

Original Research Article

Pulp alterations and internal root resorption in teeth of ovariectomized rats submitted to experimental extrusive luxation and orthodontic movement

ABSTRACT

Aims:The objective of this study was to evaluate the effects of ovariectomy associated with dentoalveolar trauma and its influence on the tooth movement, root resorption and histological structure of the pulp dentin complex during induced tooth movement in rats.

Study design: Experimental research.

Methodology:Forty-eight Wistar rats were separated into eight experimental groups (n=6/group): Control group (CLT); animals submitted to dentoalveolar trauma (DT); animals submitted to ovariectomy (OVX); animals submitted to dentoalveolar trauma and ovariectomy (DT + OVX); animals submitted to induced tooth movement (ITM); animals submitted to induced tooth movement and trauma (ITM + DT); animals submitted to tooth movement and ovariectomy (ITM + OVX) and animals submitted to tooth movement, trauma and ovariectomy (ITM+DT+OVX). At the end of the experimental period, animals were euthanized, the maxillary were removed, fixed in 10% formaldehyde, decalcified, embedded in paraplast, sectioned to 5µm and stained with hematoxylin and eosin.

Results:The tooth movement rate was significantly higher in animals in the ITM+DT+OVX group when compared to the other groups (p<0.05). There were no changes in the cellularity pattern of the pulp tissue in the different experimental groups. In the analysis of dystrophic changes, only the ITM + OVX and ITM+DT+OVX groups presented hyalinization areas. Regarding hemodynamic changes, vascular congestion and thrombosis were similar in all groups. The only alteration in dentin observed was the internal root resorption, in the animals of the DT+OVX, ITM, ITM+OVX and ITM+DT+OVX groups. The

ITM group presented larger resorption areas than the ITM+DT and ITM+DT+OVX groups and the ITM+OVX group presented the largest amounts of root resorption areas when compared with all experimental groups.

Conclusion: Induced tooth movement associated with ovariectomy, and experimental extrusive luxation causes an increase in the rate of tooth movement, pulp hyalinization and larger areas of internal root resorption.

Keywords: Tooth Movement Techniques, Estrogens, Dentoalveolar trauma, Dental pulp devitalization.

1. INTRODUCTION

With the greater accessibility to orthodontic treatment today, the number of adults seeking orthodontics has increased. Consequently, the probability of having patients with systemic diseases resulting from the aging process, such as osteoporosis, also increases [1]. Osteoporosis resulting from menopause or estrogen deficiency affect orthodontic tooth movement, causes pathological bone loss, accelerated bone resorption, tooth loss, affects odontogenesis e the pulp-dentin complex including dental pulp stem cells[2,3,4,5].

Another factor of great interest in dental clinic is dentoalveolar trauma (DT), considered an important public health problem not only because of its high prevalence, but also because it is associated with functional disorders and aesthetic sequelae that impact the patient's life [6,7]. It is extremely important the definition of the conducts to be adopted by the orthodontist in relation to the treatment of patients who suffered dentoal trauma, predicting the complications that may occur during orthodontic treatment, for exemple, changes in the periodontal and pulp tissues are observed due to the exposure of forces that vary according to the magnitude, frequency and duration [8,9].

The DT should be investigated as the main suspected cause of pulp changes during and after orthodontic treatment. Investigations reveal the occurrence of several biological reactions during induced tooth movement (ITM), including increased or reduced blood flow and angiogenesis [10]. Vascular changes in pulp caused by orthodontic movement are related to disorders in the

odontoblastic layer, pulp obliteration, early pulp hyperemia, inflammation, internal root resorption and pulp necrosis [11].

Considering the high prevalence of patients with osteoporosis and dentoalveolar trauma who seek orthodontic treatment nowadays, the aim of this study was to evaluate the effects of ovariectomy on the tooth movement, in the organization of pulp tissue and root resorption in traumatized teeth and submitted to induced tooth movement.

2. METHODOLOGY

A sample of 48 rats ($n = 6/\text{group}$) was calculated considering the variables dentoalveolar trauma, ovariectomy and induced tooth movement, with α of 5% and test power of 80% (GPower 3.1 software, Heinrich Heine University Düsseldorf, Faul et al., 2007, 2009 [12,13]).

All experimental procedures are in accordance with the Ethical Principles in Animal Experimentation and were approved by the Ethics Committee on the Use of Animals (protocol 2411/2017). Forty-eight 60-day-old Wistar rats were maintained in collective polyethylene cages (43x30x15), accommodated individually or in pairs, under controlled temperature ($22^{\circ} \sim 25^{\circ} \text{C}$) and 12/12 hours photoperiod (7:00 - 19:00h), and free access to water and standard rodent rations Nuvital® (Nuvilab CR-1, Colombo, PR, Brazil).

2.1 Experimental design

Animals were divided into eight experimental groups ($n = 6/\text{group}$): Control group (CTL); dentoalveolar trauma (DT); ovariectomy group (OVX); dentoalveolar trauma and ovariectomy (DT+OVX); induced tooth movement (ITM); induced tooth movement and dentoalveolar trauma (ITM+DT); induced tooth movement and ovariectomy (ITM+OVX) and induced tooth movement, dentoalveolar trauma and ovariectomy (ITM+DT+OVX).

2.2 Ovariectomy

At 60 days of age, animals in the OVX, DT+OVX, ITM+OVX and ITM+DT+OVX groups were subjected to bilateral ovariectomy, by Khajuria et al. 2012 [14]. After intraperitoneal anesthesia with ketamine (75 mg/Kg) and xylazine (15 mg/Kg), animals were placed in a surgical plane, the skin and musculature were incised longitudinally, the ovaries were identified and exposed. Hemostasis was

performed by connecting the upper part of the uterus with #4 Ethicon - Johnson & Johnson (São Paulo, SP, Brazil) silk thread and ovarian excision together with the surrounding fat, the uterine tube and a small part of the uterus. Planes were sutured with absorbable catgut N° 4 and the skin with silk thread #4. A sham operation was also performed for another groups, in which all procedures were the same, except for the removal of the ovaries.

2.3 Application of extrusive luxation

At 90 days of age, dentoalveolar trauma was induced according to the methodology proposed by Costa et al. (2018) [15]. After intraperitoneal anesthesia with ketamine (75 mg/Kg) and xylazine (15 mg/Kg), asepsis of the region was performed with povidone-iodine 1% (Riodeine[®], São José do Rio Preto, SP, Brazil). Animals of the DT, DT+OVX, ITM+DT and ITM+DT+OVX groups were subjected to extrusive luxation (EL). For EL, the following protocol was used: a 0.25 mm ligaturewire (Morelli[®]; Brazil) was inserted in the palatal to buccal direction between the first and second upper right molars. The two ends of the wire were placed on the mesial surface of the upper first molar and twisted with the aid of a 17.0 cm Mathieu needle holder (Quinelato[®], Rio Claro, São Paulo, Brazil), to fix the wire around the tooth. At the distal end of the inserted wire, a loop was made to connect the tensiometer (Morelli[®]; Sorocaba, São Paulo, Brazil). The tensiometer was positioned at an angle of 60 ° in relation to the vertical plane and a force of 1500cN was applied for 15 seconds. After the procedure, the ligature wire was removed.

2.4 Installation of the device for induced tooth movement (ITM)

At 105 days of age, ITM device was installed in animals in the ITM, ITM + DT, ITM + OVX, ITM+DT+ OVX groups, as described by Heller & Nanda (1979) [16], with a total tooth movement period of 7 days. The device consists of a nickel-titanium (NiTi) closed-coil springs (Sentalloy[®], GAC, NY, USA), with release of 50cN force magnitude (Tensiometer Zeusan[®] Exporting LDTa Campinas, São Paulo, Brazil). In addition, two segments of ligaturewire, 0.25 mm thick (Morelli, Sorocaba, SP, Brazil) were connected at each end of the spring, one bypassing the maxillary right first molar and the other the upper right central incisor of the animal. For stability of the ligature wire on the buccal face of the incisor, a groove was made in the cervical region and a lock was made with a photopolymerizable

composite resin (Filtek™ Z350 XT, 3M ESPE) (3M of Brazil Ltda, Sumaré, SP, Brazil). The maxillary left first molars with no orthodontic force application served as the negative controls.

2.5 Euthanasia and collection of biological material

At the end of the experimental period (112 days of age), all animals were weighed and euthanized by decapitation with guillotine. The maxillas were removed and fixed in 10% buffered formalin for 24 hours, washed in water for 48 hours and decalcified in acid solution (Allkimia®, Campinas, SP, Brazil) for 19 hours and stored in 70° alcohol.

2.6 Quantitative analysis of tooth movement

After euthanasia, the experimental groups were blind, the amount of tooth movement was obtained by the difference between the distances from the mesial face of the upper 1st molar to the distal face of the upper 3rd molar on the moved right side and unmoved left side, according to the methodology proposed by Gameiro et al. (2008) [17]. Measurements were obtained in millimeters (mm) using a digital caliper (Mitutoyo, São Paulo, SP, Brazil), the measures were repeat after two weeks and performed by two properly calibrated evaluators.

2.7 Histological processing

The maxilla were dehydrated in an increasing series of alcohols, cleared in xylol and embedded in Paraplast Plus® (Sigma-Aldrich, USA). For histological analysis, serial sections were made in the longitudinal plane of the mesio-buccal and distal-buccal roots of the right upper 1st molar, from mesial to distal, 5 µm thick, using a manual rotary microtome (Olympus® 4060, Miami, FL, USA), equipped with steel razor. Sections were deparaffinized with xylol, hydrated with distilled water and stained with hematoxylin-eosin (HE) for analysis.

For the histological analysis, an optical microscope (Olympus BX60) was used. To obtain photomicrographs at 200x and 400x magnification, an Olympus DP71 digital camera was used with the DP Controller 3.2.1.276 software.

2.8 Descriptive analysis of histological slides

The first upper right molars were examined to diagnose pulpal changes for morphological signs or morphological criteria that indicate premature aging, according to Massaro et al. (2009) [10] and Cuogui et al. (2018) [18]. The following histological characteristics were evaluated: Presence or absence of inflammatory infiltrate, reduced cellularity, increased fibrosis, pulp hyalinization, vacuolization, pulp nodules, diffuse calcification, necrosis, vascular congestion, hemorrhage, thrombosis, reactionary dentin, tubules with nucleus and internal root resorption.

2.9 Morphometric analysis of internal root resorption

For quantitative analysis of internal root resorption, photomicrographs at 400X magnification were analyzed in 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), the experimental groups were blind. When the root region showed more than one area of root resorption, the areas were added to obtain the total area of resorption per animal. The analysis was performed by two properly calibrated evaluators, and the measures were repeat after two weeks.

2.10 Statistical analysis

The data were submitted to the Shapiro-Wilk normality test, and os groups were compared by analysis of variance (One-Way ANOVA) followed by Tukey's post-hoc test. Differences were considered statistically significant when $p < 0.05$. Statistical analyses and graphs were performed using SigmaPlot 12.0 software (Systat Software Inc., San Jose, CA, USA).

2.11 Method error

Inter-examiner and intra-examiner concordance (Kappa test) for measurements of tooth movement area and root resorption were assessed by the intraclass correlation test and demonstrated, respectively, high reproducibility (0.892) and reliability (0.923). Kappa test were made in the BioEstat 5.3 program (Instituto Mamirauá, Belém, Pará, Brazil).

3. RESULTS

3.1 Tooth movement analysis

The rate of tooth movement was significantly higher in animals in the ITM+DT+OVX group when compared to the other groups ($P=.05$). There was no significant difference in the movement rate when comparing the ITM+DT and ITM+OVX groups, however both groups showed greater tooth movement when compared to the ITM group ($P=.05$) (Figure 1).

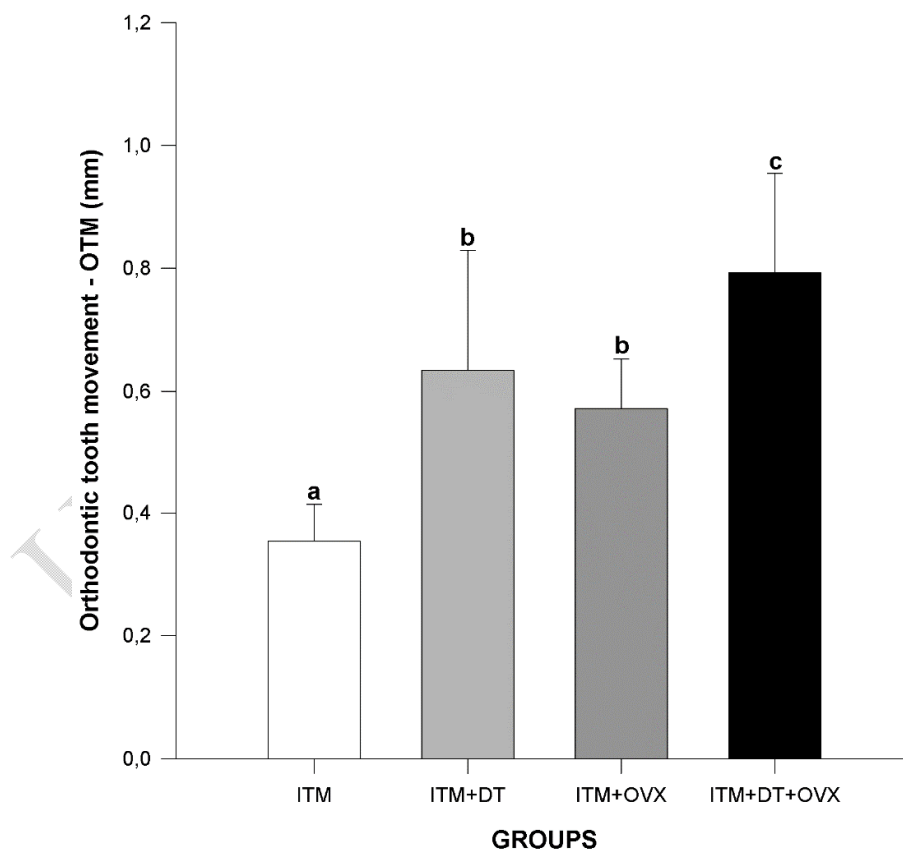


Figure 1. Rate of orthodontic tooth movement (OTM) in the different experimental groups undergoing induced tooth movement (ITM). Values expressed as mean \pm SD. N=6 animals/group. Analysis of variance (One-Way ANOVA) followed by Tukey's test. Different letters ^{a, b, c} indicate significant differences between groups ($P = .05$).

3.2 Descriptive analysis of the pulp structure

The CTL group revealed uniform dental pulp morphology. Blood vessels were usually congested (Figure. 2A-D) and full of blood components. In the internal dentinal wall, facing the dental pulp, there was no thickening of the predentin layer, nor morphological signs of restorative dentin.

There were no changes in the pattern of cellularity, presence of vacuolization, nodule and necrosis of the pulp tissue in the animals of the different experimental groups. Areas of hyalinization were observed in ITM+OVX and ITM+DT+OVX animals (Figure 2E-F). Hemodynamic changes (vascular congestion and thrombosis) were observed in all experimental groups (Figure 2C-D), while hemorrhage was not observed in any group. Changes in dentin, such as the presence of tubules and formation of reactionary dentin, were not verified in any group analyzed. However, internal root resorption was found in the DT + OVX, ITM, ITM + OVX and ITM+DT+ OVX groups (Figure 2 E-F) (Table 1).

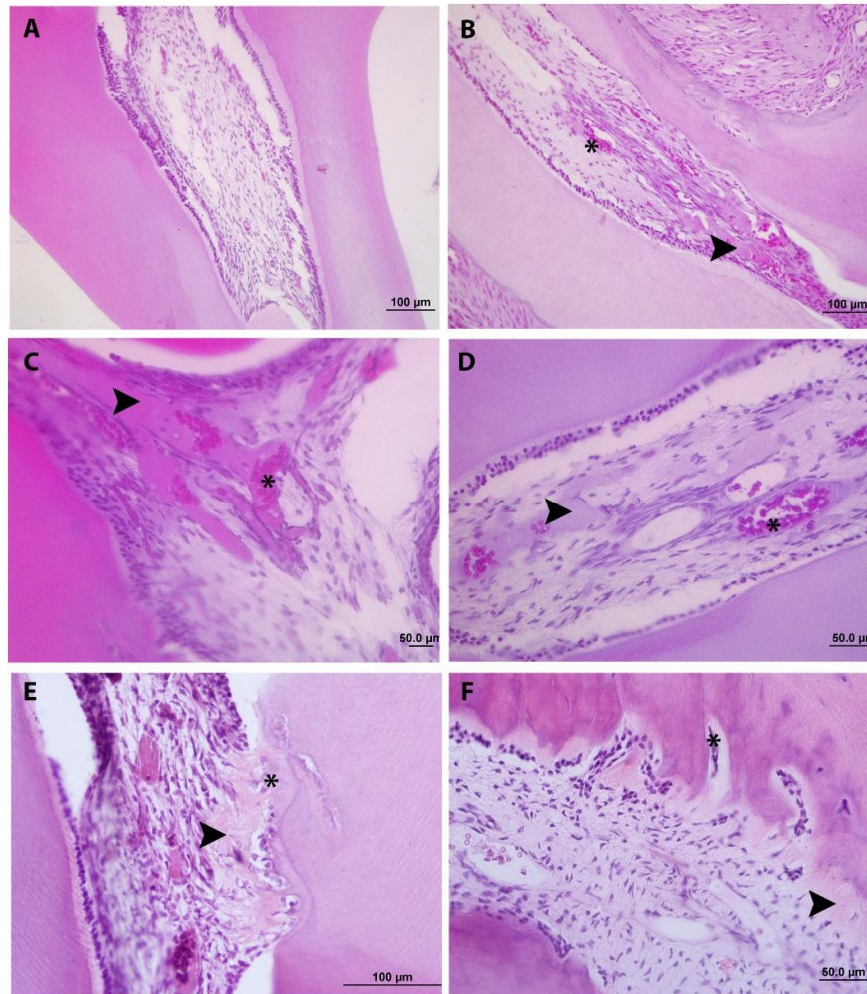


Figure 2. Photomicrograph of the dental pulp of animals from different experimental groups. A. Dental pulp of the first upper right molar showing a normal aspect; B- D. Dental pulp with areas of congested vessels (arrowhead) and thrombosis (asterisk); E and F. Presence of hyaline areas (arrowhead) and internal root resorption (asterisk); Staining = Hematoxylin and Eosin.

Table 1. Frequency of changes in the dentin-pulp complex observed in the different experimental groups. N = 6/group.

PULPAR ALTERATIONS	CTL	DT	OVX	DT+OVX	ITM	ITM+DT	ITM+OVX	ITM+DT+OVX
Cellularity pattern								

Inflammatory infiltrate	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Reduced cellularity	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Increased fibrosis	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Dystrophic alterations								
Hyalinization of pulp	0/6	0/6	0/6	0/6	0/6	0/6	2/6	2/6
Cell vacuolization	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Nodules of pulp	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Diffuse calcification	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Necrosis	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Hemodynamic alterations								
Vascular congestion	6/6	6/6	6/6	6/6	6/6	6/6	4/6	6/6
Hemorrhage	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Thrombosis	6/6	6/6	6/6	6/6	6/6	6/6	5/6	6/6
Dentin alterations								
Reactionary dentin	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6
Tubules with nucleuses	0/6	0/6	0/6	0/6	0/6	0/6	0/6	0/6

Internal root resorption	0/6	0/6	0/6	1/6	2/6	0/6	1/6	1/6
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3.3 Morphometric analysis of internal root resorption

Among the animals in the groups with no tooth movement device, the DT+OVX group had the highest rate of internal root resorption areas when compared with the CTL, DT and OVX groups ($P=.05$) (Table 2).

In the analysis of the groups with a tooth movement device, the ITM and ITM+OVX groups had larger areas of internal root resorption than all groups without a tooth movement device ($P=.05$). The ITM group showed larger resorption areas than the ITM+DT and ITM+DT+OVX groups ($P=.05$). The ITM+OVX group showed the largest amounts of root resorption areas when compared to all experimental groups ($P=.05$) (Table 2).

Table 2. Internal root resorption area in the different experimental groups.

PARAMETERS	CTL	DT	OVX	DT +OVX	ITM	ITM +DT	ITM +OVX	ITM +DT+OVX
Internal root resorption area(μm^2)	0.00 $\pm 0.00^a$	0.00 $\pm 0.00^a$	0.00 $\pm 0.00^a$	667.43 $\pm 8.37^b$	2891.19 $\pm 794.92^c$	0.00 $\pm 0.00^a$	8564.74 $\pm 170.83^d$	780.36 $\pm 22.46^{be}$

Values expressed as mean \pm SEM. N=6 animals/group. Analysis of variance (One-Way ANOVA) followed by Tukey's test. Different letters ^{a,b,c,d,e} indicate significant differences between groups ($P=.05$).

4. DISCUSSION

Thus, effects of ovariectomy on orthodontic movement have attracted great attention in studies, since orthodontic tooth movement depends on alveolar bone remodeling in the area of pressure and resorption and bone formation in the tension area. The ovariectomy model in rats is widely used to simulate human osteoporosis in the post-menopause [19,20,21].

In the present study, the animals of ovariectomized groups (ITM+OVX and ITM+DT+OVX) showed greater tooth movement when compared to the ITM group. It is known that, in ovariectomized rats, osteogenesis and chondrogenesis decrease because estrogen deficiency alters the production of osteoinductive proteins, such as osteogenin, and bone morphogenetic protein, resulting in the disruption of the bone matrix in formation [22]. This suggests that the effect of ovariectomy is related to the rate of bone turnover caused by the reduction of estrogen levels in the OVX group. Tooth movement rate in ovariectomized groups was higher than in non-ovariectomized groups, suggesting that the ovariectomy probably increased bone turnover and led to an acceleration of tooth movement, as already reported by other studies [3,23,24,25,26].

Another important aspect about the increased rate of tooth movement in ovariectomized rats is related to the differential expression of osteoprotegerin (OPG) and the Receptor activator of nuclear factor kappa-B ligand (RANKL), which are the main factors that regulate the differentiation of osteoclasts. These factors may have been modulated by the absence of estrogen, caused by ovariectomy, which possibly increased osteoclastogenesis that promotes bone resorption during orthodontic movement, resulting in accelerated tooth movement in ovariectomized rats [20,21].

Animals in the group with movement and trauma (ITM+DT) showed a higher movement rate when compared to the ITM group in the present study. Dentoalveolar trauma is associated with an inflammatory process, deterioration of the microstructure and increased alveolar bone resorption, induced by increased osteoclast activity, especially in the compression area[21,26]. Thus, the higher rate of tooth movement in animals in the ITM+DT group is associated with greater activity of osteoclasts, which induce greater alveolar bone resorption, favoring orthodontic movement.

Orthodontic movement can cause inflammatory or degenerative responses in the dental pulp. The impact of orthodontic movement on the pulp occurs mainly in the neurovascular system, causing the release of specific neurotransmitters (neuropeptides) that influence blood flow and cell metabolism. Responses induced in these pulps may have an impact on the onset and perpetuation of apical root remodelling or resorption during tooth movement. The incidence and severity of these changes can be influenced by injuries prior to the dental pulp, such as dentoalveolar trauma [27].

Neuropeptides, such as the calcitonin gene-related peptide (CGRP), can be triggered by caries, trauma and the action of orthodontic forces. CGRP increases the expression of bone morphogenetic protein in human pulp cells, stimulating the deposition of dentin by odontoblasts as a

defense mechanism. This event, together with hypoxia, induces degenerative calcification of the dental pulp and can cause the obliteration of the root pulp [18]. Nevertheless, changes in dentin, such as deposition of reactionary dentin, the presence of dental pulp calcifications were not observed in the present study.

The results obtained reinforce the findings of the research carried out by Massaro et al. (2009) [10], when the dental histological characteristics of the control group revealed uniform pulp morphology, blood vessels congested. Furthermore, in the internal dentinal wall, facing the dental pulp, there was no thickening of the predentin layer, nor morphological signs of restorative dentin.

In this study was observed hemodynamic changes in all groups, except hemorrhage. Mild forces can cause a small release of CGRP, leading to initial vascular congestion. Nevertheless, vascular congestion can be compensated for by the release of angiogenic factors, preventing irreversible damage to the pulp. Young dental pulps are larger, with a higher number of cells and with little or no fibrosis. Over time, dental pulp reduces its volume due to the deposition of secondary or reactionary dentin, increasing fibrosis and cell density, in addition to reducing blood vessels. These dynamic changes may justify the pulp changes observed in this study, since the animals were young and the dental pulp had a greater capacity to react to environmental variations, such as stress due to ITM. Also, pulp nodules and calcifications are part of the natural pulp "aging" process, but they can occur earlier in the face of traumatic processes in the tooth structure. These events were not observed in the present study, suggesting that the age of the animals and the applied variables were biologically acceptable [18].

Among dystrophic changes, hyalinization was found in the ITM+OVX and ITM+DT+OVX groups. Hyalinization can be understood as an exacerbated inflammation that results in damage to the structural components of pulp tissue with transformation of intra- and extracellular proteins into homogeneous, vitreous and pinkish material, being considered as an undesirable tissue reaction of ITM [28].

Among the animals in the groups without a tooth movement device, the DT+OVX group presented the highest rate of internal root resorption areas when compared with the CTL, DT and OVX groups. Dental resorption represents the process of dismantling mineralized odontogenic tissues by the action of clastic cells, when the protective structures of teeth in relation to bone remodeling are eliminated, especially cementoblasts, odontoblasts and epithelial rests of Malassez [29]. Dental surface

internal is protected from the action of clasts by odontoblasts, because this cell has no receptors for bone resorption mediators. The resorption process will begin when a factor acts on the tooth structure, eliminating these cells. Dentoalveolar trauma can damage this layer of odontoblasts, making traumatized teeth more prone to tooth resorption in the face of a new cause, such as induced tooth movement [30].

Studies reveal that root resorption is involved not only in osteoclastogenesis, but also in odontoclastogenesis through the OPG/RANK/RANKL system. This system covers the balance between OPG and RANKL in tension and on the compression side of the tooth during orthodontic movement. In this study, these factors may have been modulated by the loss of estrogen by ovariectomy and possibly increased osteoclastogenesis. The ITM+OVX group had the largest amount of areas of internal root resorption when compared to all experimental groups. Thus, can be affirm that ovariectomy affects orthodontically induced root resorption, as well as tooth movement through the hormonal system, by altering bone metabolism through some biomarkers and biological pathways [3].

It is worth mentioning that there is a predominance of women in the search for orthodontic treatment. Therefore, aiming at personalized service for this public, it is of fundamental importance the knowledge of systemic factors and how they can influence the result of the orthodontic treatment offered, as well as long-term post-treatment dental stability [31].

5. CONCLUSION

It can be concluded that the induced tooth movement associated with ovariectomy and/or experimental extrusive luxation increases the rate of tooth movement, hyalinization of the pulp tissue and internal 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.

REFERENCES

1. Salazar M, Hernandez L, Ramos AL, Salazar BO, Micheletti KR, Paranhos LR, et al. Effect of alendronate sodium on tooth movement in ovariectomized rats. *Arch Oral Biol.* 2015; 60:776-81.
2. Seifi M, Eslami B, Saffar AS. The effect of prostaglandin E2 and calcium gluconate on orthodontic tooth movement and root resorption in rats. *Eur J Orthod.* 2003; 25: 199-204.
3. Sirisoontorn I, Hotokezaka H, Hashimoto M, Gonzales C, Luppapanornlarp S, Darendeliler MA, et al. Tooth movement and root resorption; the effect of ovariectomy on orthodontic force application in rats. *AngleOrthod.* 2011; 81:570–77.
4. Wang Y, Yan M, Yu Y, Wu J, Yu J, Fan Z. Estrogen deficiency inhibits the odonto/osteogenic differentiation of dental pulp stem cells via activation of the NF-kappaB pathway. *Cell Tissue Res.* 2013; 352:551–59.
5. Alhodhodi A, Alkharobi H, Humphries M, Alkhafaji H, El-Gendy R, Feichtinger G, et al. O estrogen receptor β (ER β) regulates osteogenic differentiation of human dental pulp cells. *J Steroid Biochem Mol Biol.* 2017; 174: 296-302.
6. Pereira ALP, Mendonça MR, Sonoda CK, Cuoghi OA, Poi WR. Histological evaluation of experimentally induced subluxation in rat molars and its implications on the management of orthodontic treatment. *Dent Traumatol.* 2010; 26(1): 37-42.

7. Pereira ALP, Mendonça MR, Sonoda CK, Bussato MCA, Cuoghi OA, Fabre AF. Microscopic evaluation of induced tooth movement in traumatized teeth: an experimental study in rats. *Dent Traumatol.* 2012; 28:114-20.
8. Glendor U. Epidemiology of traumatic dental injuries - a 12 year review of the literature. *Dent Traumatol.* 2008. 24: 603-11.
9. Tondelli PM, Mendonça MR, Cuoghi OA, Pereira ALP, Busato MCA. Knowledge on dental trauma and orthodontic tooth movement held by a group of orthodontists. *Braz Oral Res.* 2010, 24:76–82.
10. Massaro CS, Consolaro RB, Santamaria MJr, Consolaro MFMO, Consolaro A. Analysis of the dentin-pulp complex in teeth submitted to orthodontic movement in rats. *J Appl Oral Sci.* 2009;17: 35-42.
11. Abi-Ramia LBP, Stuani AS, Stuani AS, Stuani MBS, Mendes AM. Effects of Low-Level Laser Therapy and Orthodontic Tooth Movement on Dental Pulps in Rats. *Angle Orthod.* 2010; 80:116-22.
12. Faul F, Erdfelder E, Lang AG, Buchner A. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods.* 2007; 39: 175-91.
13. Faul F, Erdfelder E, Buchner A, Lang AG. Statistical power analyses using G*Power 3.1: Tests for correlation and regression analyses. *Behavior Research Methods.* 2009; 41:1149-60.
14. Description of a new method of ovariectomy in female rats Khajuria DK, Razdan R, Mahapatra DR *Rev. Bras. Reum.* 2012; 52: 462-70
- 15. Costa LA, Cantanhede LM, Pereira EM, Crivelini MM, Cuoghi OA, Pereira ALP, et al. Validation of a new experimental model of extrusive luxation on maxillary molars of rats: a histological study. *Clinical Oral Investigations.* 2018; 22:1985-94.

16. Heller IJ, Nanda R. Effect of metabolic alteration of periodontal fibers on orthodontic tooth movement. An experimental study. *Am J Orthod.* 1979; 75: 239-58.
17. Gameiro GH, Nouer DF, Pereira-Neto JS, Siqueira VC, Andrade ED, Novaes PD, et al. Effects of short- and long-term celecoxib on orthodontic tooth movement. *Angle Orthod.* 2008; 78:860-65.
18. Cuoghi OA, Faria LP, Ervolino E, Barioni SRP, Topolski F, Arana-Chaves VE, et al. Pulp analysis of teeth submitted to different types of forces: a histological study in rats. *J. Appl. Oral Sci.* 2018; 26: e20170626.
19. Hashimoto M, Hotokezaka H, Sirisoontorn I, Nakano T, Arita T, Tanaka M, et al. Effect of bone morphometric changes on orthodontic tooth movement in an osteoporotic animal model. *Angle Orthod.* 2013; 83:766-73.
20. Dahhas FY, El-Bialy T, Afify A, Hassan AH. Effects of low-intensity pulsed ultrasound on orthodontic tooth movement and orthodontically induced inflammatory root resorption in ovariectomized osteoporotic rats. *Ultrasound in Med. & Biol.* 2016; 42: 808-14.
21. Dai Q, Zhou S, Zhang P, Ma X, Han Y, Yang X, et al. Force-induced increased osteogenesis enables accelerated orthodontic tooth movement in ovariectomized rats. *Sci Rep.* 2017; 7: 3906.
22. Cesnjaj M, Stavljenic A, Vukicevic S. Decreased osteoinductive potential of bone matrix from ovariectomized rats. *Acta Orthop Scand.* 1991; 62:471–75.
23. Tanaka M, Ejiri S, Toyooka E, Kohno S, Ozawa, H. Effects of ovariectomy on trabecular structures of rat alveolar bone. *J Periodontal Res.* 2002; 37:161–65.
24. Arslan SG, Arslan H, Ketani A, Hamanci O. Effects of estrogen deficiency on tooth movement after force application: an experimental study in ovariectomized rats. *Acta Odontol Scand.* 2007; 65:319-23.

25. Sirisoontorn I, Hotokezaka H, Hashimoto M, Gonzales C, Luppapornlarp S, Derendeliler MA, et al. Orthodontic tooth movement and root resorption in ovariectomized rats treated by systemic administration of zoledronic acid. *Am J Orthod Dentofacial Orthop.* 2012;141: 563-73.
26. Xu T, Zhao T, Xu W, Ding Y. Periodontal microstructure change and tooth movement pattern under different force magnitudes in ovariectomized rats: An in-vivo microcomputed tomography study. *Am J Orthod Dentofacial Orthop.* 2013; 143: 828-36.
27. Hamilton RS, Gutmann JL. Endodontic orthodontic relationships: a review of integrated treatment planning challenges. *Int Endod J.* 1999; 32: 343-60.
28. Krishnan V, Davidovich Z. Cellular, molecular and tissue-level reactions to orthodontic forces. *Am J Orthod Dentofacial Orthop.* 2006; 129: 469.e1-32.
29. Consolaro A, Cardoso LB, Kinoshita AM, Francischone LA, Santamaria JR, Fracalossi AC, et al. Indirect bone resorption in orthodontic movement: when does periodontal reorganization begin and how does it occur? *Dental Press J Orthod.* 2011; 16:25-31.
30. Bauss O, Röhling J, Meyer K, Kiliaridis S. Pulp vitality in teeth suffering trauma during orthodontic therapy. *Angle Orthod.* 2009; 79:166-71.
31. Haruyama N, Igarashi K, Saeki S, Otsuka-Isoya M, Shinoda H, Mitani, H. Estrous-cycle-dependent variation in orthodontic tooth movement. *J Dent Res.* 2002; 81:406-10.