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Departamento de Cirurgia e Clínica Integrada

CINTIA VANESSA ALVES DO NASCIMENTO

A influência do gênero no efeito da oclusão traumática no periodonto

Araçatuba

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Trabalho de conclusão de curso apresentado à Faculdade de Odontologia de Araçatuba da Universidade Estadual Paulista "Júlio de Mesquita Filho" – UNESP, como parte dos requisitos para obtenção do título de Bacharel em Odontologia

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2018

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“Que os vossos esforços desafiem as impossibilidades, lembrai-vos de que as grandes coisas do homem foram conquistadas do que parecia impossível.”

Charles Chaplin

NASCIMENTO, C.V.A. Influência do gênero no efeito da oclusão traumática no periodonto. 2018. Trabalho de Conclusão de Curso (Bacharelado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2018.

RESUMO

Hormônios sexuais esteróides desempenham um papel importante no crescimento, maturação e remodelação do tecido ósseo, incluindo o osso alveolar. Este estudo, objetivou avaliar o fator gênero no efeito da oclusão traumática no periodonto. Para isto, ratos Wistar com 12 semanas de idade foram divididos em quatro grupos: controle masculino (CM, n = 20) e controle feminino (CF, n = 20); oclusão traumática masculino (TOM, n = 20); oclusão traumática feminino (TOF, n = 20). A OT foi induzida experimentalmente através de restaurações diretas de resina composta e fio metálico na superfície oclusal dos primeiros molares inferiores direitos. Os espécimes foram coletados aos 7 e 30 dias de pós-operatório, cortes histológicos foram corados pelos métodos HE e picrosirius, seguido de avaliação histológica em microscopias de luz e polarização e, análise estatísticas através do teste Mann Whitney, considerando $p>0.05$ como significante. O grupo TOF apresentou alterações significativas como a diminuição da área óssea aos 7 dias e 30 dias; e aumento na matriz extracelular aos 30 dias. O grupo TOM apresentou alterações significativas com diminuição da área da matriz extracelular e aumento dos perfis celulares nucleares totais no grupo de 7 dias. Além disso, foi observado aumento significante da espessura do ligamento periodontal e de fibras colágenas do tipo I; e diminuição da área de fibras colágenas tipo III e osso aos 7 e 30 dias. A oclusão traumática causou degradação óssea do osso alveolar em ratos machos e fêmeas; entretanto, alguns parâmetros foram expressivos nos animais do sexo masculino, tais como aumento da espessura do ligamento periodontal, dos perfis celulares nucleados totais e fibras colágenas do tipo I e diminuição da área de fibas colágenas do tipo III do ligamento periodontal.

Palavras Chave: Oclusão dentária traumática. Periodonto. Dimorfismo sexual.

NASCIMENTO, C.V.A. Gender influence in the effect of traumatic occlusion on the periodontium. 2018. Trabalho de Conclusão de Curso (Bacharelado) - Faculdade de Odontologia, Universidade Estadual Paulista, Araçatuba, 2018.

ABSTRACT

Steroid sex hormones are believed to play an important role in the growth, maturation and turnover of the skeleton, including the alveolar bone. This study aims to evaluate the gender factor in the effects of traumatic occlusion on the periodontium. Eighty Wistar rats aged 12 weeks were divided into four groups: a male (CM, n=20) and female (CF, n=20) Control group, and a male (TOM, n=20) and female (TOF, n=20) Traumatic Occlusion group. TO was created with a direct composite resin filling and metallic wire on the occlusion surface of the Right Mandibular First Molar. Specimens were collected at 7 and 30 postoperative days, histological sections were stained by HE and picrosirius methods, followed by histological evaluation in light or polarization microscopy, and statistical analysis using the Mann Whitney test, considering $p > 0.05$ as significant. TOF group show significative changes in the decrease of bone area at 7 days and 30 days; and increase in the extracellular matrix at 30 days. TOM group show significative changes in the decrease of extracellular matrix area and an increase of nuclear profile at 7 days. Also show, an increase of periodontal ligament thickness and collagen fibers type I; and a decrease in the area of collagen fibers type III and bone at 7 and 30 days. Traumatic occlusion caused bone degradation in female and male rats; however changes such as an increase of periodontal ligament thickness, increase of nuclear profile and collagen fibers type I; and a decrease in the area of collagen fibers type III of the periodontal ligament were expressive only in the male gender.

Keywords: Dental Occlusion, Traumatic. Periodontium. Sex Characteristics.

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PDL Periodontal Ligament

CM Control Male

CF Control Female

TOM Traumatic Occlusion Male

TOF Traumatic Occlusion Female

RMFM Right Mandibular First Molar

PBS Phosphate Buffered Saline

EDTA Ethylenediamine Tetraacetic Acid

COF Collagenous Fibres

BV Blood Vessels

AFS Amorphous Fundamental Substance

BA Bone Area

ABS Alveolar Bone Septum

PR Picrosirius Red

TA Total Area

ERβ Estrogen Receptor β

VEGF Vascular Endothelial Growth Factor

SUMÁRIO

1 INTRODUCTION

Excessive mechanical load on the tooth can cause a narrowing of the periodontal ligament space. The organism's first reaction involves remodelling of the bone to reestablish the periodontal ligament space.[1,2] In cases where bone remodelling alone isn't sufficient to handle the destruction caused by traumatic occlusion, some changes in the periodontium occur with the objective to create a structural relation where the load will not be seen as damage. This results in a larger space of the periodontal ligament (PDL), like a funnel in the crest without a bone pocket.[3, 4]

Histological studies have revealed that several morphofunctional alterations occur when excessive occlusal force is applied, such as: disorientation and a decrease in collagenous fibres,[5, 6] changes in the alignment of periodontal fibres,[7] an increase in the number of fibroblasts,[5] elevated osteoclast activity,[8,7,9,10] decreased osteoblast activity,[14,16] venous thrombosis,[16] cell necrosis in the periodontal ligament,[10] and absence of inflammatory cell infiltration.[8,11]

In the face of a normal or traumatic occlusion, the most important function of the alveolar wall around the tooth socket is to provide mechanical support to the tooth. It interacts with collagen fibres of the periodontal membrane, which transmit mechanical strain from the tooth to the alveolar bone. Therefore, unlike bone at other sites, the alveolar wall has a unique pattern of continuous remodelling that may respond to systemic as well as local factors like tooth movement and mechanical stress.[12]

Although oestrogens are thought of as female sex hormones and androgens are considered male hormones, both men and women make hormones in both groups, with different ratios depending on gender. Testosterone is important in women for muscle and bone strength, and also for maintaining a healthy sex drive. Oestrogen may play an important role in preventing heart disease in men. Younger men generally have higher levels of testosterone and lower levels of oestrogen. With ageing, oestrogen levels often increase, while testosterone levels decrease.[13]

Steroid sex hormones are believed to play an important role in the growth, maturation and turnover of the skeleton, including the alveolar bone.[14,15] Oestrogen deficiency can therefore affect alveolar bone turnover following tooth extraction[16] and

increase the number of osteoclasts.[15] In the case of low testosterone levels, some researchers have observed an increased risk of osteopenia and osteoporosis,[17] and others were unable to find a difference in the number of osteoclasts in the alveolar bone between experimental and control groups.[15] In the case of traumatic occlusion, females seem to produce a higher number of osteoclasts (20.7)[18] than males (4.1)[15] under similar experimental conditions and analyses.

Specific knowledge and systemic differences can lead to a precise approach in the treatment of occlusal trauma.

2 OBJECTIVES

This study aims to evaluate the gender factor in the effects of traumatic occlusion on the periodontium.

3 MATERIAL AND METHODS

Following approval by the Animal Care Committee of the Dentistry School of Araçatuba (UNESP), 80 Wistar rats (*Rattus Norvegicus Albinus*) aged twelve weeks was selected. They were be kept in cages with five animals each and given granulated food and water ad libitum. The environment was kept at a constant temperature of 22 °C (±2 ºC) and 50% (±10%) humidity, and light/dark cycles of 12/12 hours.

Prior to the experimental section, the animals were intramuscularly anaesthetised with a solution of ketamine hydrochloride (25 mg/kg, Vetanarcol, Laboratorios König, Argentina) and xylazine (10 mg/kg Coopazine, Coopers, Brazil).

To evaluate the gender factor in the effect of traumatic occlusion on the periodontium, the animals were divided into 4 groups: a male (CM, n=20) and female (CF, n=20) Control group, and a male (TOM, n=20) and female (TOF, n=20) Traumatic Occlusion group. The control group consists of rats with the same age as those in the experimental group, which was not submitted to any experimental conditions. In the traumatic occlusion groups excessive mechanical loads were induced by increasing the height of the right mandibular first molar (RMFM) by direct filling using 37% phosphoric acid etchant for enamel and dentin (FGM, Brazil), microbrushes (Microbrush® International, Grafton, USA), scotchbond multi-purpose light adhesive (3M ESPE, Saint Paul, USA), Estelite Σ Quick composite resin (Tokuyama Dental Corp, Japan) and photopolymerizer (Dabi Atlante, Ribeirão Preto, Brazil), following manufacturers' instructions. A flat high occlusal table was created at the highest occlusal cusp. A piece of ligature wire 0.20 mm (.008´´) (Morelli, Sorocaba, Brazil) was attached to the surface of the composite filling. Prior to the restoration, microretentions were made with Carbide burs FG ¼ (Beavers Dental, Canada) at high speed, using a handpiece with water[19].

The study periods were 7 and 30 days, and all groups were equally divided. Animals were excluded in case they die of natural causes or if they lose the occlusal composite filling.

After anaesthesia, transcardial perfusion was performed. An intraventricular injection of heparin (0.1 ml / 5,000 U.I/ml) was administered. After 1

minute 100 ml saline was perfused via the aorta, followed by a mixed solution of 300 ml paraformaldehyde fixation at 4% (Sigma Chemical Co., St. Louis, MO, USA) and 200 ml phosphate buffered saline (PBS) at 0.1M, pH 7.4, 4ºC (Sigma Chemical Co., St. Louis, MO, USA).

After dissection, the specimens were washed in PBS and kept in 4% paraformaldehyde (Sigma Chemical Co., St. Louis, MO, USA) for 24 hours before decalcification with ethylenediamine tetraacetic acid disodium (EDTA) at 10% for 20 days. The specimens were processed with progressive dehydration in ethyl alcohol, cleared with xylene, impregnated with paraffin at a low fusion temperature (56-58 ºC) for 3 hours and embedded according to standard protocols.

Of each specimen, three longitudinal histological cuts 4µm thick with a distance of 40 µm in between of the lower first molar and surrounding tissues were obtained with an automated microtome (Leica SMR 2000), transferred to a bain-marie (40-50 ºC) and then collected using slides. The selected cuts were used to perform histomorphological analysis and group comparison at the selected intervals throughout the experimental period.

3.1 Histological analyses

The cuts were observed using an Aristoplan light microscope (Leica - Aristoplan, Solms, Germany) and micrographed with an Axiocam MRc digital camera (Carl Zeiss, Oberkochen, Germany). The picrosirius red images were obtained using a polarised light microscope (data from the microscope and camera) and analyzed by QWin program (Leica QWin V3; Leica Microsystems).

Visual fields of the RMFM and surrounding tissues (Figure 1) of each animal were collected and analysed with the program Axionvision Rel 4.0 (Carl Zeiss, Oberkochen, Germany).

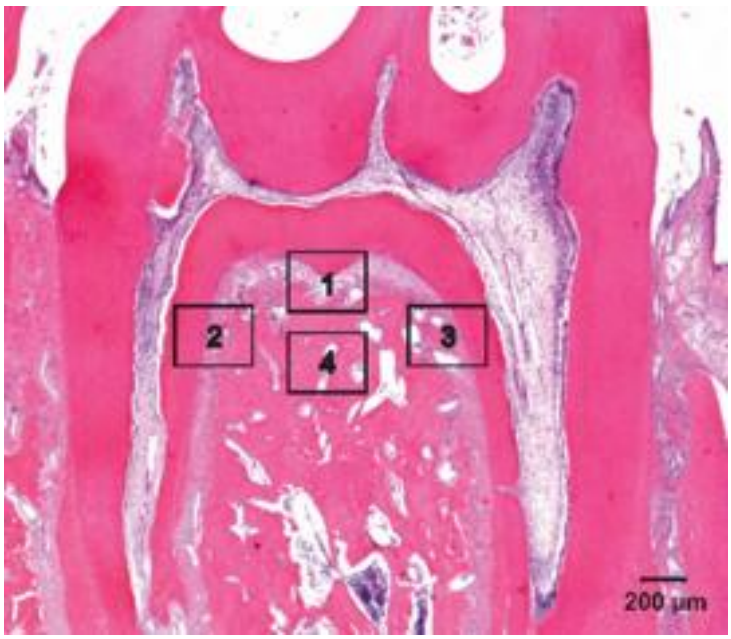
The following variables were considered for quantitative analysis: relative percentage of the area occupied by collagenous fibres (COF); blood vessels (BV); amorphous fundamental substance (AFS) of the PDL; number of nuclear profiles, insertion level of the periodontal ligament, and bone area (BA) of the central region of the alveolar bone septum (ABS).

The slides stained with hematoxylin and eosin were used for the analysis of blood vessels, amorphous fundamental substance, nuclear profiles, PDL width and bone area, also the insertion level and inflammatory process of the periodontal ligament. The slides counterstained with picrosirius red (PR) were used to analyse the organisation and/or heterogeneity of collagen fibre orientation, collagen fibre area and collagen type (Type I e III**)** of these fibres.

The images were processed by the programme Image J (National Institute of Health, USA) for measurement in a pre-determined area (mm^2) in the periodontal ligament (Figura 2A-2F) and/or alveolar bone of the furca region (Figure 3A-3C), according to the case, for quantitative and qualitative analysis.

The average width of the PDL in all RMFM roots was calculated by adding the PDL widths on the mesial, distal and upper surfaces and dividing this number by 3 (Figure 1).

Figure 1 - Regions of analysis in the RMFM submitted to histomorphometric analysis. 1-Region of furca of the PDL; 2-Cervical region PDL of the distal root; 3-Cervical region of the PDL of the mesial root; 4-Inter-radicular septum region.



Source: Author

To calculate the area occupied by blood vessels, these structures were delimited and then calculated as a percentage of the total area (TA) (Figure 2C).

For the quantitative analysis of the percentage amorphous fundamental substance (Figure 2D), an ImageJ function was used that provides these percentages by contrasting colours.

The nuclear profiles in the PDL were counted. Endothelial cells were not included. To count the cells, an ImageJ function called “Multi-point” was used, which inhibits the double counting of cells (Figure 2F).

For quantification of the bone area (BA) in the central portion of the interradicular septum of the RMFM, non-osseous regions were removed (Figura 3B) and mineralised bone and osteoid were included (Figura 3C).[20] The bone percentages were obtained by contrasting colours.

The sections counterstained with picrosirius red (PR) were used to analyse the organisation and/or heterogeneity of collagen fibre orientation, collagen fibre area and collagen type (Type I e III**)** of these fibres. The collagen fibers will be quantified by how much the coloration, being the yellowish red ones are type I collagen and the yellowish green ones, type III (Figure 4A – 4C).

The histomorphological parameters used in the present study are based on the criteria described by Panzarini et al.[21] The specimens were examined and scores from 1 to 4 were assigned to each parameter, with 1 indicating the best result and 4 the worst. The histological events analyzed at the epithelial reattachment site were the intensity and extent of inflammatory processes.

- Area of epithelial insertion

1- Cementoenamel junction
2- Ligament below the cementoenamel junction
3- Much below the cementoenamel junction (near medium third)
4- Absence of epithelial insertion

- Inflammatory process- Intensity

1- Absence or occasional presence of inflammatory cells
2- Small number of inflammatory cells. Up to 10 inflammatory cells
3- Moderate number of inflammatory cells. From 11 of 50 inflammatory cells
4- Large number of inflammatory cells. More than 50 inflammatory cells

- **Inflammatory process extension**

1- Absence or occasional presence of inflammatory cells
2- Infammatory process restrieted to the lamina propria of the internal aspect of the internal aspect of the epithelium
3- Inflammatory process extending apically toward the small portion of connective tissue
4- Inflammatory process reaching the area of the alveolar bone crest

In order to avoid bias, the examiner was not informed as to which group the assessed images belonged.

Figure 2 - Image J program to calculate the percentage area of the strutures and number of nuclear profile of the periodontal ligament. A: Original photo of periodontal ligament. B: Photo edited (bone area removed): Total area without blood vessel. C: Area of blood vessels. D: Extracellular matrix. E: Ligament thickness. F: Nuclear profile.

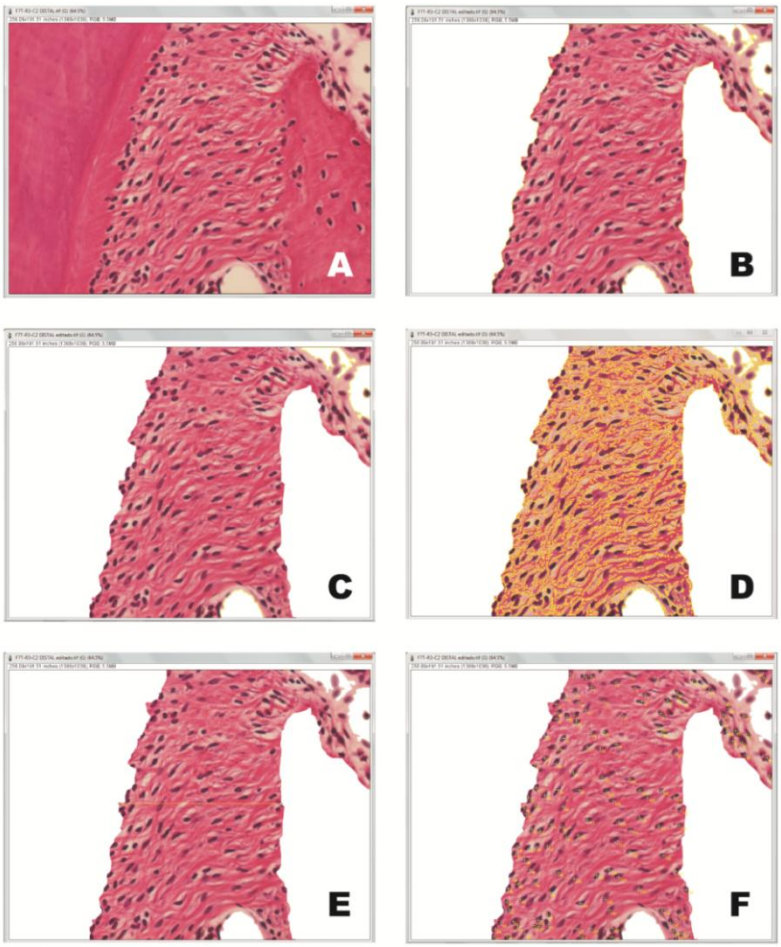


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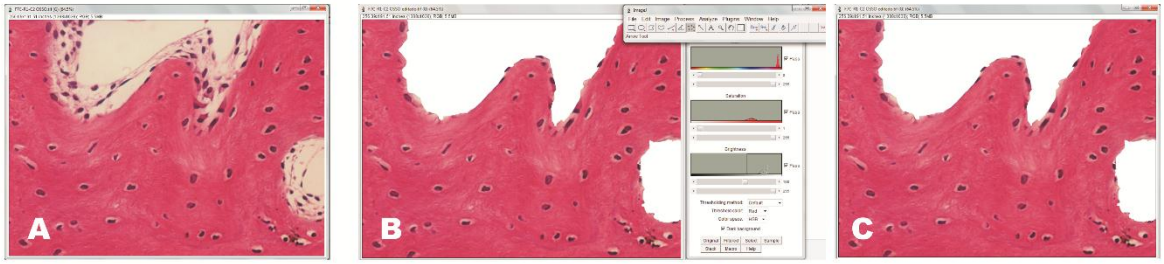
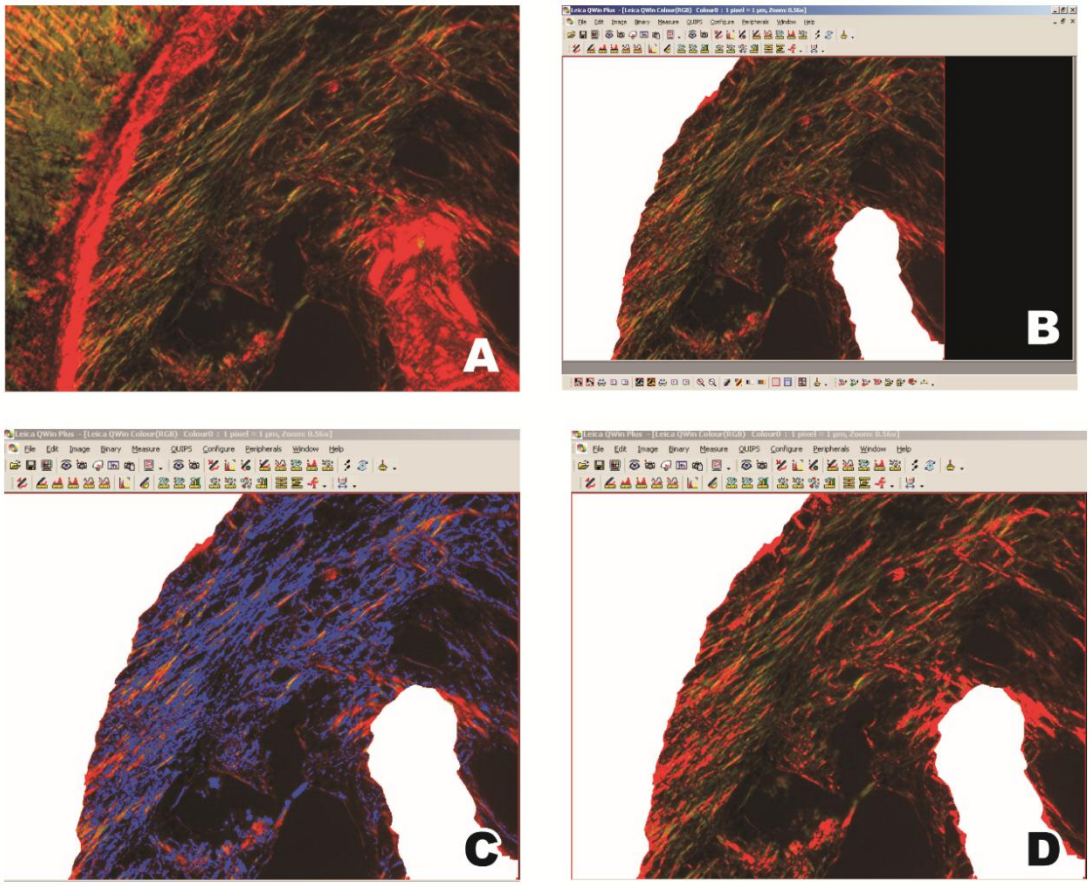
Figure 3 - Image J program to calculate the percentage of bone area. A: Original photo of bone area analysed. B: Photo edited with ligament removal and open program. C: Bone area without ligament.



Source: Author

Figure 4 - Analyze of collagen fiber area and type (Type I and III) in the cuts stained with. picrosirius red. A: Initial ligament photo, without editing. B: Photo edited (removal of bone area). C: Young fiber area. D: Area of mature fiber.



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3.2 Statistical Analysis

The data were analysed using IBM SPSS 20.0 (IBM, Armonk, NY, USA) at $\alpha=0.05$. The Mann Whitney test was used for group comparison. Data was expressed as mean ± SD.

4 RESULTS

Samples of the animals were lost due to technical difficulties in the laboratory processing, being 7 of the CF group, 2 TOF and 2 TOM, reducing the sample to 3, 8 and 8 specimens respectively. The CM group remained with 10 specimens.

Representative histological observations are shown in Figures 5, 6 and 7. These analyzes were limited to the furcation region of the alveolar bone of the lower right first molars. Table 1 and 2 present the histomorphometric events data associated with the periodontal ligament (Figure 5 and 7) and the interradicular septum bone (Figure 6) of the RMFM. Tables 3 and 4 show, respectively, the semi-quantitative data of the histomorphometric changes of the location of the epithelial insertion, and intensity and extention of the inflammatory process of the epithelial insertion region of the periodontal ligament.

4.1 Female

In the TOF groups were observed that thickness of periodontal ligament, area of blood vessel (Figure 5), collagen fibers type III and I (Figure 7) and number of nuclear profile (Figure 5) did not show significant changes in the experimental periods (7 and 30 days). However, the bone area shows a significant decrease to more than ¼ at 7 days (27.2%) and more than ⅓ at 30 days (34.9%) (Table 1), with a increase in bone loss by the time (Figure 6). The extracellular matrix, at 30 days (Figure 5), shows a significant increase (28.75%) (Table 1). The periodontal ligament presented uniform organization and absence of inflammatory process in females (CF and TOF groups) in all specimens, and it was not possible to submit the data to statistical tests (Table 3). Small and few changes in the insertion level of the periodontal ligament were observed in the TOF group at 7 and 30 days (Table 3).

4.2 Male

In the TOM groups just the area of blood vessels did not show any significant alterations at 7 and 30 days (Table 2). It was noted that thickness of periodontal ligament, area of extracellular matrix, collagen fibers type III and I, bone and number of nuclear profile showed significant changes in one or both experimental periods (7 and 30 days) (Table 2) .

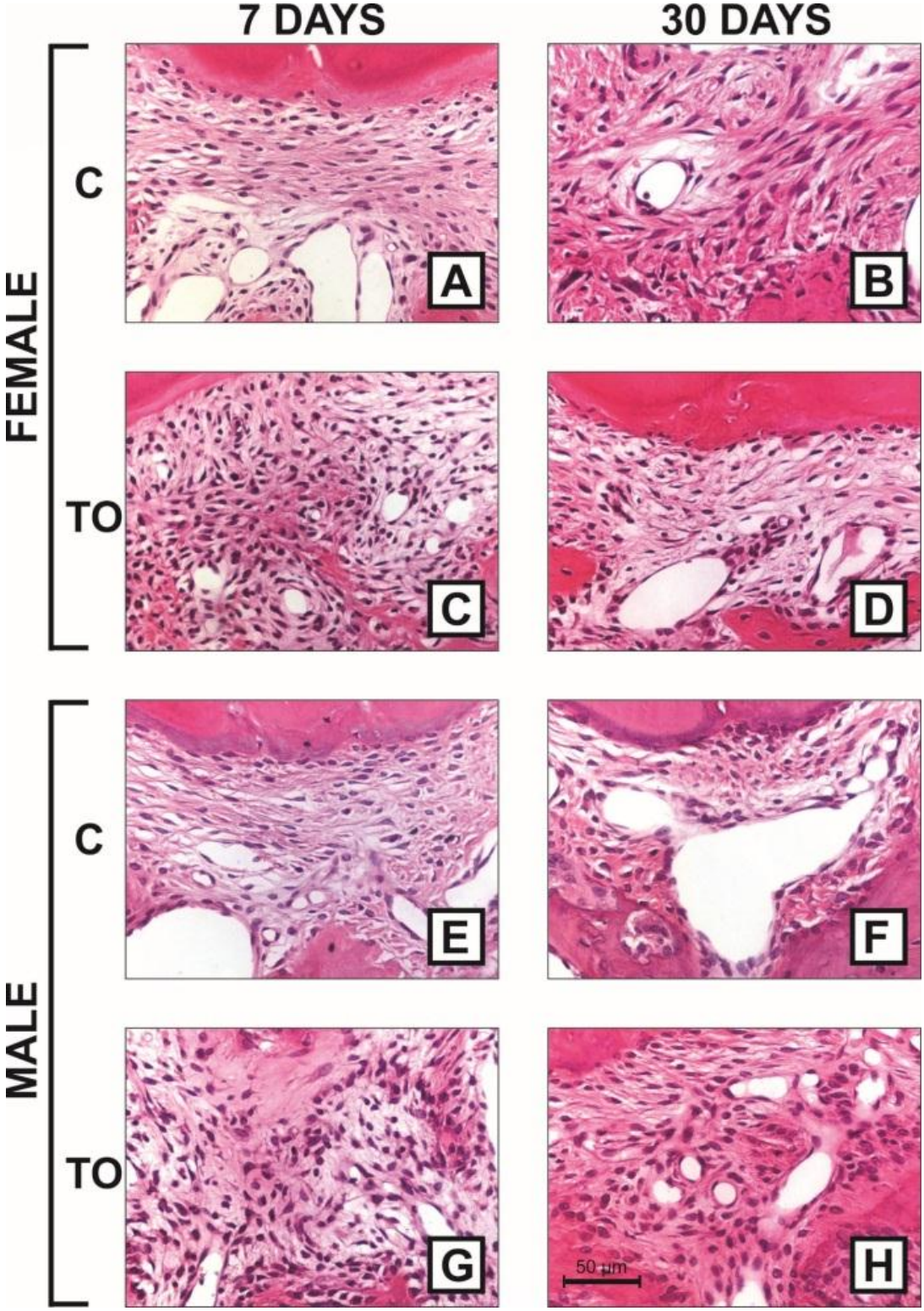
Comparing the significant changes occurred in the periods of 7 and 30 days, respectively; the thickness of periodontal ligament showed an increase of 23% and remained increased about 16.5% (Figure 5); the area of collagen fibers type III (Figure7) decreased 66.8% and 54.5%, while the area of collagen fibers type I (Figure7) increased 108.5% and 253.9% (Table 2).

The area of extracellular matrix (Figure 5) show a significant decrease (27.1%) and the number of nuclear profile (Figure 5) an increase (12.7%) just at 7 days (Table 2).

The resorption of alveolar bone occurred significantly in the TOM groups at 7 and 30 days, with a reduction of 36.4% and 39.4% respectively; showing an increase in this loss of bone area over time (Table 2) (Figure 6).

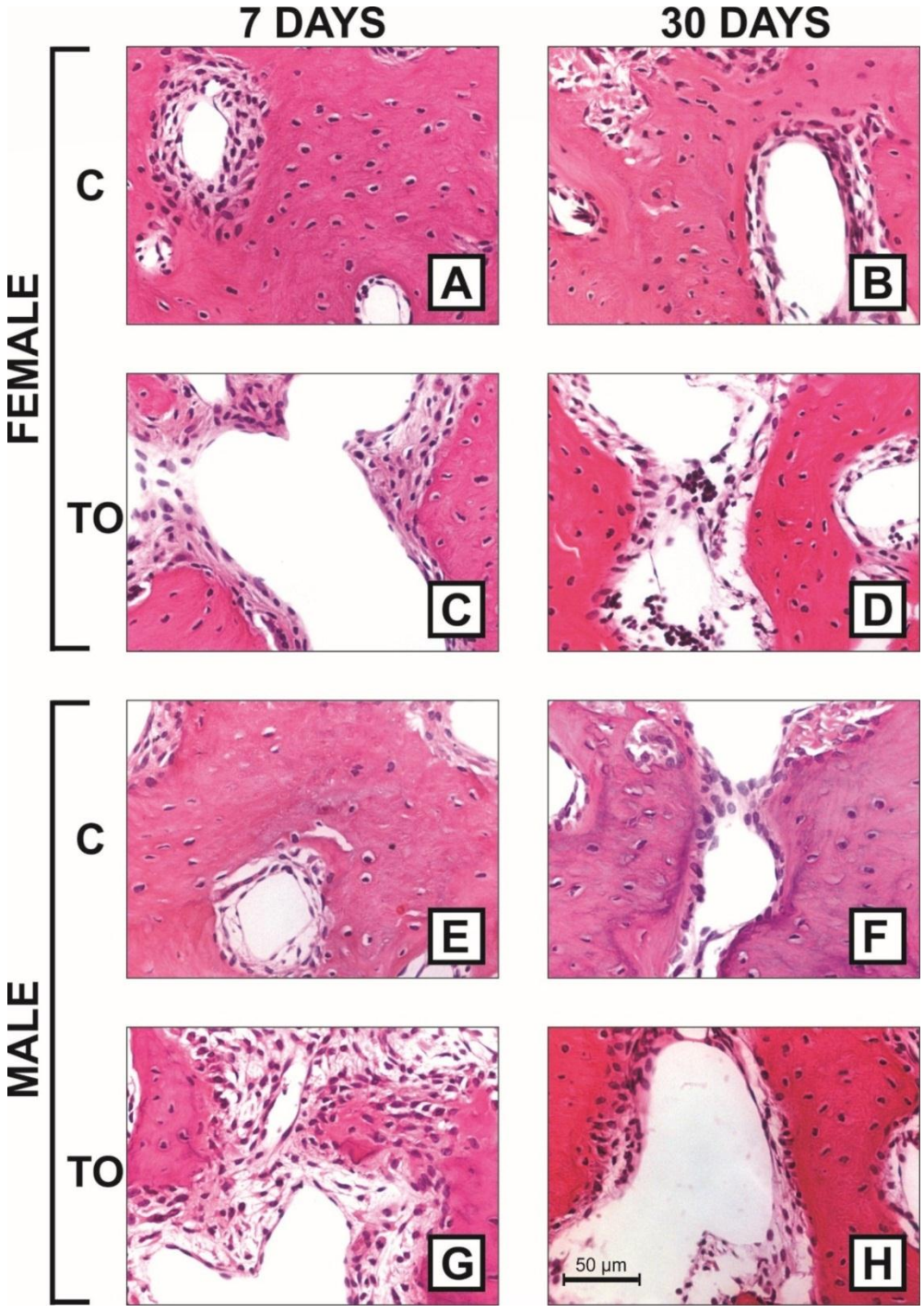
The epithelial insertion in the male groups (TOM) at 7 and 30 days show a small change in the insertion level of the periodontal ligament, however some inflammatory process appears at 30 days, even thought not statistically significant (Table 4).

Figure 5 - Longitudinal histological cuts of the first lower right molar, HE staining on day 7 and 30. The periodontal ligaments contains.



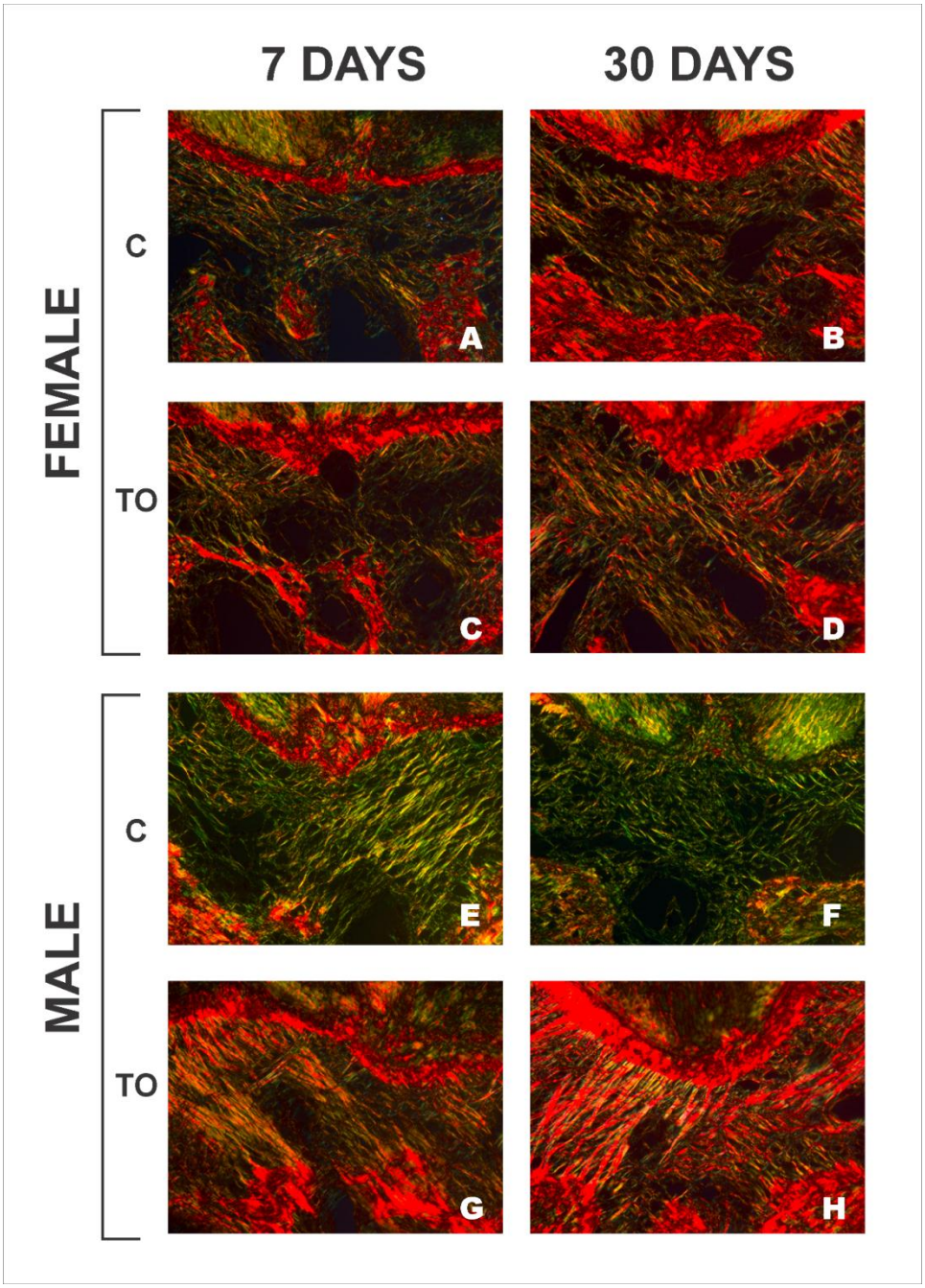
Source: Author

Figure 6 - Longitudinal histological cuts of the cervical part of the inter-radicular septum of the right lower first molar, stained with hematoxylin and eosin. The presence of bone resorption (2C, 2D, 2G and 2H) in the Traumatic occlusion groups (TOF and TOM), compared with bone area in the Control group (2A, 2B, 2E e 2F) on days 7 and 30, respectively; the bone resorption can be seen in the TOF (2C and 2D) and TOM (2G and 2H) groups.



Source: Author

Figure 7 - Longitudinal histological cuts of the periodontal ligamento of the cervical part of the right lower first molar, stained with picrocirius red.



Source: Author

Table 1. Statistical analysis of histomorphometric events associated with the periodontal ligament and alveolar bone in the experimental groups of the females by Mann-Whitney with 5% level of significance.

FEMALE						
PERIOD	**VARIABLES**	**CONTROL**		**TRAUMATIC OCCLUSION**		**P VALUE**
		Mean	**SD**	**Mean**	**SD**	
7 DAYS	Thickness ligament (μm)	152.3	23.91	165.3	21.44	Ns
	Area of blood vessels (%)	8.67	1.84	7.35	4.39	Ns
	Area of extracellular matrix (%)	26.96	7.49	30.72	4.38	Ns
	Area of collagen fibers type III (%)	56.16	9.35	57.65	10.34	Ns
	Area of collagen fibers type I (%)	43.84	9.35	42.34	10.34	Ns
	Nuclear profile (n)	580.8	41.93	597.7	156.0	Ns
	Bone area (%)	63.38	3.41	46.17	14.38	0.024
30 DAYS	Thickness ligament (μm)	158.3	12.61	153.4	11.18	Ns
	Area of blood vessels (%)	8.03	2.46	8.32	3.27	Ns
	Area of extracellular matrix (%)	29.63	2.04	38.15	6.39	0.036
	Area of collagen fibers type III (%)	56.50	11.02	54.23	11.56	Ns
	Area of collagen fibers type I (%)	43.49	11.02	45.76	11.56	Ns
	Nuclear profile (n)	361.9	47.21	411.9	64.57	Ns
	Bone area (%)	75.11	9.77	48.90	11.65	0.036

Source: Author

Table 2. Statistical analysis of histomorphometric events associated with the periodontal ligament and alveolar bone in the experimental groups of the males by Mann-Whitney with 5% level of significance.

MALE						
PERIOD	**VARIABLES**	**CONTROL**		**TRAUMATIC OCCLUSION**		**P VALUE**
		Mean	**SD**	**Mean**	**SD**	
7 DAYS	Thickness ligament (µm)	128.8	17.57	158.4	11.07	0.001
	Area of blood vessels (%)	5.53	1.73	6.81	2.26	Ns
	Area of extracellular matrix (%)	30.29	5.94	22.08	1.48	0.0001
	Area of collagen fibers type III (%)	76.6	5.1	51.2	13.3	0.0001
	Area of collagen fibers type I (%)	23.4	5.1	48.8	13.3	0.0001
	Nuclear profile (n)	403.9	47.18	455.1	33.12	0.021
	Bone area (%)	76.77	11.65	48.79	12.81	0.001
30 DAYS	Thickness ligament (µm)	133.2	21.44	155.2	13.53	0.017
	Area of blood vessels (%)	6.84	3.70	8.51	4.54	Ns
	Area of extracellular matrix (%)	26.69	3.11	32.68	6.63	Ns
	Area of collagen fibers type III (%)	84.7	4.1	46.2	7.4	0.0001
	Area of collagen fibers type I (%)	15.2	4.1	53.8	7.4	0.0001
	Nuclear profile (n)	440.5	61.63	425.5	36.74	Ns
	Bone area (%)	84.50	6.55	51.23	10.10	0.0001

Source: Author

Table 3. Statistical analysis of histomorphometric events associated with periodontal ligament insertion in the experimental groups of the females by Mann-Whitney with 5% level of significance.

EPITHELIAL INSERTION FEMALE								
			MESIAL			DISTAL		
		SCORE	C	TO	P value	C	TO	P value
7 DAYS	AREA OF EPITELIAL INSERTION (%)	1	100	88.9		100	100	
		2	0	11.1		0	0	
		3	0	0		0	0	
		4	0	0	0.86	0	0	US
	INFLAMMATORY PROCESS - INTENSITY (%)	1	100	100		100	100	
		2	0	0		0	0	
		3	0	0		0	0	
		4	0	0	US	0	0	US
	INFLAMMATORY PROCESS – EXTENSION (%)	1	100	100		100	100	
		2	0	0		0	0	
		3	0	0		0	0	
		4	0	0	US	0	0	US
30 DAYS	AREA OF EPITELIAL INSERTION (%)	1	100	44.4		100	66.7	
		2	0	55.6		10	33.3	
		3	0	0		0	0	
		4	0	0	0.20	0	0	0.48
	INFLAMMATORY PROCESS - INTENSITY (%)	1	100	100		100	100	
		2	0	0		0	0	
		3	0	0		0	0	
		4	0	0	US	0	0	US
	INFLAMMATORY PROCESS – EXTENSION (%)	1	100	100		100	100	
		2	0	0		0	0	
		3	0	0		0	0	
		4	0	0	US	0	0	US

US - Unable to statistics - the same scores
Source: Author

Table 4. Statistical analysis of histomorphometric events associated with periodontal ligament insertion in the male experimental groups by Mann-Whitney with 5% level of significance.

EPITHELIAL INSERTION MALE								
			MESIAL			DISTAL		
		SCORE	C	TO	P value	C	TO	P value
7 DAYS	AREA OF EPITELIAL INSERTION (%)	1	88.9	71.4		100	100	
		2	11.1	28.6		0	0	
		3	0	0		0	0	
		4	0	0	0.60	0	0	US
	INFLAMMATORY PROCESS - INTENSITY (%)	1	100	100		100	100	
		2	0	0		0	0	
		3	0	0		0	0	
		4	0	0	US	0	0	US
	INFLAMMATORY PROCESS – EXTENSION (%)	1	100	100		100	100	
		2	0	0		0	0	
		3	0	0		0	0	
		4	0	0	US	0	0	US
30 DAYS	AREA OF EPITELIAL INSERTION (%)	1	100	75		90	62.5	
		2	0	25		10	37.5	
		3	0	0		0	0	
		4	0	0	0.40	0	0	0.36
	INFLAMMATORY PROCESS - INTENSITY (%)	1	100	87.5		100	100	
		2	0	12.5		0	0	
		3	0	0		0	0	
		4	0	0	0.70	0	0	US
	INFLAMMATORY PROCESS – EXTENSION (%)	1	100	87.5		100	100	
		2	0	12.5		0	0	
		3	0	0		0	0	
		4	0	0	0.70	0	0	US

US - Unable to statistics - the same scores
Source: Author

5 DISCUSSION

The influence of gonadal hormones in the periodontal tissues has showed a significant link to both normal function and pathophysiology of disease[15]. This study shows that traumatic occlusion induced bone degradation[19] in both male and female young and health rats.

In relation to the action of the gonadal hormones in the bone, it is known that estrogen and testosterone present an anabolic effect in the formation and maintenance of bone tissue[17]. Estrogen increases the activity of alkaline phosphatase[22,23] (marker enzyme for calcification and differentiation of osteoinductive cells) and in the production of osteocalcin (marker of bone formation) and the osteoblastic differentiation of PDL cells and in bone formation, by way of ERβ (Estrogen Receptor β), and may influence the maintenance of alveolar bone mass[22] . The testosterone stimulate osteoblast proliferation and differentiation and presents a inhibitory effect on osteoclastic function[14]. The estrogen may play a significant role in osteoblastic function human periodontal ligament cells. However, these hormonal actions do not happen in a direct and isolated way[17], there is an interaction between the 3 main sex hormones; since estrogen stimulates the synthesis of testosterone, whereas progesterone inhibits this synthesis, and this progesterone effect is reduced when it is associated with estrogen[24].

Bone degradation is more likely related to female genus because of the large influence of the absence of estrogen on osteopenia in the menopause stage[25]. However, in young rats, this research show a larger reduction of bone area in male than in female in 7 days (36,4% and 27%) and 30 days (39,4% and 34,9%), respectivly. Also the thickness of periodontal ligament just has a significant increase in male rats, in a short (7 days) and long (30 days) experimental period, probably owing to bone remodeling. Kaku et al. [18] show that the periodontal ligament thickness return to normality on day 7 in female rats. These findings may suggest that the anabolic action of bone estrogen is more significant than that of testosterone, which favors the regeneration of bone tissue, by a turnover cellular faster[22,23].

5.1 Periodontal Ligament

The literature show that some changes happen in the periodontal ligament when the tooth is submitted to traumatic occlusion. Until the 5th day there is an increase in the number [5] and a disorientation[7,5] of fibroblasts, cell necrosis and venous thrombosis in the periodontal ligament[5], a decrease in collagenous fibers[5,6]. There were also a significant increasing of Ruffini endings and free nerves endings in the PDL [26].

The changes related to periodontal ligament itself were more prevalent and significant in male rats, compared to female rats, in face of a traumatic occlusion.

Blood vessels area were not significant for the changes of the periodontal ligament front a traumatic occlusion, in both genders. The literature has cited the presence of venous thrombosis in the PDL. Although the estrogen is known as one responsable factor of VEGF (Vascular Endothelial Growth Factor) production[22], the literature clarifies that sex hormones are not determinant in vascular neoformation, being this association with metabolic precursors, such as fatty acids. These are more involved in the process of endothelial cell proliferation and angiogenesis than the sex hormones.[27]

The action of gonadal hormones in the periodontal ligament is known. The estrogens, in general, tend to promote cell division, particularly in hormone-sensitive tissues, such as the periodontal ligament. In front of traumatic occlusion were expected on 7 days a cell necrosis in the periodontal ligament[11]. However the number of nuclear profile had significant increase only in the male rats front a traumatic occlusion, probably because endogenous testosterone promotes wound healing[28] by stimulate a positive growth on periodontal cells[14].

There was significant change in the area of extracellular matrix between groups C and OT in the male on day 7 with a decrease and in the female on 30 days with a increase.Different from what was expected by other literary findings that show that testosterone acts on the periodontal tissues increasing the synthesis of extracellular matrix[29] and progesterone is able to lower glycosaminoglycans synthesis, but not collagen synthesis in a physiological hormone doses[30]. This difference can be by the method of analysis, gender or stimuli.

The area of collagen fibers type I and III show a signicative change in front of traumatic occlusion in the male samples. On day 7 and 30 there was a larger amount of collagen fibers type I and a decrease of collagen fiber type III.

Mechanical tensile loads at low intensity increase the production of type I collagen fibers [31]. And in healing processes, there is an increase of type III collagen in the early stages, and later type III immature collagen is replaced by type I collagen[6]. This may explain a possible delay in the repair of the periodontal ligament of males that at 7 and 30 days present a higher proportion of type I and lower type III collagen fibers.

5.2 Area of epithelial insertion and inflammation

The results obtained regarding the influence of traumatic occlusion on the epithelial insertion area were as expected, since occlusal trauma does not present alterations in this region of the periodontium [3,8], the testosterone may protect this strutures by a inhibitory effect on the production and presence of mediators of inflammation[14]. However, some specimens presented a different behavior in both genders.

The increase in the distance between the cells of the insertion of the periodontal ligament in the men can favor the entrance of microorganisms, justifying the presence of inflammatory infiltrate in the region of insertion of the periodontal ligament. Opening the possibility of primary trauma may evolve into secondary trauma depending on the external presence of pathogenic microorganisms.

Estrogen also show inhibitory effect on the activity of neutrophils during the normal menstrual cycle, not only during pregnancy, and influence inflammation [32]. Future studies should identify the age and stage of the menstrual cycle of rats [24], in order to more accurately obtain the influence of gonadal hormones on the periodontal repair process through traumatic occlusion. As well as, identify if there is difference of the repair process in the different phases of the cycle and its influence on the prognosis.

Probably the factors that may alter the periodontal response to the incidence of gender-related traumatic occlusion may be related to cellular turnover,

adaptation of the periodontal ligament mechanoreceptors, the induction of proliferation and rearrangements of the collagen fibers, and the change in the distance between the cells of the junctional epithelium.

However, in women the cellular turnover is faster, the speed of adaptation of the mechanoceptors may be slower and muscle alterations more sensitive than in men. These mechanisms can respectively accelerate the repair process, maintain the protective reflex arc for longer, and reduce the strength of the mandibular closure force.

6 CONCLUSION

Traumatic occlusion caused bone degradation in female and male rats; however changes such as an increase of periodontal ligament thickness, increase of nuclear profile and collagen fibers type I; and a decrease in the area of collagen fibers type III of the periodontal ligament were expressive only in the male gender.

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