmenopausal women. Its decrease accelerates bone resorption, increasing resorption more than remodeling [20]. In the present study, the animals in the OVX+ITM group showed a higher rate of tooth movement than those in the ITM group. Estrogen deficiency causes an increase in the number of osteoclasts, with an increase in the resorption area and increased tooth movement [20]. [13] also stated that estrogen withdrawal in OVX rats affects the alveolar bone and promotes periodontal diseases. In orthodontic treatments, the increase in bone turnover, induced by the lack of estrogen, results in a significantly faster progression of tooth movement in an unstable pattern.

Estrogen deficiency increases the expression of the nuclear factor ligand receptor (RANKL). The binding of RANKL to its RANK receptor on preosteoclasts is necessary for the differentiation and proliferation of preosteoclasts into mature osteoclasts [12]. [21] found that on the tension side, the cells of the periodontal ligament had a large amount of osteoprotegerin (OPG) and little RANKL; on the pressure side, there was greater expression of RANKL, especially in osteoclasts along the alveolar surface, and OPG expression was very weak; it was found that the decrease in estrogen levels increased the production of PGE2 (prostaglandin E2), which subsequently activated prostaglandin receptors in osteoblasts and increased RANKL expression, also increasing the expression of cytokines of resorption such as interleukin (IL)-1, IL-6 and transforming growth factor (TGF)-β.

Among the histological results of our study, it was possible to observe that only the CTL group had the LP with normality; in the other groups, a disorganized LP was observed. In the ITM and OVX+ITM groups, there were vascular changes in the interradicular septum, according to [19], and the forces used in dentoalveolar trauma are capable of generating stress that results in vascular changes, such as bleeding and inflammation of the periodontal ligament, resulting in its disorganization.

Our results showed that the ITM and OVX+ITM groups achieved greater external root resorption. Orthodontically induced root resorption is a pathological consequence; tooth

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movement, as an essential component of orthodontic treatment, is achieved by bone remodeling during the application of force. The cells of the periodontal ligament on the pressure side undergo a process of necrosis, and after the formation of the hyaline zone, tooth movement stops root resorption. During the process of shedding the hyaline zone, mononuclear macrophages and multinucleated giant cells may damage the outer layer of the root, which consists of cementoblasts[13].

5. CONCLUSION

We conclude that induced tooth movement associated with ovariectomy induces a higher rate of tooth movement and a higher occurrence of root resorption.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experimental procedures were in accordance with the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation (COBEA) and were analyzed by the Committee on Ethics in the Use of Animals (CEUA) of UNIOESTE.

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