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The Impact of Ovariectomy and Dental Trauma on Dental Pulp, on Tooth Movement and Internal Root Resorption: Findings from a Rat Model

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background/Aim: A patient's hormonal status significantly impacts the orthodontist's conduct, because it can directly influence the success of the treatment. Estrogen reduction is associated with a decrease in alveolar bone mineral density, increased tooth movement, root resorption and changes detinopulpar complex. This study aimed to evaluate the effects of ovariectomy associated with dentoalveolar trauma during induced tooth movementand its influence on the histological structure of the pulp dentin complex 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, and the maxillae were removed, fixed in 10% formaldehyde, decalcified, embedded in paraplast, sectioned to 5µm and stained with hematoxylin and eosin. The first upper right molars were examined to diagnose pulpal changes. The data were compared by analysis of variance (One-Way ANOVA) followed by Tukey's post-hoc test.
Results: The tooth movement rate was significantly higher in animals in the ITM+DT+OVX group when compared to the other groups ($p<0.05$). The cellularity pattern of the pulp tissue did not change in any of the 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 increased in the rate of tooth movement, pulp hyalinization and larger areas of internal root resorption. These findings are important for guiding personalized orthodontic for women experiencing hormonal imbalances and dental trauma.

Keywords: Tooth movement techniques; estrogens; dentoalveolar trauma; dental pulp devitalization.

1. INTRODUCTION

Greater accessibility to orthodontic treatment today has, increased the number of adults seeking orthodontics. 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 affects orthodontic tooth movement, causes pathological bone loss, accelerates bone resorption, and tooth loss, and affects odontogenesis and the pulp-dentin complex, including dental pulp stem cells [2,3,4,5].

Another factor of great interest in dental clinics is dentoalveolar trauma (DT), which is 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]. Defining the conduct to be adopted by the orthodontist in relation to the treatment of patients who suffered dental trauma is extremely important to predict 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].

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 currently seek orthodontic treatment, this study aimed to evaluate the effects of ovariectomy on tooth movement, in the organization of pulp tissue and root resorption in traumatized teeth and subjected to induced tooth movement.

2. METHODOLOGY

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

All experimental procedures were 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 (22º ~ 25º 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/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, as described by Pasa et al. 2024 [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, and the ovaries were identified and exposed. Hemostasis was achieved 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 Nº 4 and the skin with silk thread #4. A sham operation was also performed for other 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. [15]. After intraperitoneal anesthesia with ketamine (75 mg/kg) and xylazine (15 mg/kg), asepsis of the region was ensured 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). The following protocol was used for EL: a 0.25 mm ligature wire (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 1500 cN was applied for 15 seconds. After the procedure, the ligature wire was removed (Fig. 1).

2.4 Installation of the Device for Induced Tooth Movement (ITM)

At 105 days of age, an ITM device was installed in animals in the ITM, ITM + DT, ITM + OVX and ITM+DT+ OVX groups, as described by Heller & Nanda [16] and Pasa et al. [14], 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 50 cN force magnitude (Tensiometer Zeusan® Exporting LDTa Campinas, São Paulo, Brazil). In addition, two segments of ligature wire, 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 bypassing the upper right central incisor of the animal. To ensure the 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). 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 maxillae were removed and fixed in 10% buffered formalin for 24 hours, washed in water for 48 hours, decalcified in acid solution (Allkimia®, Campinas, SP, Brazil) for 19 hours and stored in 70% alcohol.

2.6 Quantitative Analysis of Tooth Movement

The experimenters were blinded to the experimental groups, and tooth movement was quantified after euthanasia based on 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. [17] and Pasa et al. [14]. Measurements were obtained in millimeters (mm) using a digital caliper (Mitutoyo, São Paulo, SP, Brazil). The measurements were repeated after two weeks and performed by two properly trained evaluators.

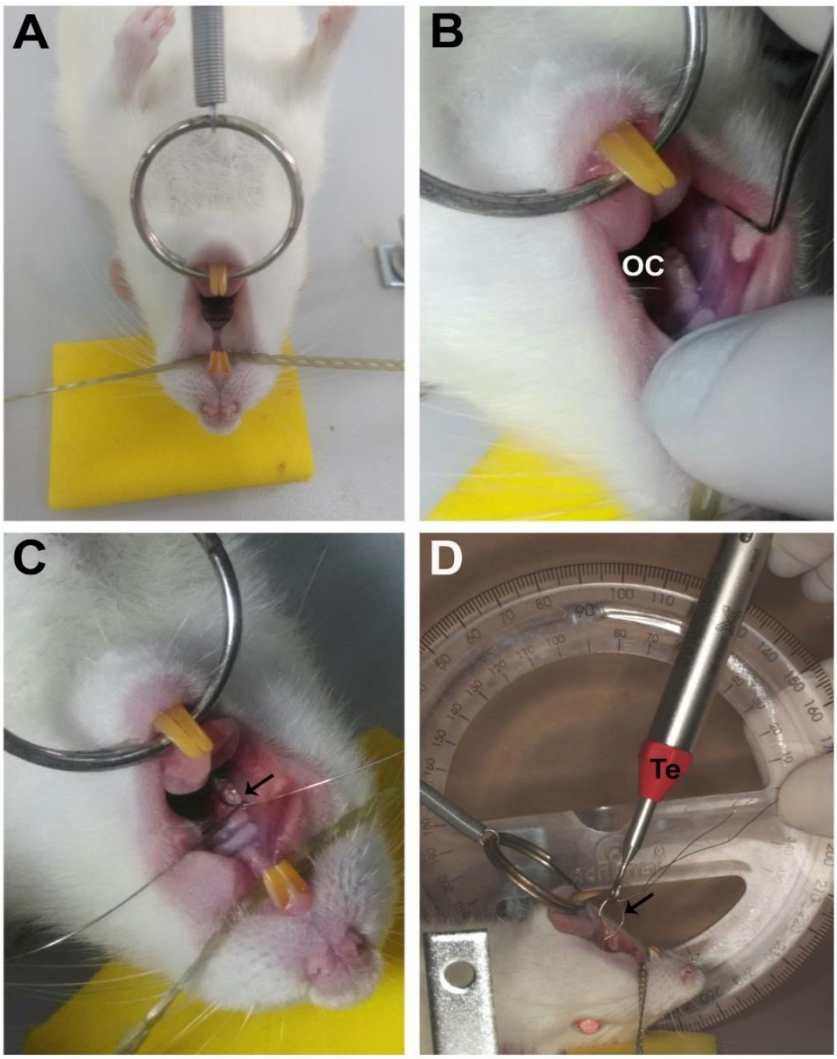

Fig. 1. Photographs of the dentoalveolar trauma procedure. A. Positioning the animal in the supine position on the operating table; B. Opening of the animal's oral cavity (CO); C. Yellow wire inserted between the upper right first and second molars (arrow); D. Tensiometer (Te) positioned on the handle (arrow) and traction at a 60° angle

2.7 Histological Processing

The maxillae were dehydrated in graded alcohols series, cleared in xylol and embedded in Paraplast Plus® (Sigma-Aldrich, USA). For histological analysis, serial sections were made in the longitudinal plane of the mesiobuccal 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 xylene, hydrated with distilled water and stained with hematoxylin-eosin (HE) for analysis.

An optical microscope (Olympus BX60) was used for the histological analysis. To obtain photomicrographs at 200x and 400x magnification, an Olympus DP71 digital camera was used with 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. [10] and Cuogui et al. [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

To quantitatively analyze 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), and researchers were blinded to the experimental groups. 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 measurements were repeated after two weeks.

2.10 Statistical Analysis

The data were submitted to the Shapiro–Wilk normality test, and the differences between groups were compared by analysis of variance (one-way ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant when $p<0.05$. SigmaPlot 12.0 software (Systat Software Inc., San Jose, CA, USA) was used for statistical analyses and to prepare graphs.

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 high reproducibility (0.892) and reliability (0.923), respectively. Kappa test were performed 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) (Fig. 2).

3.2 Descriptive Analysis of the Pulp Structure

The CTL group revealed uniform dental pulp morphology. Blood vessels were usually congested (Fig. 3 A-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.

The pattern of cellularity, presence of vacuolization, nodule or necrosis of the pulp tissue did not change in the animals of the different experimental groups. Areas of hyalinization were observed in ITM+OVX and ITM+DT+OVX animals (Fig. 3 E-F). Hemodynamic changes (vascular congestion and thrombosis) were observed in all experimental groups (Fig. 3 C-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 (Fig. 3 E-F) (Table 1).

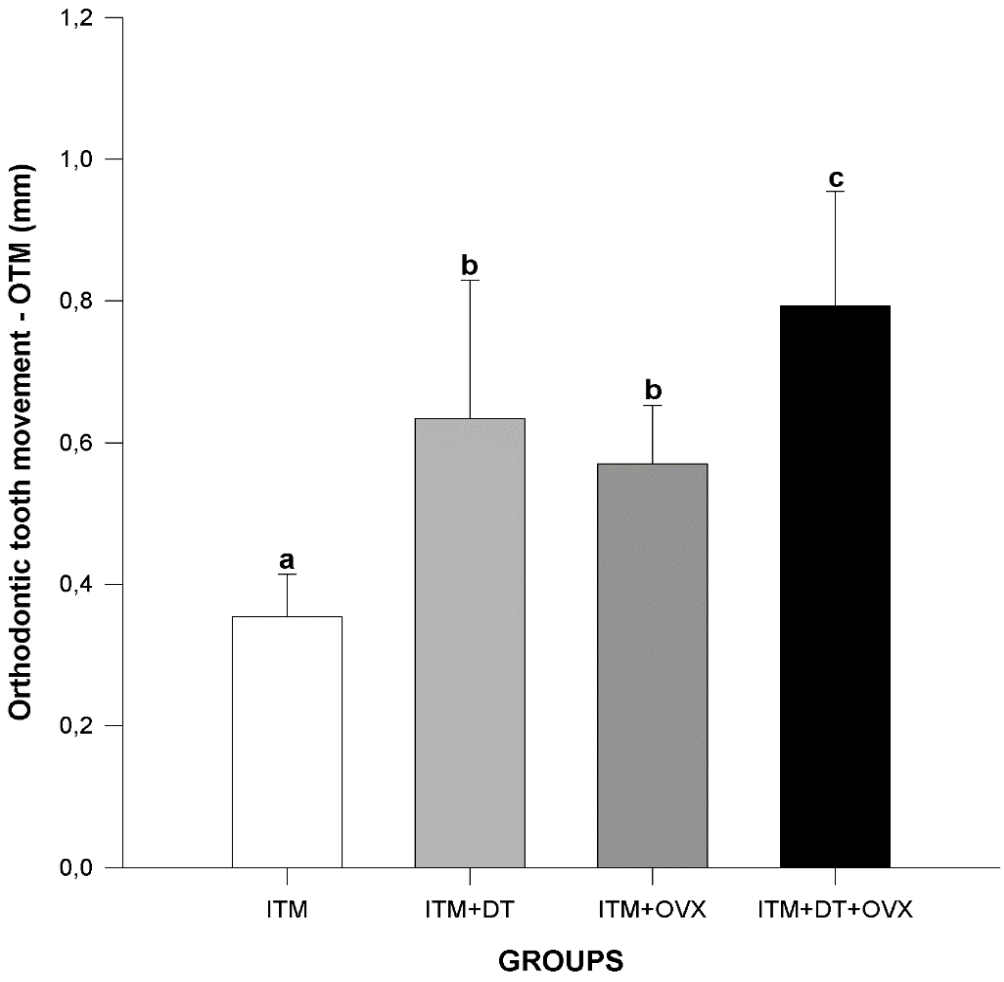

Fig. 2. Rate of orthodontic tooth movement (OTM) in the different experimental groups undergoing induced tooth movement (ITM). Values expressed as mean ± SD. N=6 animals/group. Analysis of variance (One-Way ANOVA) followed by Tukey's test. Different letters [a, b, c] indicate significant differences between groups (*P* =.05)

3.3 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 showed 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 root resorption areas when compared to all experimental groups (*P* =.05) (Table 2).

4. DISCUSSION

"Thus, the 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 postmenopausale period" [19,20,21].

"In the present study, animals in the ovariectomized groups (ITM+OVX and ITM+DT+OVX) showed greater tooth movement than those in 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].

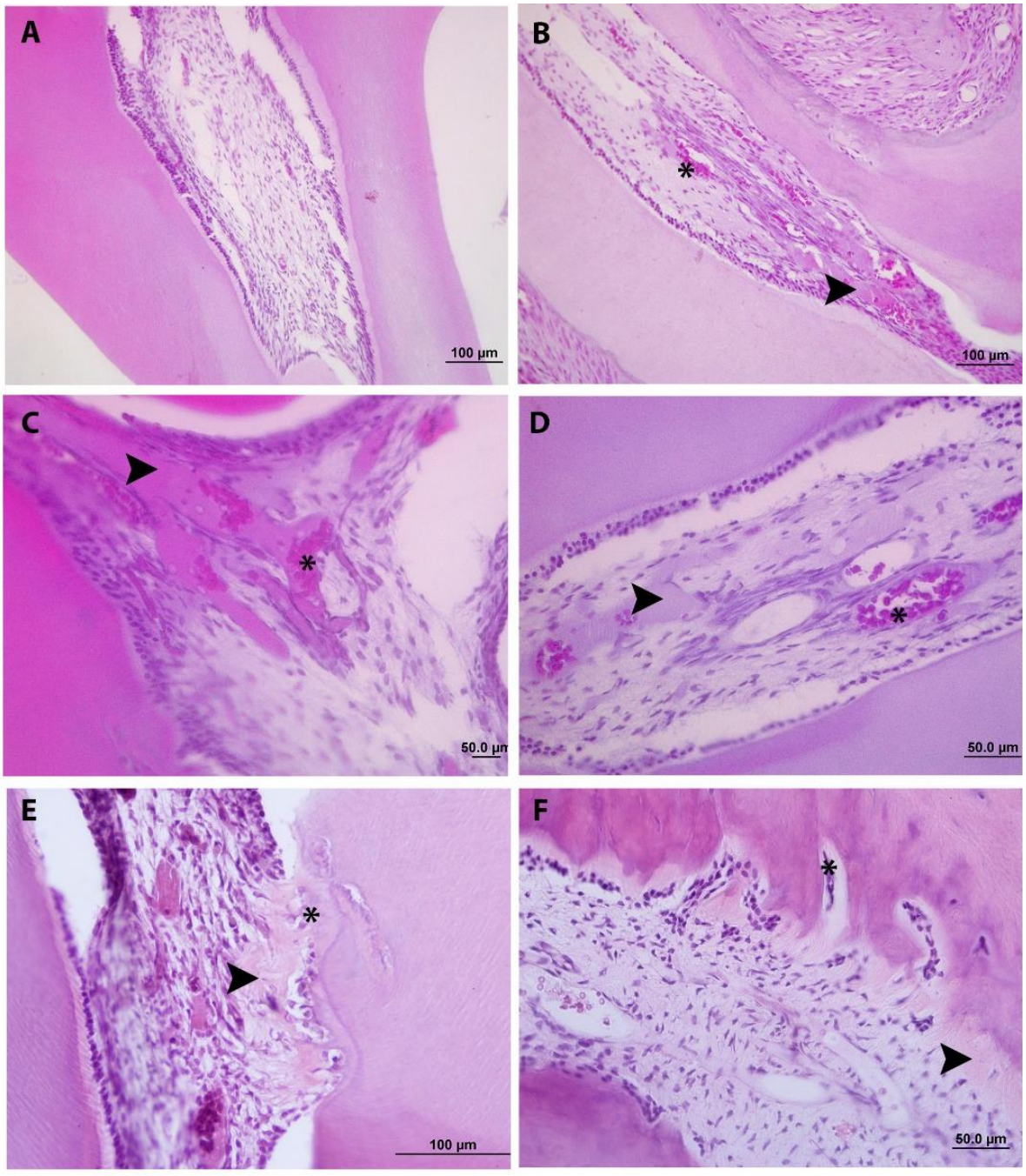

Fig. 3. Photomicrograph of the dental pulp of animals from different experimental groups. A. Dental pulp of the first upper right molar showing a normal aspect; B- D. Dental pulp with areas of congested vessels (arrowhead) and thrombosis (asterisk); E and F. Presence of hyaline areas (arrowhead) and 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

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 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	667.43 ± 8.37^{b}	2891.19 ± 794.92^{c}	0.00 ± 0.00^{a}	8564.74 ± 170.83^{d}	780.36 ± 22.46^{be}

Values expressed as mean ± 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)

“Another important aspect of the increased rate of tooth movement in ovariectomized rats is related to the differential expression of osteoprotegerin (OPG) and 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” [21,26,27]. 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 impact the onset and perpetuation of apical root remodeling 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].

“The release of neuropeptides, such as 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 the degenerative calcification of dental pulp and can cause the obliteration of root pulp” [18]. Nevertheless, changes in dentin, such as the deposition of reactionary dentin and 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. [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, hemodynamic changes were observed in all groups, but hemorrhage was not observed. Mild forces can cause a small release of CGRP, leading to initial vascular congestion. Nevertheless, the release of angiogenic factors can compensate for vascular congestion, 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. Additionally, 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 exacerbated inflammation that results in damage to the structural components of pulp tissue with the transformation of intra- and extracellular proteins into homogeneous, vitreous and pinkish material and is considered 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]. The internal dental surface 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 to eliminate 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 area of internal root resorption when compared to all experimental groups. Thus, it can be affirmed that ovariectomy affects orthodontically induced root resorption, as well as tooth movement through the hormonal system, by altering bone metabolism through various biomarkers and biological pathways" [3].

Notably, women are more likely to seek orthodontic treatment. Therefore, understanding systemic factors and their influence on the result of the orthodontic treatment offered and understanding long-term posttreatment dental stability are fundamentally important to provide personalized service for this population [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. These findings are important for guiding personalized orthodontic for women experiencing hormonal imbalances and dental trauma.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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