Histological analysis of the pulp tissue of rats with testosterone deficiency submitted to orthodontic tooth movement induced

Análise histológica do tecido pulpar de ratos com deficiência de testosterona submetidos à movimentação dentária ortodôntica induzida

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ABSTRACT

Considering the increased demand for orthodontic treatments in adult and elderly patients, with possible hormonal changes caused by age, this study carried out a histological analysis of pulp alterations in the molars of testosterone-deficient rats submitted to induced tooth movement (ITM), aiming at a better understanding of the pulp's biological aspects. Thirty-two Wistar rats were used in four experimental groups, with 8 animals in each: G1 –control group animals, without experimental manipulation; G2 – animals submitted to bilateral orchidectomy; G3 – animals submitted to ITM technique; G4 –

castrated animals submitted to ITM. We used a nickel-titanium (NiTi) spring to induce tooth movement, exerting a force of 50cN magnitude on the first upper molar. Seven days after the installation of the ITM device, the animals were euthanized, the jaws were dissected and stored in 10% formaldehyde for 24 hours and processed with routine histological techniques. We evaluated the efficiency of the force and verified tooth movement by measuring the distance between the first and second molars, at the cementoenamel junction level. The groups were histologically evaluated in terms of cellularity pattern, presence of dystrophic and hemodynamic alterations in the dental pulp. The ITM device succeeded in inducing tooth movement in the animals of groups G3 and G4, which differed from each other, with a higher movement rate in the group submitted to castration associated with ITM (G4). In the comparative analysis of groups, the animals' pulp in groups G1 and G2 (not submitted to ITM) presented histological characteristics typical of the pulp tissue. We found no dystrophic changes such as necrosis, fibrosis, nodules, or calcifications in the pulp of experimental animals that could be attributed to ITM. The animals with orthodontic movement, castrated or not, presented dilated and congested vessels, in addition to hyalinized vessels in the root and coronary pulp. However, we observed no histological changes that could be attributed to testosterone deficiency. Thus, in this experimental model, testosterone deficiency did not cause morphological and degenerative changes in the pulp during tooth movement. The vascular alterations observed in the animals resulted from the orthodontic process.

Keywords: Orthodontic movement, orchidectomy, dental pulp, rats.

RESUMO

Considerando o aumento da procura de tratamentos ortodônticos em pacientes adultos e idosos, com possíveis alterações hormonais causadas pela idade, este estudo realizou uma análise histológica das alterações da polpa nos molares de ratos com deficiência de testosterona submetidos ao movimento dentário induzido (ITM), visando uma melhor compreensão dos aspectos biológicos da polpa. Trinta e dois ratos Wistar foram utilizados em quatro grupos experimentais, com 8 animais em cada um: G1 - animais do grupo de controlo, sem manipulação experimental; G2 - animais submetidos a orquidectomia bilateral; G3 - animais submetidos à técnica ITM; G4 - animais castrados submetidos à técnica ITM. Utilizámos uma mola de níquel-titânio (NiTi) para induzir o movimento dentário, exercendo uma força de 50cN de magnitude sobre o primeiro molar superior. Sete dias após a instalação do dispositivo ITM, os animais foram eutanizados, as mandíbulas foram dissecadas e armazenadas em formaldeído a 10% durante 24 horas e processadas com técnicas histológicas de rotina. Avaliámos a eficiência da força e verificámos o movimento dentário medindo a distância entre o primeiro e o segundo molares, ao nível da junção cemento-esmalte. Os grupos foram avaliados histologicamente em termos de padrão de celularidade, presença de alterações distróficas e hemodinâmicas na polpa dentária. O dispositivo ITM conseguiu induzir movimento dentário nos animais dos grupos G3 e G4, que diferiram entre si, com uma maior taxa de movimento no grupo submetido à castração associada ao ITM (G4). Na análise comparativa dos grupos, a polpa dos animais dos grupos G1 e G2 (não submetida a ITM) apresentou características histológicas típicas do tecido da polpa. Não encontramos alterações distróficas tais como necrose, fibrose, nódulos, ou calcificações na polpa de animais experimentais que pudessem ser atribuídas à ITM. Os animais com movimento ortodôntico, castrados ou não, apresentavam vasos dilatados e congestionados, para além de vasos hialinizados na raiz e na polpa coronária. No entanto, não observámos alterações histológicas que pudessem ser atribuídas à deficiência de testosterona. Assim, neste

modelo experimental, a deficiência de testosterona não causou alterações morfológicas e degenerativas na polpa durante a movimentação dentária. As alterações vasculares observadas nos animais resultaram do processo ortodôntico.

Palavras-chave: Movimento ortodôntico, orquidectomia, polpa dentária, ratos.

1 INTRODUCTION

Testosterone is an important regulator of bone metabolism, and some studies suggest that this hormone also affects pulp cells (Haouri *et al.*, 2016; Inaba *et al.*, 2013; Dale *et al.*, 2002). Dale *et al*. (2002) points out that pulp cells would be as responsive to androgens as osteoblasts in bone tissue, regulating dentinogenesis during development and/or in response to injury. According to these authors, the decrease in testosterone concentrations that occurs with age can alter androgenic regulation in the pulp and pulp viability.

Testosterone deficiency (or hypogonadism) is an age-related physiological change and is associated with decrease in mineral bone density, deterioration of trabecular bone microarchitecture, and increased risk of fracture. Men reach peak bone mass at the age of 35 when a slow process of bone loss begins due to an imbalance between bone resorption/formation (Armada *et al.*, 2018). Some authors associate the deficiency of sex hormones during orthodontic movement to an increase in alveolar bone loss and a higher risk for root resorption (Zhou *et al.*, 2018; Iglesias-Linares *et al.*, 2016). However, studies on pulp alterations in organisms with testosterone deficiency are still scarce.

With the increase in life expectancy, more and more elderly patients have undergone orthodontic treatment. During orthodontic tooth movement, tissue changes may occur in the pulp: rupture of the odontoblast layer; changes in blood flow and/or pulp vascular pressure; hypoxia; calcifications; vacuolization; hemorrhage; fibrosis and even necrosis (Von Böhn *et al.*, 2016; Han *et al.*, 2013; Abi-Ramia *et al.*, 2010; Ramazanzadeh *et al.*, 2009; Sano *et al.*, 2002). The alteration may be reversible or irreversible depending on the type, magnitude, and duration of the applied force, as well as the physiological degree of tolerance of the dental pulp (Cuoghi *et al.*, 2018), impairing tooth vitality.

Testosterone deficiency due to bilateral orchidectomy in rodents alters bone microarchitecture, causing degenerative changes similar to osteoporosis in humans. Several studies have used this model to evaluate the impact of hypogonadism on periodontal tissues and pulp tooth complex during orthodontic movement. Thus, our

objective was to carry out a histological analysis of the molars dental pulp of castrated rats submitted to induced tooth movement.

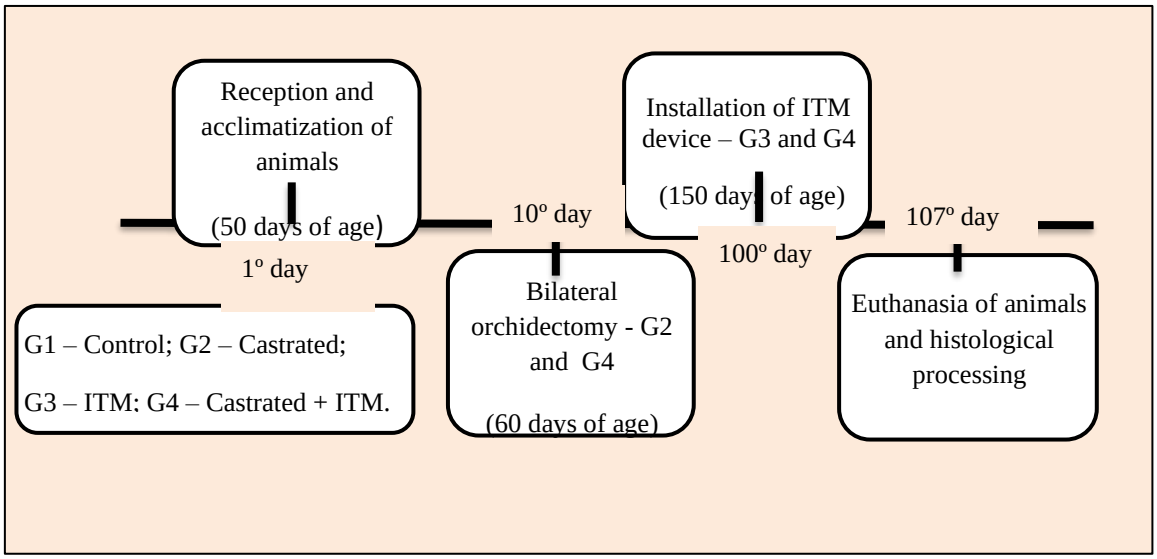
2 MATERIALS AND METHODS

2.1 ANIMALS

32 male Wistar rats (50 days of age; weighing approximately 230g) were acquired from the Central Bioterium of the Western Paraná State University. The animals were kept in the Sectorial Bioterium of the Physiology Laboratory, Center for Biological and Health Sciences (CCBS – *Centro de Ciências Biológicas e da Saúde*), UNIOESTE, Cascavel-PR, in collective polyethylene cages (43×30×15), with three animals in each, under controlled conditions of temperature (22° and 25 °C), relative humidity of approximately 55% and photoperiod of 12 hours (light period from 7 a.m.to 7 p.m.). The animals received food and water *ad libitum*. The experimental procedures were approved by the Ethics Committee on the Use of Animals (CEUA – *Comitê de Ética no Uso de Animais*) of UNIOESTE.

2.2 EXPERIMENTAL GROUPS

The animals were randomly divided into 4 experimental groups, with 8 animals in each: Group 1 – control (CON), without experimental manipulation; Group 2 (CAST) – animals submitted to bilateral orchidectomy; Group 3 (ITM) – animals submitted to induced tooth movement (ITM); Group 4 (CAST+ITM) – animals submitted to ITM and bilateral orchidectomy. Surgical and experimental procedures: Figure 1 shows the chronological order of this research's procedures.



2.3 BILATERAL ORCHIDECTOMY

At 60 days of age, groups G2 and G4 underwent bilateral orchidectomy. All animals were left fasting for a period of 12 hours, starting the night before the procedure. The next morning, five minutes before the surgery, a single dose of antibiotic prophylaxis was administered with intramuscular ceftriaxone (50mg/kg) (EMS, Brazil) and analgesia with subcutaneous sodium dipyrone (50mg/kg) (EMS, Brazil). Surgical and experimental procedures were carried out under general anesthesia, by applying ketamine hydrochloride (DOPALEN, Sespo Indústria e Comércio, Paulínia-SP), a 75 mg/kg dosage, and Xylazine Hydrochloride (muscle relaxant) (ANASEDAN, Sespo Indústria e Comércio, Paulínia-SP), a 15mg/Kg dosage, both intraperitoneally. After anesthesia, the animals were positioned in a surgical plane in supine position on an operating table and the testicular pouch was opened in the midline with an incision of 2 cm and dissected until the exposure of the testicles. The testicles were removed after previous ligation of the spermatic cord with cotton thread. In the non-castrated groups (G1 and G3), the testicles were exposed, manipulated, and reinserted into the testicular pouch, under the same experimental conditions of the castrated animals. All groups had the testicular pouch sutured with simple stitches of 4-0 nylon thread.

2.4 INSTALLATION OF THE INDUCED TOOTH MOVEMENT (ITM) DEVICE

90 days after castration, the orthodontic device for induced tooth movement was installed in groups G3 and G4 (Figure 2). The device used in this study was similar to the one proposed by Heller & Nanda (1979), and the total period of ITM was 7 days, similar to other studies (Consolaro, 2005; Massaro *et al.*, 2009). This modified device consisted of a closed-section nickel-titanium spring (Morelli, Sorocaba, SP, Brazil), 7mm long and with a release force of 50 cN magnitude. The magnitude of the spring force was previously verified with a dynamometer (Zeusan Exporting Ltda Campinas, São Paulo, Brazil). In addition, two segments of tie wire, 0.25 mm thick (Morelli, Sorocaba, SP, Brazil), were connected at each end of the spring: one bypassing the animal's first right upper molar, and the other the right upper central incisor. To stabilize the wire, a chamfer was made on the vestibular face of the incisor in the cervical region, and fluid light-curing composite resin locked the wire (Oppalis FGM) to avoid displacement. Rodent incisors are monoradicular and present continuous risogenesis during life to compensate for the constant wear caused by their gnaw function. Its long root and exuberant bone

implantation base allow it to be used as anchorage to move the first molar (Consolaro, 2005).

2.5 BIOLOGICAL MATERIAL COLLECTION AND HISTOLOGICAL PROCESSING

At the end of the experimental period (seven days after the installation of the ITM device) all animals were weighed, received a high dose of anesthetic, and were subsequently sacrificed in the guillotine. The jaws were removed and stored in 10% buffered formaldehyde for 24 hours, washed under running water for 48 hours, and then decalcified in a descaling acid solution (Allkimia®) for 24 hours. After decalcification, the pieces were washed under running water for 15 minutes, dehydrated in an increasing series of alcohols, diaphanized in xylol and embedded in Paraplast® (Sigma Aldrich). Serial cuts were made in the longitudinal plane of the mesio- and distal-vestibular roots of the right upper first molar, from mesial to distal, 5µm thick, using a manual rotary microtome (Olympus 4060), equipped with a steel razor. The sections obtained were deparaffinized with xylol, hydrated with distilled water and submitted to the hematoxylin-eosin (HE) staining technique (Ren et al., 2004).

2.6 QUANTITATIVE ANALYSIS OF ITM

The dental movement rate was obtained by measuring the distance between the first upper molar (mesial face) and the second (distal face), at the cementoenamel junction level, using 10× lens. The measurement average was calculated in 3 histological sections per animal/group.

2.7 MICROSCOPIC ANALYSIS

Three sections of each animal were selected, in which at least one complete root was present, including the pulp chamber and the apical foramen. The slides were observed under a common light microscope (Carl Zeiss Microscopy GmbH, Axio Lab, Jena, Germany), with magnification power of 200 and 400 times, evaluating the occurrence of the following pathological changes: presence or absence of inflammatory infiltrate, reduced cellularity, increased fibrosis, pulp hyalinization, pulp nodules, diffuse calcification, vascular congestion, hemorrhage, thrombosis, reactionary dentin, tubules with nuclei and necrosis. Both the coronary and root pulp of the animals' first upper molar

were analyzed. A single observer analyzed the slides, and they were coded so that the evaluator did not know to which group the histological material belonged.

2.8 DATA ANALYSIS

The data were evaluated qualitatively by the presence or absence of pathological and vascular alterations; quantitatively by the percentage of slides with or without alterations, using descriptive statistics and the nonparametric tests of Kruskal-Wallis and Dunn. The weight comparison between the groups was performed through the variance analysis of a classification criterion and Tukey's post-hoc test. In all analyses, a significance level of 5% was considered, and statistical analyses were performed with Instat (version 3.0; GraphPad, Inc., San Diego, CA, USA).

2 RESULTS

2.1 ANALYSIS OF TOOTH MOVEMENT

No movement was observed in groups G1 and G2. There was a significant increase in interdental distance in the animals of groups G3 and G4. Comparing the groups submitted to ITM, the movement rate was higher in G4 than G3 (table 1).

Table 1. Evaluation of tooth movement (µm) in animals from different experimental groups.

	G1	G2	G3	G4
Tooth movement (µm)	0,00±0,00 [a]	0,00±0,00 [a]	357,25±31,25 [b**]	403,00±29,50 [b**;c*]

G1: control; G2: castrated; G3: ITM; G4: castrated + ITM. Different letters indicate statistically significant differences between groups. ** p <0.001, when comparing the groups; * p <0.05, compared to G3. Tukey-Kramer test.

2.2 HISTOLOGICAL ANALYSIS OF THE PULP TISSUE

In all groups evaluated, we observed no significant changes in the pulp morphology – both in the coronary and root pulp. All groups presented morphological uniformity in the dental pulp. Some of the odontoblastic layers presented rupture points in its continuity, associated with technical artifacts. The cellularity pattern and organization of the extracellular matrix remained unchanged, with absence of inflammatory infiltrate in all groups. We also found no dystrophic alterations (necrosis, diffuse calcification, and pulp nodules) or changes in dentin (presence of tubules with cellular nuclei and reactionary dentin formation) in the experimental groups. All the

animals submitted to ITM (G3 and G4) presented vascular alterations (vascular congestion, increase in vessel diameter, and vascular hyalinization) in both the coronary and root pulp (Figures 2 and 3). Table 2 shows the frequency of the histological findings.

Table 2. Percentage of animals in the experimental groups with the presence of pathological changes, observed microscopically.

PULP CHANGES	G1	G2	G3	G4
Inflammatory infiltrate	0%	0%	0%	0%
Decreased cellularity	0%	0%	0%	0%
Fibrosis	0%	0%	0%	0%
Edema	0%	0%	0%	0%
Pulp nodules	0%	0%	0%	0%
Calcification	0%	0%	0%	0%
Necrosis	0%	0%	0%	0%
Cell vacuolization	0%	0%	0%	0%
Dentin reactionary	0%	0%	0%	0%
Vascular congestion	0%	0%	100%	100%
Vascular hyalinization	0%	0%	100%	100%
Hemorrhage	0%	0%	0%	0%
Thrombosis	0%	0%	0%	0%

Data expressed as a percentage. G1: control; G2: castrated; G3: ITM; G4: castrated + ITM.

Figure 2. Photomicrograph of the coronary pulp of the experimental animals: G1 (A and B); G2 (C and D), G3 (E and F) and G4 (G and H). p: dental pulp; d: dentin. Arrow: indicates the layer of odontoblasts. Arrowhead: vascular congestion. Dashed arrow: blood vessel hyalinization.

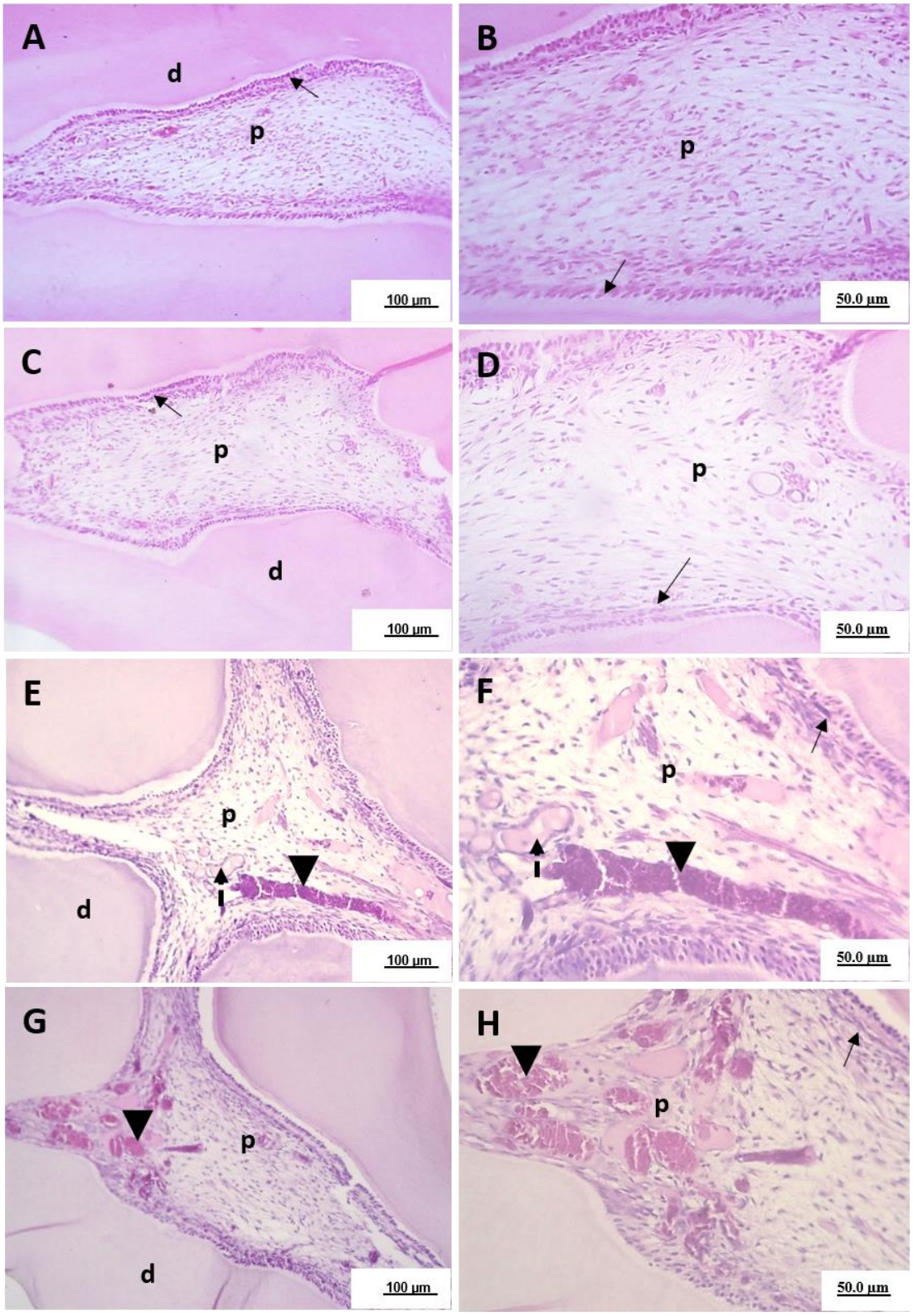
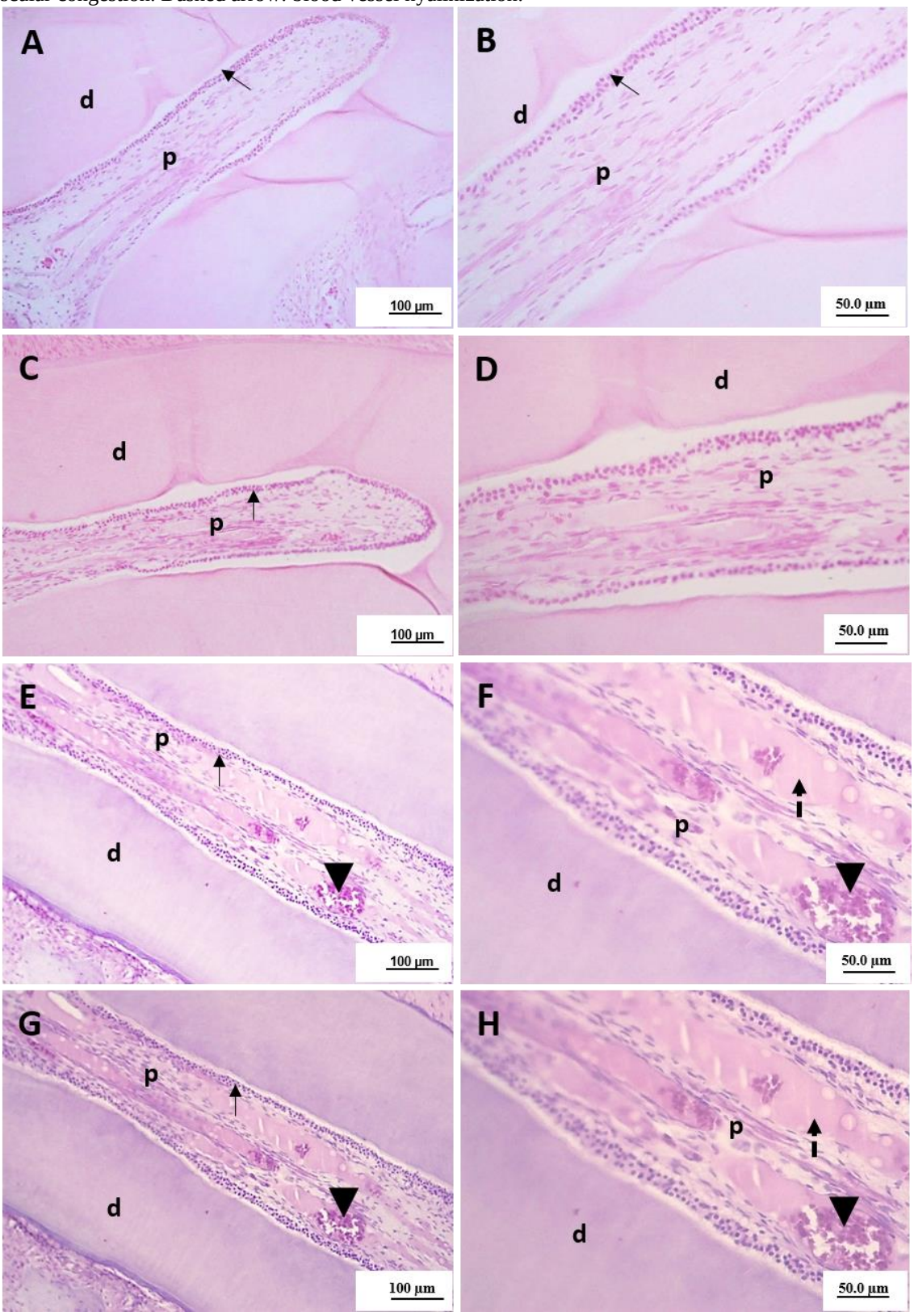


Figure 3. Photomicrograph of the root pulp of the experimental animals: G1 (A and B); G2 (C and D), G3 (E and F) and G4 (G and H). p: dental pulp; d: dentin. Arrow: indicates the layer of odontoblasts. Arrowhead: vascular congestion. Dashed arrow: blood vessel hyalinization.



3 DISCUSSION

Corroborating other experimental studies (Zhou *et al.*, 2018; Gonzales *et al.*, 2008), the device used in our study (50cN for 7 days) succeeded in inducing tooth movement in the animals. The rate of tooth movement was higher in the castrated animals (G4), when compared with the non-castrated ones (G3).

Orthodontic tooth movement (TM) is a complex process that depends on alveolar bone remodeling during strength application and consists basically of bone tissue formation in the area of bone tension, and resorption in the pressure area (El-Bialy *et al.*, 2020; Dai *et al.*, 2017; Seifi *et al.*, 2015). The stability and safety of TM are based on the balance between catabolic bone resorption and anabolic bone formation (Dai *et al.*, 2017). Bone remodeling is a physiological process that maintains normal skeletal structure and is also a key factor in orthodontic tooth movement (Singh *et al.*, 2018). In experimental animals, bilateral orchidectomy may significantly decrease the volume, density and number of bone trabeculae (Zhou *et al.*, 2018). Orchidectomy in rats induces osteoporotic effects that are related to a decrease in the expression of osteogenic genes, causing alterations in the tissue's microarchitecture. The change in bone structure and quality – caused by orchidectomy and the consequent decrease in testosterone concentrations – creates an imbalance in the bone remodeling process, through the combination of decrease in bone formation and preserved activity of bone resorption (Chin *et al.*, 2014). During orthodontic movement, this change in bone remodeling (inflicted by the deficiency of sex hormones) increases the response of the alveolar bone to the tension and compression forces, accelerating dental movements (Arslan *et al.*, 2007).

Several studies evaluated the pulp tissue's reactions in response to orthodontic forces in humans (Javed *et al.*, 2015; Jena *et al.*, 2018; Lazaretti *et al.*, 2013; Han *et al.*, 2013) and in experimental animals (Cuogui *et al.*, 2018; Von Böhl *et al.*, 2016; Massaro *et al.*, 2009; Grünheid *et al.*, 2007); while some studies relate the occurrence of calcification, nodules, fibrosis, necrosis, loss of tooth movement vitality (Jena *et al.*, 2018; Bernard-Granger and Gebeile-Chauty 2015; Lazaretti *et al.*, 2013), others suggest that tooth movement alone does not induce degenerative changes in the pulp (Consolaro and Consolaro, 2018; Massaro *et al.*, 2009). This divergence in the literature is due partially to the methodological differences used, mostly regarding intensity and type of the force applied on the tooth, type of induced movement, age and species used, time of movement, presence of previous traumatic factors during or after orthodontic movement,

the occurrence of local or systemic changes, all of which impairs the comparison between them.

In the experimental model used in our study, we observed no dystrophic changes such as necrosis, fibrosis, nodules, or calcifications in the pulp of the experimental animals. The control and castrated animals without ITM presented pulp tissue with normal histological aspects. Animals submitted to tooth movement, castrated or not, presented dilated and congested vessels, in addition to hyalinized vessels in the root and coronary pulp. However, no histological changes could be attributed to testosterone deficiency.

Vascular hyalinization is characterized by the presence of homogeneous, eosinophilic material of light pink color, which indicates an accumulation of plasma proteins within blood vessels. Several studies have demonstrated vascular changes in the dental pulp – such as vascular congestion and increased angiogenesis – in response to orthodontic forces (Cuogui *et al.,* 2018; Santamaria *et al.,* 2007; Derringer *et al.,* 1996). The increase in the number of blood vessels and vascular congestion observed in experimental studies is a reversible phenomenon during the first three to seven days after the force application (Abi-Ramia *et al.,* 2010; Nixon *et al.,* 1993; Shigehara, Matsuzaka & Inoue, 2006) – the pulp restores to its normal pattern even in cases with root resorption (Tripuwabhrut *et al.*, 2010).

Unlike our results, some studies reported a decrease in pulp blood flow in response to orthodontic movement (Hamersky *et al.*, 1980; Anstendig and Kronman, 1972). This contradiction suggests us that the type of force, type of induced dental movement, and age, may affect the pulp tissue differently, increasing or decreasing blood flow in the tissue. In addition, age is an important factor since decreased blood supply to pulp cells commonly occurs with aging and may alter the pulp response to orthodontic tooth movement (Ersahan and Sabuncuoglu, 2018).

The process of tissue remodeling occurs around the tooth during appositional and resorptive processes (with orthodontic movement) and requires a high cellular energy demand. Thus, it is biologically acceptable that blood flow in the pulp may increase to supply the metabolic demand of cells in the tissue (Nixon *et al.*, 1993). According to Massaro *et al.* (2009), changes in the metabolic rates of pulp tissue (evidenced by an increase or decrease in blood flow during tooth movement in various studies) are unrelated to histological changes, loss of viability, or vitality of pulp tissue.

4 CONCLUSION

In the experimental model used, testosterone deficiency did not cause morphological and degenerative changes to the pulp during tooth movement. The vascular alterations observed in the animals result from the orthodontic process and are reversible.

REFERENCES

Abi-Ramia LBP, Stuani AS, Stuani MBS, Mendes AM. Efeitos da Laserterapia de Baixa Intensidade e do Movimento Ortodôntico do Dente nas Polpas Dentárias em Ratos. The Angle Orthodontist, p. 116-122, 2010.

Anstendig HS, Kronman JH. A histological study of pulpal reaction to orthodontic tooth movement in dogs. Angle Orthod, p. 50-55, 1972.

Armada L, de Castro Brasil S, Armada-Dias L, Bezerra J, Pereira RMR, Takayama L, Moreira Morais Dos Santos R, Gonçalves LS, Nascimento-Saba CCA. Effects of aging, gender, and hypogonadism on mandibular bone density. J Investig Clin Dent, p.123-130, 2018.

Arslan, S.G., Arslan, H., Ketani, A., Hamamci, O. Effects of estrogen deficiency on tooth movement after force application: an experimental study in ovariectomized rats. Acta Odontol Scand, p. 319-323, 2007.

Bernard-Granger C, Gebeile-Chauty. Perte de vitalité: influence des procédures orthodontiques. Orthod Fr, p. 161–167, 2015.

Chin, K.Y., Ima-Nirwana, S. The effects of orchidectomy and supraphysiological testosterone administration on trabecular bone structure and gene expression in rats. Aging Male, p. 60-66, 2014.

Consolaro A, Consolaro RB. There is no pulp necrosis or calcific metamorphosis of pulp induced by orthodontic treatment: biological basis. Dental Press Journal Of Orthodontics,, p. 36-42, 2018.

Cuoghi OA, Faria LP, Ervolino E, Barioni SRP, Topolski F, Arana-Chavez VE, Mendonça MR. Pulp analysis of teeth submitted to different types of forces: a histological study in rats. Journal of Applied Oral Science, p. 1102-1116, 2018.

Dai Q, Zhou S, Zhang PMA, Ha N, Yang X, Yu Z, Fang B, Jiang L. Force-induced increased osteogenesis enables accelerated orthodontic tooth movement in ovariectomized rats. Scientific Reports, p. 1-18, 2017.

Dale JB, Sarich SL, Bretz TM, Hatton JF, Zachow RJ. Hormonal Regulation of Androgen Receptor Messenger Ribonucleic Acid Expression in Human Tooth Pulp. Journal Of Dental Research, p. 360-365, 2002.

Derringer, K.A., Jaggers, D.C., Linden R.W. Angiogenesis in human dental pulp following orthodontic tooth movement. J Dent Res, p. 1761-1766, 1996.

El-Bialy T, Farouk K, Carlyle TD, Wiltshire W, Drummond R, Dumore T, Knowlton K, Tompson B. Effect of Low Intensity Pulsed Ultrasound (LIPUS) on Tooth Movement and Root Resorption: A Prospective Multi-Center Randomized Controlled Trial. J Clin Med, p. 1-14, 2020.

Ersahan, S., Sabuncuogl, F.A. Effect of age on pulpal blood flow in human teeth during orthodontic movement. J Oral Sci, p.446-452, 2018.

Gonzales. C., Hotokezaka H, Yoshimatsu M, Yozgatian JH, Darendeliler MA, Yoshida N. Force magnitude and duration effects on amount of tooth movement and root resorption in the rat molar. Angle Orthod, p. 502-509, 2008.

Grünheid, T., Morbach, B.A., Zenter, A. Pulpal cellular reactions to experimental tooth movement in rats, p. 434-441, Oral Surg, 2007.

Hamersky PA, Weimer AD, Taintor JF. The effect of orthodontic force application on the pulpal tissue respiration rate in the human premolar. Am J Orthod Dentofac Orthop, p.368-378, 1980.

Han G, Hu M, Zhang Y, Jiang H. Pulp vitality and histologic changes in human dental pulp after the application of moderate and severe intrusive orthodontic forces. American Journal Of Orthodontics And Dentofacial Orthopedics, p. 518-522, 2013.

Houari S, Loiodice S, Jedeon K, Berdal A, Babajko S. Expression of Steroid Receptors in Ameloblasts during Amelogenesis in Rat Incisors. Frontiers In Physiology, p. 327-345, 2016.

Iglesias-Linares, A., Morford, L.A., Hartsfield, J.K. Bone Density and Dental External Apical Root Resorption. Curr Osteoporos Rep, p. 292–309, 2016.

Inaba T, Kobayashi T, Tsutsui TW, Ogawa M, Uchida M, Tsutsui T. Expression status of mRNA for sex hormone receptors in human dental pulp cells and the response to sex hormones in the cells. Archives of Oral Biology, p. 943-950, 2013.

Javed F, Al-Kheraif AA, Romanos EB, Romanos GE. Influence of orthodontic forces on human dental pulp: a systematic review. Arch Oral Biol. p. 347-56, 2015.

Jena D, Balakrishna K, Singh S, Naqvi ZA, Lanje A, Arora N. A Retrospective Analysis of Pulp Stones in Patients following Orthodontic Treatment. J Contemp Dent Pract, p. 1095-1099, 2018.

Lazzaretti DN. Avaliação histológica do tecido pulpar humano pós-intrusão ortodôntica. Dissertação (Mestrado) - Curso de Odontologia, Universidade Estadual de Santa Maria, 2013.

Massaro CS, Consolaro RB, Santamaria M, Consolaro MFM, Consolaro A. Analysis of the dentin-pulp complex in teeth submitted to orthodontic movement in rats. Journal Of Applied Oral Science, p. 35-42, 2009.

Nixon, CE, Saviano JA, King GJ, Keeling SD. Histomorphometric study of dental pulp during orthodontic tooth movement. J Endod, p. 13-6, 1993.

Ramazanzadeh BA, Sahhafian A, Mohtasham N, Hassanzadeh N, Jahanbin A, Shakeri MT.Histological changes in human dental pulp following application of intrusive and extrusive orthodontic forces. Journal Of Oral Science, p. 109-115, 2009.

Sano Y. The effect of continuous intrusive force on human pulpal blood flow. The European Journal Of Orthodontics, p. 159-166, 2002.

Santamaria M. Milagres D, Iyomasa MM, Stuani MBS, Ruellas ACO. Initial pulp changes during orthodontic movement: histomorphological evaluation. Braz Dent J**,** p. 34-39, 2007.

Seifi M, Ezzati B, Saedi S, Hedayati M. The Effect of Ovariectomy and Orchiectomy on Orthodontic Tooth Movement and Root Resorption in Wistar Rats. J Dent (Shiraz), p. 302-309**,** 2015.

Singh A, Gill G, Kaur H, Amhmed M, Jakhu H. Role of osteopontin in bone remodeling and orthodontic tooth movement: a review. Prog Orthod, p. 1-8, 2018.

Shigehara S, Matsuzaka K, Inoue T. Morphohistological change and expression of HSP70, osteopontin and osteocalcin mRNAs in rat dental pulp cells with orthodontic tooth movement. The Bulletin of Tokyo Dental College, p.117–124 2006.

Tripuwabhrut P, Brudvik P, Fristad I, Rethnam S. Experimental orthodontic tooth movement and extensive root resorption: periodontal and pulpal changes. European Journal of Oral Sciences, p.596–603, 2010.

Von Bönl M, Ren Y, Kuijpers-Jagtman AM, Fudalej PS, Maltha JC. Age-related changes of dental pulp tissue after experimental tooth movement in rats. Peerj**,** p. 1625-1641, 2016.

Zhou J, Yang F, Xu X, Feng G, Chen J, Song J, Dai H. Dynamic Evaluation of Orthodontically-Induced Tooth Movement, Root Resorption, and Alveolar Bone Remodeling in Rats by in Vivo Micro-Computed Tomography. Med Sci Monit, p.8306–8314, 2018.