

ALFREDO FRANCO QUEIROZ

**INFLUÊNCIA DO USO DA TETRACICLINA OU DA
AMOXICILINA NO REPARO PULPAR PÓS
REPOSICIONAMENTO OU REIMPLANTE
DENTÁRIO MURINO**

**ARAÇATUBA
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Tese apresentada à Faculdade de Odontologia do
Campus de Araçatuba – Universidade Estadual
Paulista “Júlio de Mesquita Filho” – Unesp, para
obtenção do Título de Doutor em Odontologia –
Área de Clínica Integrada

Orientador:

Prof. Dr. Wilson Roberto Poi

Coorientadora:

Prof^ª. Dra. Mirian Marubayashi Hidalgo

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À Departamento de Odontologia da UEM

RESUMO

Queiroz, AF. Influência do uso da tetraciclina ou da amoxicilina no reparo pulpar pós reposicionamento ou reimplante dentário murino. [Tese]. Araçatuba: Universidade Estadual Paulista Júlio de Mesquita Filho – UNESP; 2018.

Objetivo: Avaliar, o reparo pulpar diante de dois antibióticos de uso sistêmico, tetraciclina e amoxicilina, em casos de reposicionamento (luxação extrusiva) ou reimplante (avulsão) dentários imediatos em ratos.

Material e Métodos: Incisivos centrais maxilares superiores de ratos foram luxados e reposicionados após 5 minutos (n=90) ou extraídos, imersos em soro fisiológico por 5min e reimplantados (n=90). Os grupos foram divididos em três subgrupos (n=30) conforme o antibiótico e o controle (soro fisiológico) administrado após o ato cirúrgico e até sete dias. Os animais foram eutanasiados após 7, 15 ou 30 dias. Cortes histológicos longitudinais foram corados com HE para análise histomorfológica da camada de odontoblastos, deposição de dentina reparativa, bainha epitelial de Hertwig mantida junto com o dente, necrose e infiltrado inflamatório periapical. Para a análise estatística, utilizaram-se os testes de Mann-Whitney, Kruskal-Wallis e de Dunns ($p < 0,05$). **Resultado:** Verificou-se haver diferença estatisticamente significativa entre os grupos amoxicilina e tetraciclina apenas na deposição de dentina reparativa no terço médio aos 15 dias após a luxação extrusiva. Para avulsão, a diferença ocorreu entre esses mesmos grupos aos 7 dias (presença da camada de odontoblastos, necrose e infiltrado inflamatório) e aos 15 dias (presença de necrose). Adicionalmente, verificaram-se diferenças entre o uso da amoxicilina ao longo do tempo tanto na avulsão como na luxação. **Conclusão:** A avaliação microscópica demonstrou a importância do uso de antibióticos de uso sistêmico na reação pulpar em casos de reposicionamento e reimplante dentários imediatos em ratos, uma vez que a amoxicilina contribuiu favoravelmente para o reparo pulpar após a realização de reimplante subsequente à avulsão dentária, porém, isto não ocorreu com a tetraciclina. No reposicionamento dentário após luxação extrusiva, o uso de antibióticos não afetou o reparo obtido.

Palavras-chave: Traumatismos dentários, tetraciclina, amoxicilina, polpa dental.

ABSTRACT

Queiroz, AF. Influence of the use of tetracycline or amoxicillin on pulp repair post murine dental repositioning or reimplantation. [Tesis]. Araçatuba: Universidade Estadual Paulista Júlio de Mesquita Filho – UNESP; 2018.

Aim: To evaluate the pulp repair in the presence of two systemic antibiotics, tetracycline and amoxicillin, in cases of immediate dental repositioning (extrusive luxation) or replantation (avulsion), in rats.

Material and Methods: Upper maxillary central incisors of rats were dislocated and repositioned after 5min (n = 90), or extracted, immersed in physiological solution for 5min, and reimplanted (n = 90). The groups were divided into three subgroups (n=30) according to the antibiotic or saline (control) administered after the surgery and up to seven days. The animals were euthanized after 7, 15 or 30 days. Longitudinal histological sections were stained with HE for histomorphological analysis of the odontoblast layer, reparative dentin deposition, Hertwig epithelial sheath maintained along with the tooth, necrosis, and periapical inflammatory infiltrate. For the statistical analysis the Mann-Whitney, Kruskal-Wallis e de Dunns ($p<0,05$). **Results:** There was a statistically significant difference between the amoxicillin and tetracycline groups only in the reparative dentin deposition in the middle third at 15 days after the extrusive luxation. For avulsion, the difference occurred between these same groups at 7 days (presence of odontoblasts layer, necrosis and inflammatory infiltrate) and at 15 days (presence of necrosis). Additionally, there were differences between the use of amoxicillin over time in both avulsion and luxation.

Conclusion: Microscopic evaluation demonstrated the importance of the use of systemic antibiotics in the pulp reaction in cases of immediate dental repositioning and reimplantation, in rats, since amoxicillin contributed favorably to the repair after reimplantation after dental avulsion, but this did not occur with tetracycline. In dental repositioning after extrusive luxation, the use of antibiotics did not affect the repair obtained.

Key words: Dental trauma, tetracycline, amoxicillin, dental pulp

LISTA DE GRÁFICOS

- Gráfico 1** Mostra as diferenças entre os grupos com os diferentes tratamentos (tetraciclina, amoxicilina e controle com soro fisiológico) que foram submetidos à luxação e avulsão decorridos os diferentes períodos de tempo. pág. 23
- Gráfico 2** Apresenta as diferenças entre os períodos de tempo (7, 15 e 30 dias) após a realização dos procedimentos luxação e avulsão com os diferentes tratamentos..... pág. 24

LISTA DE FIGURAS

- Figura 1** Aspectos morfológicos observados na seção longitudinal dos incisivos centrais superiores direitos de ratos, 7 dias após o procedimento traumático. LE= luxação extrusiva e reposicionamento e A= avulsão e reimplante, ambos com tratamentos de T= tetraciclina, A= amoxicilina e C= soro fisiológico, considerado controle negativo. H.E. pág. 26
- Figura 2** Principais achados histomorfológicos observados aos 7 dias nos grupos Luxação extrusiva (superior) e Avulsão (inferior). H. E. pág. 27
- Figura 3** Aspectos morfológicos observados na seção longitudinal dos incisivos centrais superiores direitos de ratos, 15 dias após o procedimento traumático. LE= luxação extrusiva e reposicionamento e A= avulsão e reimplante, ambos com tratamentos de T= tetraciclina, A= amoxicilina e C= soro fisiológico, considerado controle negativo. H.E. pág. 28
- Figura 4** Principais achados histomorfológicos observados aos 15 dias nos grupos Luxação extrusiva (superior) e Avulsão (inferior). H. E. pág. 29
- Figura 5** Aspectos morfológicos observados na seção longitudinal dos incisivos centrais superiores direitos de ratos, 30 dias após o procedimento traumático. LE= luxação extrusiva e reposicionamento e A= avulsão e reimplante, ambos com tratamentos de T= tetraciclina, A= amoxicilina e C= soro fisiológico, considerado controle negativo. H.E. pág. 30
- Figura 6** Principais achados histomorfológicos observados aos 30 dias nos grupos Luxação extrusiva (superior) e Avulsão (inferior). H. E. pág. 31

LISTA DE ABREVIATURAS, SIGLAS E SÍMBOLOS

%	= Porcentagem
µm	= Micrometro
AA	= Avulsão Amoxicilina
AC	= Avulsão Controle
AT	= Avulsão Tetraciclina
EDTA	= Ácido etileno diamino tetra acético
et al.	= E outros
Farmac	= Farmacêutica
Fig.	= Figura
FOA	= Faculdade de Odontologia de Araçatuba
g	= Gramas
h	= Horas
HE	= Hematoxilina Eosina
IADT	= Associação Internacional de Traumatismo Dentário
Ind	= Indústria
Kg	= Quilograma
LEA	= Luxação Extrusiva Avulsão
LEC	= Luxação Extrusiva Controle
LET	= Luxação Extrusiva Tetraciclina
Ltda	= Limitada
mg	= Miligramas
mL	= Mililitros
mm	= Milímetros
n°	= Número
pH	= Potencial hidrogeniônico
PVP-I	= Polivinilpirrolidona Iodada
RJ	= Rio de Janeiro
SP	= São Paulo
UNESP	= Universidade Estadual Paulista

SUMÁRIO

Introdução	pág. 13
Objetivo	pág. 15
Material e Métodos	pág. 17
Resultados	pág. 21
Discussão	pág. 32
Conclusão	pág. 36
Referências	pág. 38
Anexo A – Certificados do Comitê de Ética no Uso de Animais	pág. 43
Anexo B – Normas do periódico Dental Traumatology.....	pág. 45
Apêndice A – Artigo apresentado na Qualificação e submetido ao periódico Dental Traumatology.....	pág. 58
Apêndice B – Artigo apresentado na Qualificação e na fase final de preparação para ser submetido ao periódico Journal of Applied Oral Science..	pág. 77

INTRODUÇÃO

INTRODUÇÃO

A resistência aos antibióticos e o declínio de sua eficácia impõem, potencialmente, grandes encargos à saúde e à economia nas sociedades¹. É necessário determinar o efeito real do uso de antibióticos nos diferentes procedimentos em saúde, incluindo aqueles subsequentes aos traumatismos dentários.

Na avulsão de dentes com rizogênese incompleta, todos os esforços devem ser realizados para manutenção do dente, mesmo quando o prognóstico não seja favorável, para preservar o osso alveolar o máximo de tempo possível permitindo, no futuro, caso seja necessário, a colocação do implante. Para melhorar o prognóstico de dente reimplantado, os estudos demonstram a importância da prescrição de antibiótico²⁻⁴, além do tempo extra-alveolar, do meio de conservação adequado, entre outros^{2, 5-7}. A antibioticoterapia evitaria a complicação mais frequente, a infecção do tecido pulpar necrosado, que pode induzir uma inflamação periapical crônica ou aguda^{8, 9} ou, nos dentes com rizogênese incompleta, pode interromper o desenvolvimento radicular resultando em ápice aberto e paredes de dentina finas, tornando o dente propenso a fratura¹⁰.

Nos casos de luxação extrusiva, o desenvolvimento de necrose pulpar está relacionado à extensão do dano causado ao complexo pulpo periodontal e o estágio de formação radicular devido à capacidade desse complexo de reparar¹¹.

Três tipos de antibióticos são indicados pelo Guia da Associação Internacional de Traumatismos Dentários (IADT) para tratamento da avulsão dentária²: a tetraciclina, como primeira escolha, e a fenoximetilpenicilina (penicilina V) ou amoxicilina, como alternativas. Para luxação extrusiva, não há indicação de antibioticoterapia por parte da IADT¹², porém, muitas instituições e profissionais utilizam, preventivamente. A Associação Europeia de Endodontia segue o Guia da IADT para os casos de traumatismos e indica, como primeira escolha, para as infecções endodônticas, os β -lactâmicos (penicilina V e amoxicilina)¹³.

Assim sendo, verificou-se a necessidade de se determinar o efeito real do uso de antibióticos em procedimentos como o reposicionamento ou reimplante dentário e a ausência, de nosso conhecimento, de manuscritos descrevendo, comparativamente, o reparo pulpar perante seu uso ou não.

OBJETIVO

OBJETIVO

O objetivo deste estudo foi avaliar o reparo pulpar diante de dois antibióticos de uso sistêmico, tetraciclina e amoxicilina, em casos de reposicionamento (luxação extrusiva) e reimplante (avulsão) dentários imediatos em ratos. A hipótese testada é que os antibióticos contribuem favoravelmente para o reparo após a realização de procedimentos subsequentes aos traumatismos dentários.

MATERIAL E MÉTODOS

MATERIAL E MÉTODOS

O projeto de pesquisa foi aprovado pela Comissão de Ética no Uso de Animais da Faculdade de Odontologia de Araçatuba – UNESP –Processos FOA 0763/2011 e 2014-00491 e (Anexo A).

Cento oitenta ratos machos Wistar (*Rattus norvegicus*, albinus), com peso corporal entre 150g e 300g foram usados. Os animais foram mantidos no biotério da Faculdade de Odontologia de Araçatuba durante o período experimental, alimentados com ração sólida e água à vontade, exceto no período das 12h antecedentes ao ato cirúrgico. Após a cirurgia, durante 3 dias, os animais foram alimentados com ração triturada.

A etapa cirúrgica foi realizada sob anestesia geral, administrando-se por via intramuscular a combinação de cloridrato de xilazina (Dopaser, Calier do Brasil Ltda. Osasco, SP, Brasil) na dosagem de 0,6 mg para cada 100g de peso corporal, para promover o relaxamento muscular. Na sequência, foi utilizado o cloridrato de ketamina (Dopalen, AgriBans Ltda. Paulínia, SP, Brasil) na dosagem de 0,7mg para cada 100g de peso corporal para a indução anestésica. Em seguida, foi realizada a antisepsia da porção anterior da maxila com polivinilpirrolidona iodada (PVP-I, Riodeine Ind. Farmac. Rioquímica Ltda. São José do Rio Preto, SP, Brasil). No grupo da luxação extrusiva (n=90), foram realizadas a sindesmotomia, luxação e deslocamento de 3 mm no sentido axial do incisivo central superior direito de cada animal. Decorridos 5 minutos, o dente foi reposicionado no seu alvéolo. No grupo da avulsão (n=90), foi realizada a extração dos incisivos centrais superiores direitos de todos os animais. Os dentes foram imersos em soro fisiológico por 5 minutos e em seguida reimplantados. Não houve necessidade da colocação de esplintagem flexível, como recomendado pelo Guia IADT^{2, 11} em ambos os grupos devido à anatomia dental murina. Os animais de ambos os grupos foram então divididos aleatoriamente em 3 subgrupos experimentais com 30 animais cada: tetraciclina (Doxy® suspensão, Cepav Pharma Ltda. São Paulo, SP, Brasil) 2,5 mg/kg; amoxicilina (Duprancil®, Duprat. Rio de Janeiro, RJ, Brasil) 25 mg/kg; e, controle 1 ml de soro fisiológico. No subgrupo tetraciclina, a ração foi removida 1h antes da administração para não interferir na absorção da droga. Os antibióticos/soro fisiológico foram administrados de 12/12h por via intragástrica,

durante 7 dias, iniciando-se imediatamente após o reposicionamento/reimplante dentário. Quinze animais foram perdidos sendo 10 do grupo da avulsão e 5 do grupo da luxação extrusiva.

Dez animais de cada subgrupo foram eutanasiados por meio de sobredose anestésica aos 7, 15 e 30 dias após o reposicionamento/reimplante. A maxila direita contendo o dente reposicionado/reimplantado foi separada da esquerda na linha mediana usando-se um escalpelo. Um corte na porção distal do 3º molar possibilitou a obtenção do espécime, que foi então fixado em solução de formalina a 10% por 24h e descalcificado com solução de EDTA (10%, pH 7,0). Após a descalcificação, as peças foram embebidas em parafina. Três cortes longitudinais semi-seriados de 6µm de espessura foram corados com Hematoxilina e Eosina para a análise histomorfológica.

A análise microscópica foi realizada com o auxílio de microscópio óptico (Olympus CX31- Tokyo-Japan) por um avaliador calibrado. Cada espécime foi examinado em todos os segmentos da polpa: coronário, médio e apical assim como a região periapical. Para análise dos achados histomorfológicos de cada grupo, foram atribuídos escores ordinários de 0 a 3 adaptados de Nishioka et al.⁸, correspondente, respectivamente, a ausente, presente em área restrita, presente parcialmente e presente na totalidade. Sete diferentes eventos, de acordo com as observações descritas por Fiane, Breivik e Vandevska-Radunovic¹⁴ e Nishioka et al.⁸, foram analisados: camada de odontoblastos, deposição de dentina terciária nos terços coronário, médio e apical, bainha epitelial de Hertwig mantida junto ao dente, necrose e infiltrado inflamatório periapical.

As imagens foram capturadas com o uso de uma câmera de alta resolução (Nikon, DS-File, Shimjuku, Japan) acoplada ao microscópio óptico (Nikon, Eclipse 80i, Shimjuku, Japan) e conectada ao computador equipado com um software de análise de imagem digital (Imaging Software Nis Elements, version 4.0). Os cortes longitudinais dos dentes inteiros foram obtidos mediante a utilização de negatoscópio e câmera de celular (iPhone 6S, Designed by Appple/California origem China).

A análise estatística dos dados obtidos foi realizada por meio do programa GraphPad Prisma v.7.05 e os resultados foram expressos como média \pm erro padrão. Em todas as análises, os dados não apresentaram distribuição normal, segundo o teste KS (Kolmogorov Smirnov). Para comparação entre dois grupos foi utilizado o teste não

paramétrico Mann-Whitney. Para as demais análises foi realizado o teste não paramétrico Kruskal-Wallis seguido de *post hoc* de Dunns. Para ambas as análises, o valor de p menor que 0,05 foi considerado estatisticamente significativo.

RESULTADOS

RESULTADOS

Os achados histomorfológicos que apresentaram diferenças estatisticamente significativas estão apresentados nos Gráficos 1 e 2. O Gráfico 1 mostra as diferenças entre os grupos com os diferentes tratamentos (tetraciclina, amoxicilina e controle com soro fisiológico) que foram submetidos à luxação e avulsão decorridos os diferentes períodos de tempo. O Gráfico 2 apresenta as diferenças entre os períodos de tempo (7, 15 e 30 dias) após a realização dos procedimentos luxação e avulsão com os diferentes tratamentos. Digno de menção, as diferenças estatísticas encontradas com o uso da amoxicilina para a camada de odontoblastos, presença de necrose e de infiltrado inflamatório para avulsão dentária e camada de odontoblastos e deposição de dentina reparativa no terço apical para luxação extrusiva.

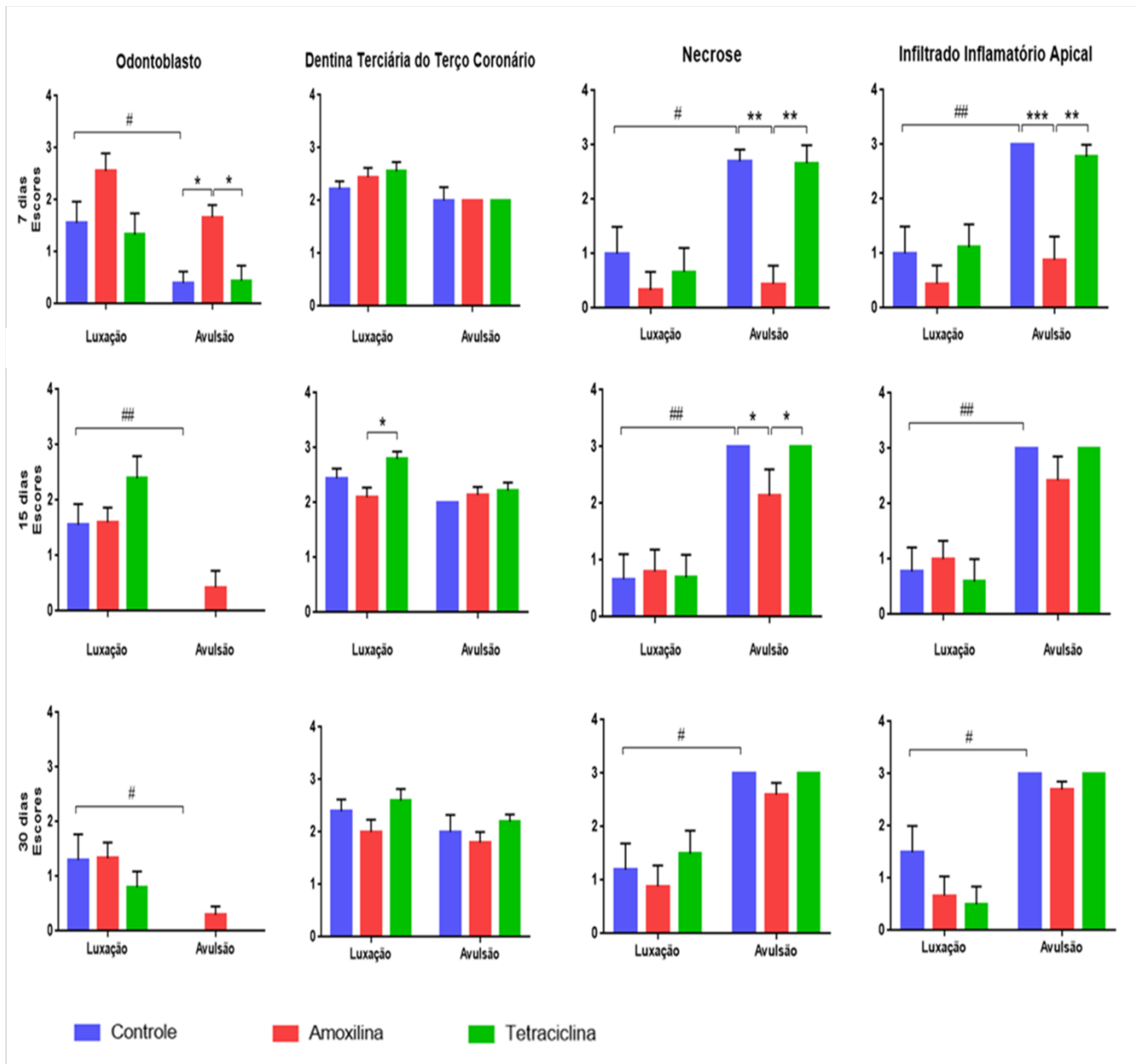


Gráfico 1. Média ± erro-padrão dos escores atribuídos aos achados histomorfológicos observados nos grupos tratados com tetraciclina, amoxicilina e soro fisiológico (controle), após luxação extrusiva/reposicionamento e avulsão/reimplante, eutanasiados após 7, 15 e 30 dias do procedimento. Testes Mann-Whitney, Kruskal-Wallis e Dunns, n=7 a 10, *=p<0,05, **=p<0,01, ***=p<0,001 entre os tratamentos e #=p<0,05 e ##=p<0,01 entre os controles da luxação extrusiva e avulsão.

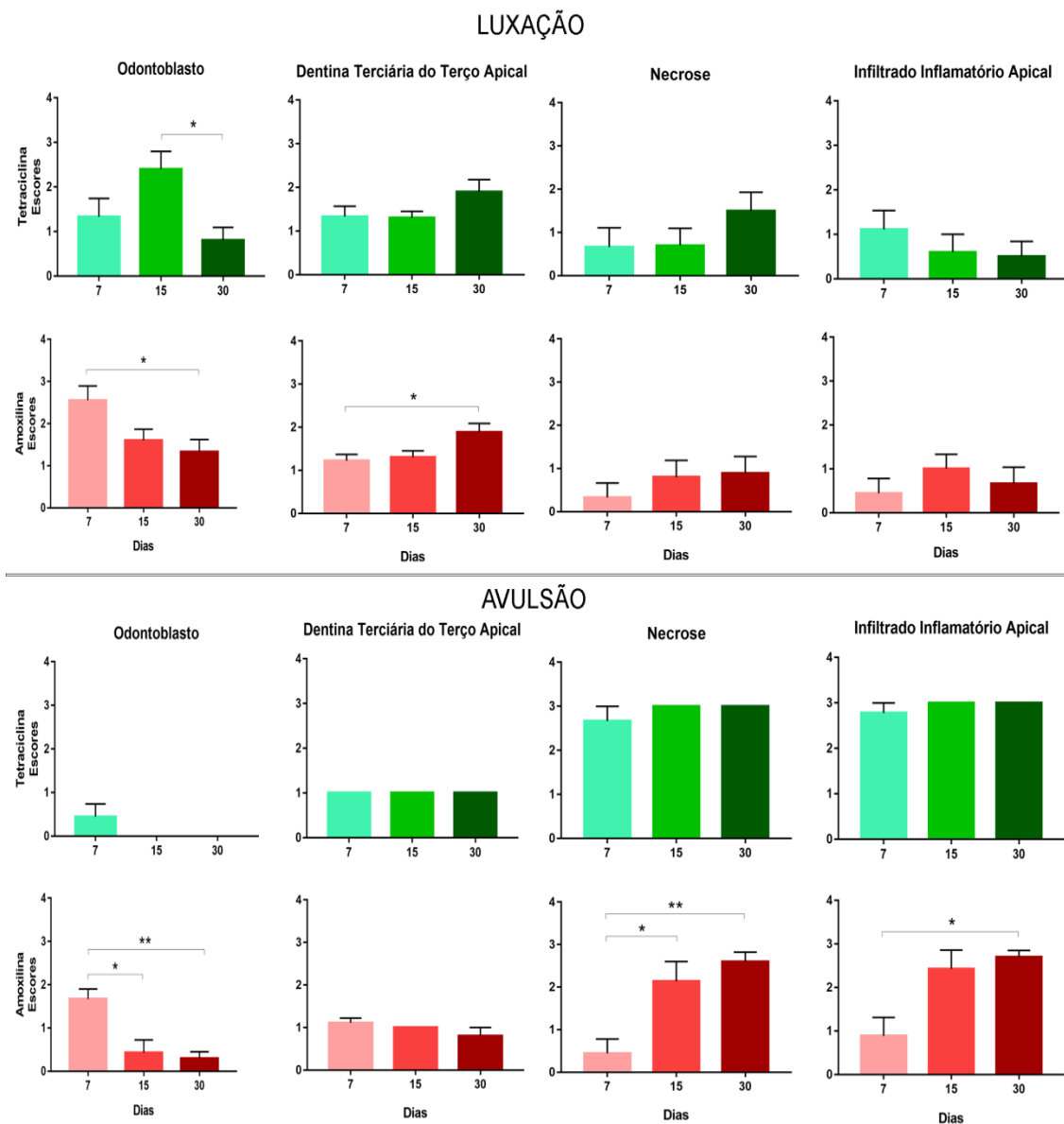


Gráfico 2. Média \pm erro-padrão dos escores atribuídos aos achados histomorfológicos observados nos animais eutanasiados após 7, 15 e 30 dias do procedimento nos grupos luxação extrusiva/reposicionamento e avulsão/reimplante com os tratamentos tetraciclina, amoxicilina e soro fisiológico (controle). Testes Mann-Whitney, Kruskal-Wallis e Dunns, $n=7$ a 10 , $*=p<0,05$, $**=p<0,01$.

Sete dias após o reposicionamento/reimplante. Na Fig. 1 observa-se o aspecto geral do que ocorreu com os diferentes tratamentos nos grupos que sofreram luxação extrusiva e avulsão. No Gráfico 1, aos 7 dias foi possível observar, numericamente, que, tanto na luxação extrusiva quanto na avulsão, a camada de odontoblastos estava mais presente no grupo da amoxicilina, com diferença estatística no grupo avulsão, assim Fig. 1 mostra que as características das células se apresentavam mais próximas da normalidade no grupo luxação. Neste, os odontoblastos apresentavam-se com formato colunar, com núcleos polarizados e dispostos como um epitélio pseudo estratificado (Fig.4LE-a, seta vermelha). Havia células no interior do tecido pulpar na periferia de ilhas de dentina reparativa neoformada, fato que não foi observado no grupo avulsão, no qual as células apresentavam características de degeneração, ou seja, células com cariorrexe e cariólise (Fig.4AV-d, setas brancas e amarelas). Não foi possível observar diferença estatística entre os grupos da tetraciclina e da amoxicilina na deposição de dentina reparativa em cada tipo de trauma. No entanto, estava bem clara a diferença entre os dois tipos de trauma, sendo que a deposição foi muito mais acentuada na luxação extrusiva do que na avulsão (Fig.3). A deposição de dentina seguia o padrão de deposição de dentina secundária, ou seja, começava e era mais intensa na porção coronária e diminuía gradativamente até o ápice. Em alguns casos, a deposição era tão intensa que muitas células ficavam aprisionadas na matriz dentinária, dando a impressão de serem tecido ósseo (Fig.4LE-b, seta azul). A presença da bainha epitelial de Hertwig não sofreu influência do uso de antibiótico, mas sim do tipo de trauma. Estava presente em alguns casos de luxação extrusiva, observado quando ocorria fratura apical e depois consolidava ou vista como apenas um simples fragmento, diferentemente dos casos de avulsão nos quais estava ausente em praticamente todos os casos e quando vista somente fragmento (Fig. 4LE-c e AV-f, setas lilás). A necrose (Gráfico 1-7 dias) ocorreu com maior intensidade no grupo da tetraciclina no trauma da avulsão, igualando ao grupo controle sem o uso do antibiótico e o grupo da amoxicilina avulsão foi comparável aos resultados de ambos antibióticos na luxação extrusiva, sendo também apresentado na Fig.4LE-c e AV-e (setas verdes). O infiltrado inflamatório periapical apresentou resultados semelhantes aos da necrose (Fig.1-7dias).

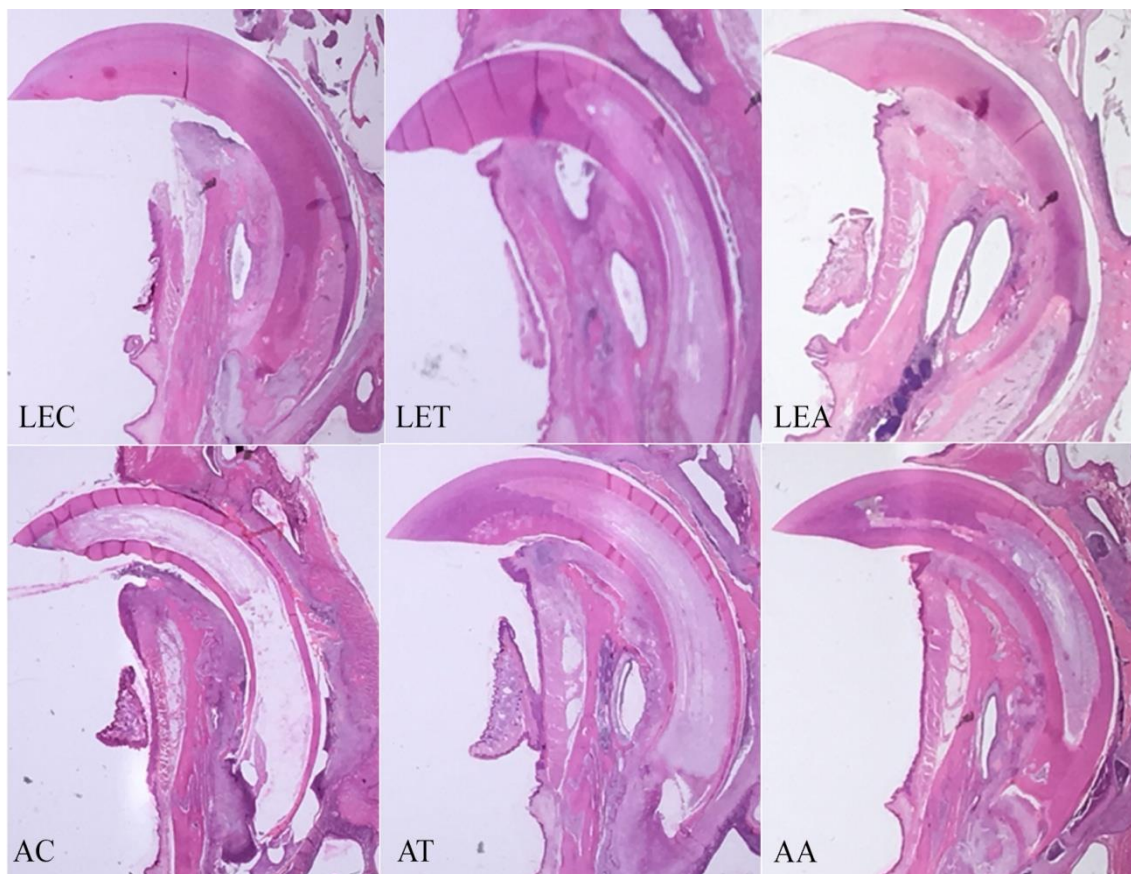


Figura 3. Aspectos morfológicos observados na seção longitudinal dos incisivos centrais superiores direitos de ratos, 7 dias após o procedimento traumático. LE= luxação extrusiva e reposicionamento e A= avulsão e reimplante, ambos com tratamentos de T= tetraciclina, A= amoxicilina e C= soro fisiológico, considerado controle negativo. H.E.

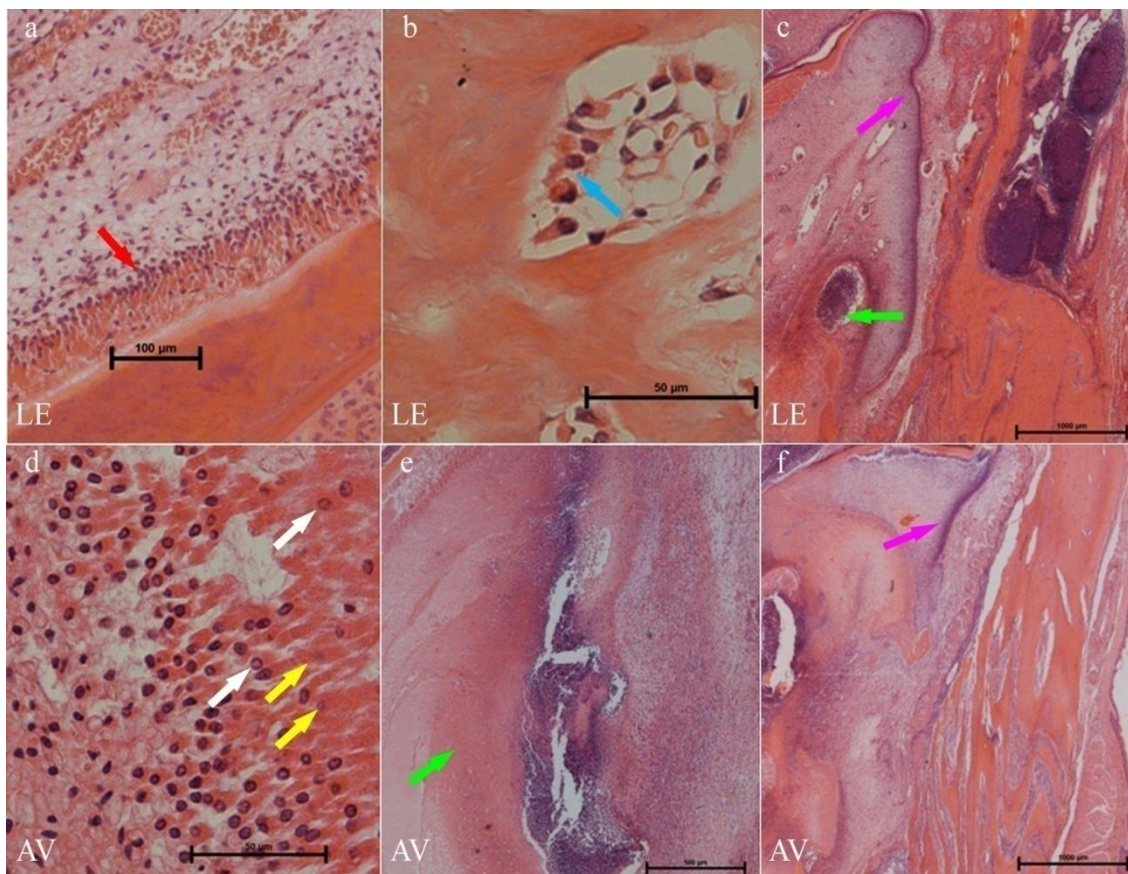


Figura 4. Principais achados histomorfológicos observados aos 7 dias nos grupos Luxação extrusiva (superior) e Avulsão (inferior). H. E.

Quinze dias após o reposicionamento/reimplante. O aspecto geral do que ocorreu com os diferentes tratamentos nos grupos luxação extrusiva e avulsão podem ser vistos na Fig. 5. A camada de odontoblastos apresentou uma distribuição semelhante, sem diferença estatística entre os grupos de antibióticos (Gráfico 1-15 dias), mas, em muitos casos da luxação extrusiva, se observava a camada de odontoblastos com grande número de células (Fig. 4LE-a, seta amarela), promovendo uma deposição irregular de dentina (Fig. 4LE-a, seta vermelha) e, em alguns casos, com formação de ilhas de dentina no interior do tecido pulpar (Fig. 4LE-b, seta azul). No grupo avulsão, mesmo com o uso da amoxicilina, a camada de odontoblastos estava completamente alterada com características de degeneração (Fig. 4AV-d, seta branca). Houve diferença estatística na dentina reparativa depositada no grupo tetraciclina (Fig.1-15 dias), com maior prevalência de deposição na luxação extrusiva (Fig. 3). A bainha epitelial de Hertwig estava ausente na quase totalidade dos dentes analisados, quando presente era devido a consolidação de fragmento radicular apical que não tinha sido deslocado na

luxação (Fig. 4LE-c, seta lilas). A necrose (Fig. 4AV-e, seta verde) estava presente em todos os grupos, com maior intensidade no trauma de avulsão, sendo o grupo amoxicilina estatisticamente diferente dos grupos tetraciclina e controle. O infiltrado inflamatório estava sempre relacionado à necrose (Fig. 4AV e-f, seta verde) acompanhado o mesmo padrão, ainda que sem diferença estatística entre os tipos de antibióticos (Gráfico 1-15 dias).

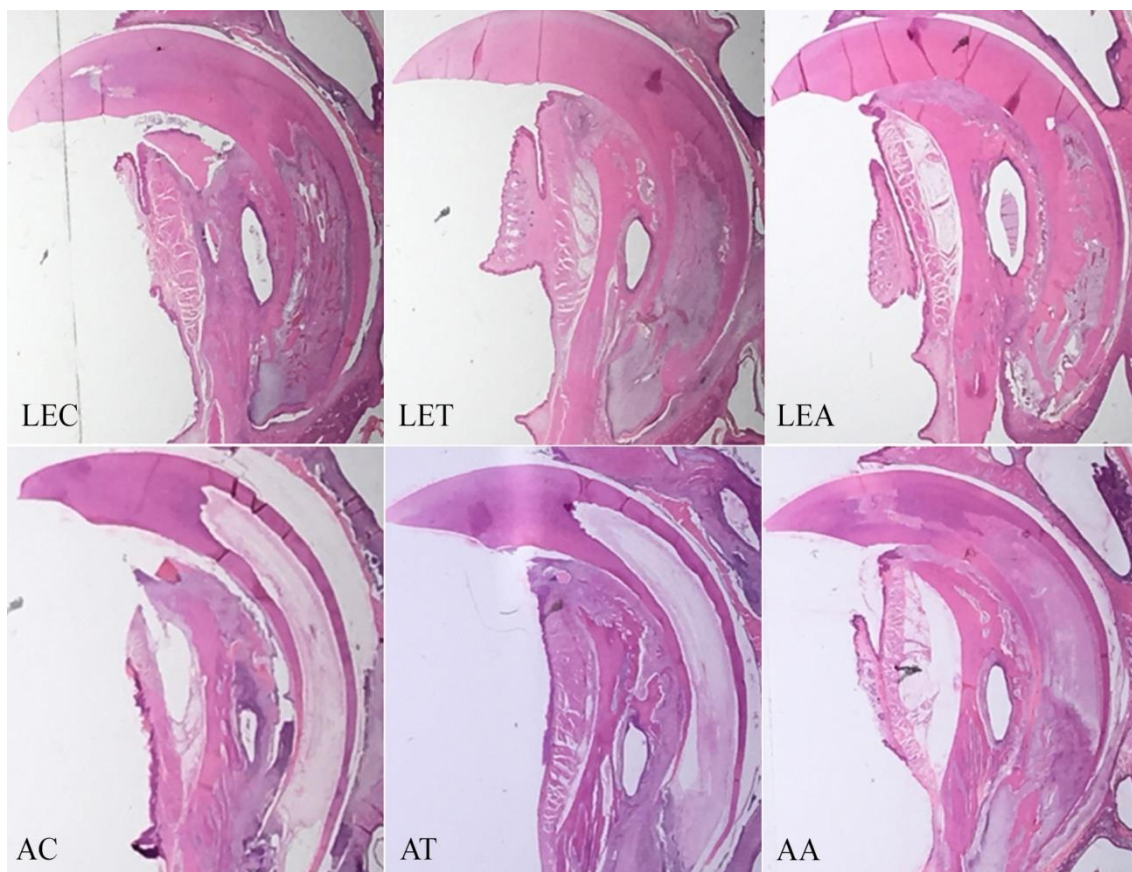


Figura 5. Aspectos morfológicos observados na seção longitudinal dos incisivos centrais superiores direitos de ratos, 15 dias após o procedimento traumático. LE= luxação extrusiva e reposicionamento e A= avulsão e reimplante, ambos com tratamentos de T= tetraciclina, A= amoxicilina e C= soro fisiológico, considerado controle negativo. H.E.

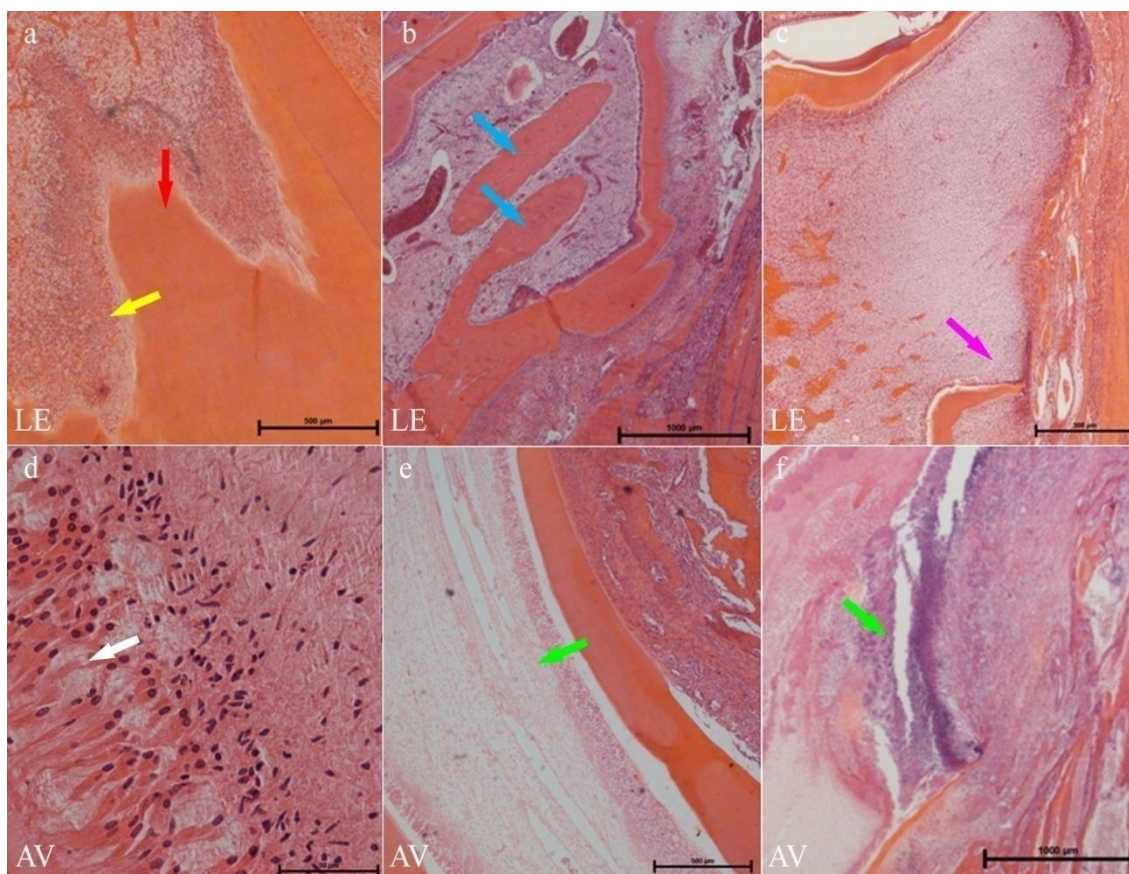


Figura 6. Principais achados histomorfológicos observados aos 15 dias nos grupos Luxação extrusiva (superior) e Avulsão (inferior). H. E.

Trinta dias após o reposicionamento/reimplante. A Fig.7-30 dias nos mostra o aspecto geral do que ocorreu com os diferentes tratamentos nos grupos, luxação extrusiva e avulsão. A camada de odontoblastos estava ausente em praticamente todos os casos de trauma de avulsão, com exceção da presença em algumas áreas restritas no grupo da amoxicilina (Fig. 6AV-d, seta amarela). Na luxação extrusiva, a camada de odontoblastos estava presente parcialmente e em área restrita (Fig. 6LE-a, seta branca), principalmente devido à formação de dentina reparativa que praticamente obliterava quase toda a polpa, não havendo diferença entre os grupos (Fig. 6LE-b, c, setas azuis). A deposição de dentina reparativa foi semelhante entre os grupos com relação ao antibiótico, mas do ponto de vista do trauma, na luxação extrusiva ocorreu maior deposição (Fig. 5-30 dias). Como nos outros tempos, a bainha epitelial de Hertwig foi encontrada somente nos casos de luxação extrusiva, naqueles casos onde ocorreu fratura apical e depois ocorria a consolidação entre as partes (Fig. 8LC-c seta lilás). O infiltrado inflamatório e a necrose não apresentaram diferença entre os grupos de antibióticos,

entre os traumas, foram mais frequentes na avulsão. A necrose observada era por coagulação e depois por liquefação devido a presença de grande quantidade de células inflamatórias (Fig.8AV-f seta verde). Na comparação entre os grupos controle (uso de soro fisiológico) da avulsão em relação ao da luxação extrusiva, foram encontradas diferenças estatisticamente diferentes para camada de odontoblastos, presença de necrose e de infiltrado inflamatório em todos os tempos de estudo (Gráfico 1).

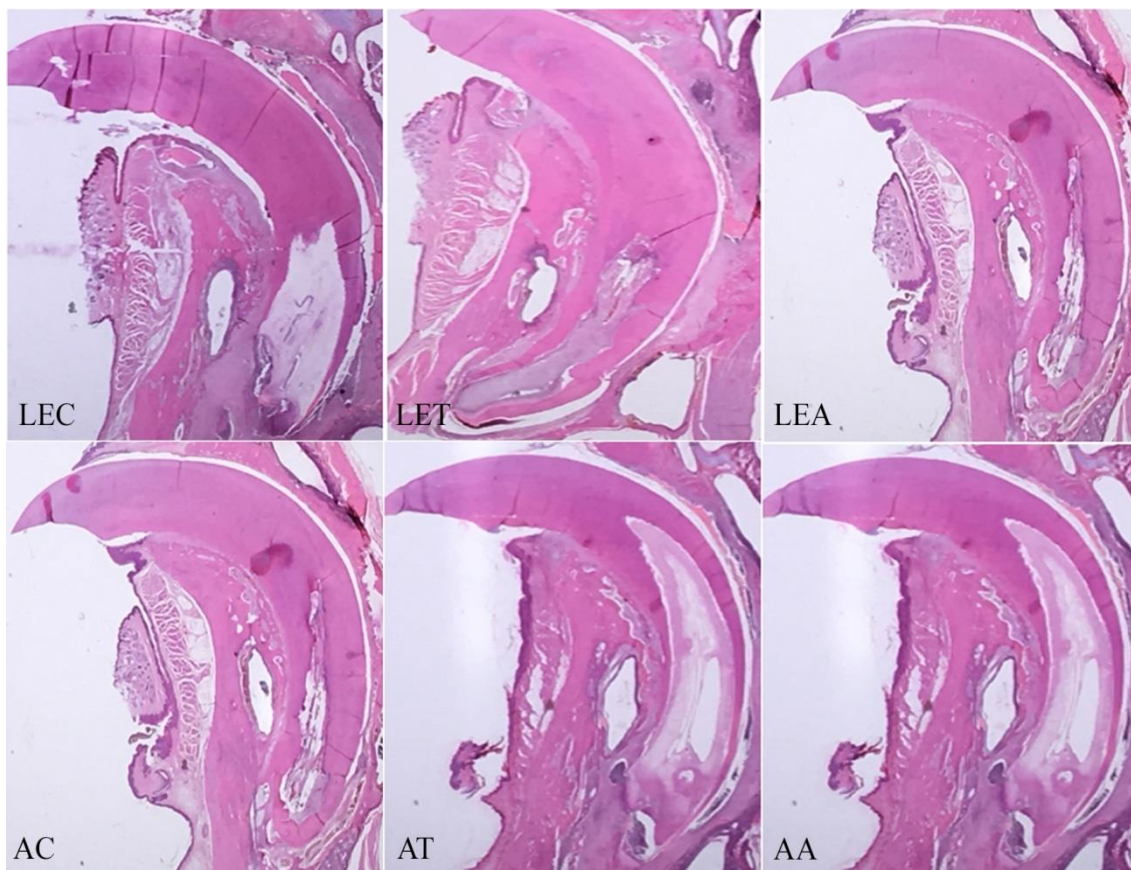


Figura 7. Aspectos morfológicos observados na seção longitudinal dos incisivos centrais superiores direitos de ratos, 30 dias após o procedimento traumático. LE= luxação extrusiva e reposicionamento e A= avulsão e replante, ambos com tratamentos de T= tetraciclina, A= amoxicilina e C= soro fisiológico, considerado controle negativo. H.E.

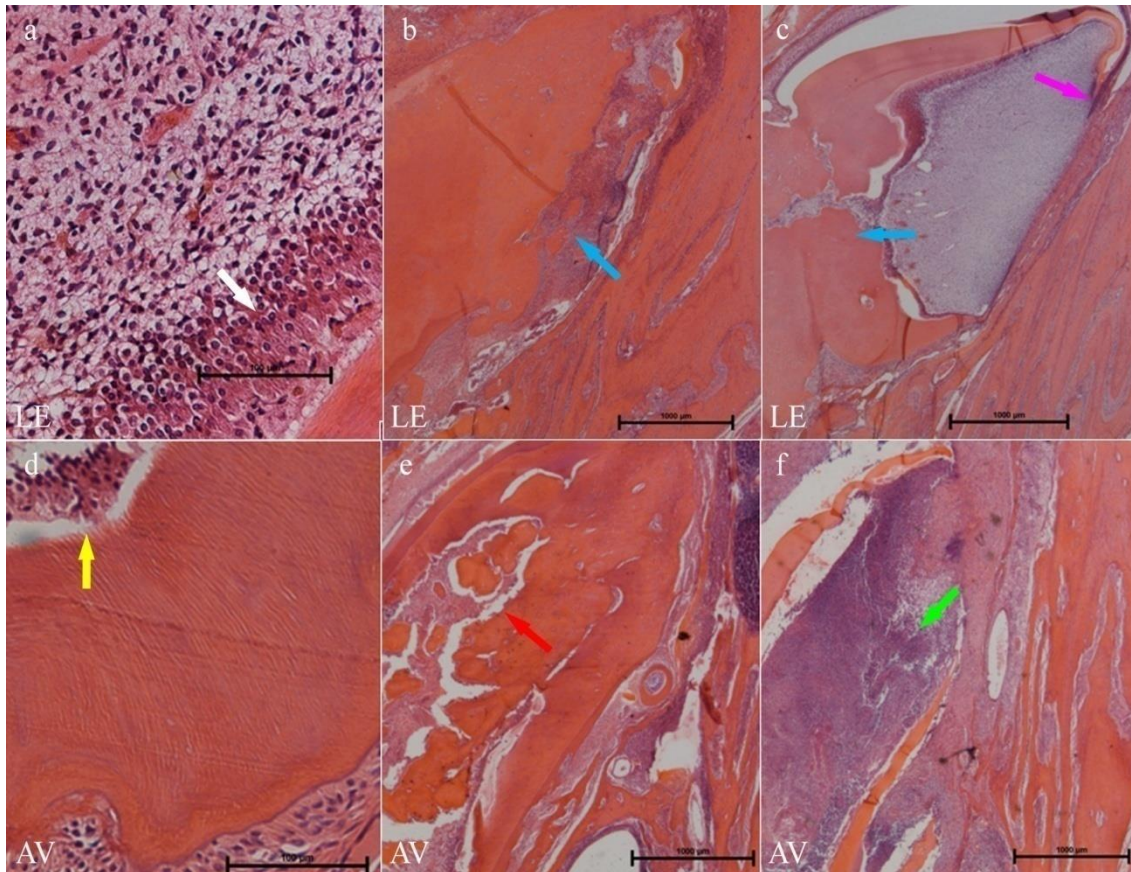


Figura 8. Principais achados histomorfológicos observados aos 30 dias nos grupos Luxação extrusivos (superior) e Avulsão (inferior). H. E.

DISCUSSÃO

DISCUSSÃO

A IADT e a Associação Europeia de Endodontia recomendam a prescrição de antibiótico quando um dente avulsionado é reimplantado, a fim de prevenir a contaminação microbiana^{2, 13}. Já para os casos de luxação extrusiva, há limitadas evidências para o uso de antibiótico sistêmico, segundo a IADT¹², sendo acompanhada pela Associação Europeia de Endodontia¹³. No entanto, esta associação indica o uso profilático padrão da amoxicilina endodontia¹³, rotina prescrita por muitos profissionais e instituições. Assim sendo, nesta pesquisa foram administrados dois tipos de antibiótico para cada tipo de trauma, de modo a verificar a efetividade dos mesmos sobre a polpa dental e a influência no reparo. Isso posto, ao considerar que a polpa dental contaminada é a principal responsável pela instalação da reabsorção radicular inflamatória, que acarreta um prognóstico desfavorável ao dente reposicionado/reimplantado^{3, 14, 16}.

Com esta conexão entre microrganismos e trauma dental, a abordagem clínica lógica seria excluir ou limitar a carga bacteriana na fase de cura para otimizar a cicatrização. Poderia se esperar que a redução da carga bacteriana com o uso de antibiótico reduziria ou mudaria a taxa ou padrão de complicações após lesões traumáticas¹⁰. No entanto, Shah e Ashley¹⁷ questionaram a efetividade da prescrição de antibiótico nos casos de avulsão dentária após avaliarem vários guias e entenderem que estas recomendações têm uma base fraca de evidências, mencionando o trabalho de Hinkfuss e Messer¹⁸ com semelhantes constatações. Este último artigo foi contestado por Andreasen, Ahrensburg e Tendal¹⁹, que demonstraram que a meta análise havia sido realizada sem a randomização dos dados e o consideraram inapropriado. Ainda assim, autores utilizaram como referência^{20, 21} nos poucos artigos publicados sobre a prescrição de antibiótico após reimplante de dentes avulsionados. Até mesmo o Guia da IADT¹² usa o trabalho das autoras como referência.

Dos antibióticos utilizados, destaca-se que a tetraciclina, além da atividade antimicrobiana bacteriostática por inibir a síntese proteica, apresenta boa difusão no interior das células tendo ótima ação contra bactérias intracelulares e ainda possui efeitos antirreabsortivos ao inibir a atividade da colagenase e dos osteoclastos^{22, 23}. A amoxicilina, que se caracteriza por possuir um anel beta em sua estrutura é de amplo

espectro de ação sendo eficaz contra bactérias gram-positivas e gram-negativas, vem sendo utilizada e testada com bons resultados desde o primeiro trabalho experimental realizado para verificar a influência do antibiótico nos casos de avulsão³.

Os resultados obtidos neste trabalho demonstram que a amoxicilina apresentou resultado numérico melhor em relação à tetraciclina nos itens avaliados. Estes achados corroboram estudos prévios de Melo et al.²⁰, que avaliaram os tecidos de sustentação do dente, e Gomes ET al.²¹, que estudaram esses mesmos antibióticos em reimplante tardio. Já Sae-Limet al.^{23,24}, que usaram os mesmos antibióticos em uma avaliação de reimplante tardio, mas em dentes com rizogênese completa, obtiveram resultados favoráveis a tetraciclina. Este fato pode ser explicado pela intensidade maior da reabsorção em dentes com rizogênese incompleta²⁶.

Aos sete dias no grupo avulsão, a camada de odontoblastos estava mais presente no grupo da amoxicilina do que nos outros dois grupos, ainda que de uma forma alterada, em estado de degeneração. Desta forma, os odontoblastos não foram capazes de exercer sua função e a dentina reparativa depositada foi semelhante para os grupos do antibiótico e pior para o controle. Na literatura, a formação de tecido mineralizado no interior da polpa é tratada como resultado da revascularização, que é dependente da ausência de bactérias no interior da polpa, alcançada pela aplicação tópica e sistêmica do antibiótico^{27, 28}. Nos demais períodos de observação, 15 e 30 dias, a camada de odontoblastos estava completamente ausente nos grupos tetraciclina e controle e no grupo amoxicilina ainda era visível em áreas restritas. Constatações de outros autores dão conta que o infiltrado inflamatório aumentava muito no período de 15 dias, o que levou à indicação de que o tratamento antibiótico prosseguisse até 14 dias²¹. No trauma da luxação extrusiva, o uso de antibiótico aparentemente não trouxe nenhuma melhora, pois todos os grupos apresentavam-se semelhantes.

A bainha epitelial de Hertwig permaneceu junto ao ápice radicular em muitos casos de luxação extrusiva, principalmente no grupo da amoxicilina, ainda que nenhuma diferença estatística tenha sido encontrada. Nos demais casos de luxação, pode ter havido o seu deslocamento do restante do dente, como descrito por Andreasen et al.²⁹, e quando o dente foi reposicionado ocorreu uma consolidação entre a raiz e o fragmento apical. Diferentemente, em todos os casos do trauma avulsão, não foi possível observar a bainha epitelial de Hertwig junto ao ápice, possivelmente em decorrência do próprio

trauma ou de dano no momento do reimplante dentário, sendo que somente fragmentos da bainha foram observados em alguns dentes, o que também foi descrito por Andreasen²⁹.

A influência da presença das bactérias no processo de reparo em dentes avulsionados, conforme relataram Nishioka et al.⁸, fica clara com a observação da presença de necrose e de infiltrado inflamatório periapical em todos os grupos controle negativo, isto é, no qual se administrou soro fisiológico, do trauma avulsão em todos os períodos de observação. O grupo da tetraciclina foi comparável ao controle negativo em todos os tempos para necrose e para o infiltrado inflamatório. Com o uso profilático da doxiciclina foi observado o mesmo efeito, ou seja, não foi possível evitar a contaminação nem eliminar microrganismos do tecido pulpar²⁷. Já no grupo da amoxicilina, o infiltrado inflamatório estava menos presente que nos demais grupos principalmente aos sete dias e ligeiramente melhor que os outros grupos nos demais períodos de observação. Isto vai de encontro com os achados Hammarstron et al.³, que demonstraram a diminuição da ocorrência de reabsorção radicular inflamatória, presumivelmente devido à redução de bactérias na superfície radicular e no interior da polpa. A necrose observada no grupo amoxicilina nos tempos de 15 e 30 dias, provavelmente está relacionada ao nível plasmático da droga que foi administrada até os sete dias, sendo alta no momento da administração e diminuindo progressivamente até a eliminação da corrente sanguínea²¹. Os resultados obtidos com o trauma de luxação extrusiva confirmaram a influência das bactérias, pois a contaminação dos tecidos envolvidos nesse tipo de trauma é menor e os resultados referentes à necrose e ao infiltrado inflamatório foram semelhantes, independentemente do uso ou não de antibiótico em todos os tempos. E o próprio trauma decorrente da luxação extrusiva é menor que aquele da avulsão, podendo ser dimensionado pela diferença estatística encontrada entre os respectivos controles negativos quando se observa a camada de odontoblastos, presença de necrose e de infiltrado inflamatório.

Os resultados deste estudo mostraram que, o uso de antibiótico é fundamental para o processo de reparo do tecido pulpar nos casos de avulsão de dentes com rizogênese incompleta e que há necessidade de mais estudos, para determinar qual o período ideal de prescrição.

CONCLUSÃO

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A avaliação microscópica demonstrou a importância do uso de antibióticos de uso sistêmico na reação pulpar em casos de reposicionamento e reimplante dentários imediatos em ratos. A hipótese testada foi parcialmente aceita, uma vez que a amoxicilina contribuiu favoravelmente para o reparo após a realização de reimplante subsequente à avulsão dentária, porém, isto não ocorreu com a tetraciclina. No reposicionamento dentário após luxação extrusiva, o uso de antibióticos não afetou o reparo obtido.

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REFERÊNCIAS

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ANEXOS


ANEXO A**APROVAÇÕES PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA
FACULDADE DE ODONTOLOGIA DE ARAÇATUBA UNESP**

unesp  UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Araçatuba

CERTIFICADO

Certificamos que o Projeto **"EFEITO DA ANTIBIOTICOTERAPIA SISTÊMICA
COM AMOXICILINA E TETRACICLINA EM REIMPLANTE DENTÁRIO IMEDIATO EM RATO"**
sob responsabilidade do(a) **Pesquisador(a) Sônia Regina Panzarini Barioni** e os
colaboradores **Moriel Evangelista Melo, Weglis Dyanne de Souza Gomes e Wilson
Roberto Poi** está de acordo com os Princípios Éticos da Experimentação Animal (CONCEA)
e foi aprovado pela CEUA em 01/07/2011 de acordo com o Processo 0763/2011.

Araçatuba, 04 de Julho de 2011


João Eduardo Gomes Filho
Presidente da CEUA

Faculdade de Odontologia e Curso de Medicina Veterinária – Departamento de Apoio, Produção e Saúde
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UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"



CAMPUS ARAÇATUBA
FACULDADE DE ODONTOLOGIA
FACULDADE DE MEDICINA VETERINÁRIA

CEUA - Comissão de Ética no Uso de Animais
CEUA - Ethics Committee on the Use of Animals

CERTIFICADO

Gerificamos que o Projeto de Pesquisa intitulado "Efeito da antibioticoterapia sistêmica com amoxicilina e tetraciclina no processo de reparo após injúria de luxação extrusiva", Processo FOA nº 2014-00491, sob responsabilidade de Sônia Regina Panzarini Barioni apresenta um protocolo experimental de acordo com os Princípios Éticos da Experimentação Animal e sua execução foi aprovada pela CEUA em 26 de Junho de 2014.

VALIDADE DESTES CERTIFICADO: 11 de Março de 2016.

DATA DA SUBMISSÃO DO RELATÓRIO FINAL: até 11 de Abril de 2016.

CERTIFICATE

We certify that the study entitled "Effect of systemic antibiotic therapy with Amoxicillin and Tetracycline in the repair after extrusive luxation", Protocol FOA nº 2014-00491, under the supervision of Sônia Regina Panzarini Barioni presents an experimental protocol in accordance with the Ethical Principles of Animal Experimentation and its implementation was approved by CEUA on June 26, 2014.

VALIDITY OF THIS CERTIFICATE: March 11, 2016.

DATE OF SUBMISSION OF THE FINAL REPORT: April 11, 2016.

Prof. Dr. Edilson Ervolino
Coordenador da CEUA
CEUA Coordinator

CEUA - Comissão de Ética no Uso de Animais
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ANEXO B

GUIA PARA AUTORES – DENTAL TRAUMATOLOGY

Author Guidelines

Content of Author Guidelines: 1. General, 2. Ethical Guidelines, 3. Submission of Manuscripts, 4. Manuscript Types Accepted, 5. Manuscript Format and Structure, 6. After Acceptance

Useful Websites: Submission Site, Articles published in Dental Traumatology, Author Services, Wiley-Blackwell's Ethical Guidelines, Guidelines for Figures

1. GENERAL

Dental Traumatology is an international peer-reviewed journal which aims to convey scientific and clinical progress in all areas related to adult and pediatric dental traumatology. It aims to promote communication among clinicians, educators, researchers, administrators and others interested in dental traumatology. The journal publishes original scientific articles, review articles in the form of comprehensive reviews or mini reviews of a smaller area, short communication about clinical methods or techniques and case reports. The journal focuses on the following areas **as they relate to dental trauma:**

Epidemiology and Social Aspects

Periodontal and Soft Tissue Aspects

Endodontic Aspects

Pediatric and Orthodontic Aspects

Oral and Maxillofacial Surgery / Transplants/ Implants

Esthetics / Restorations / Prosthetic Aspects

Prevention and Sports Dentistry

Epidemiology, Social Aspects, Education and Diagnostic Aspects.

Please read the instructions below carefully for details on the submission of manuscripts, the journal's requirements and standards as well as information concerning the procedure after a manuscript has been accepted for publication in *Dental Traumatology*. Authors are encouraged to visit Wiley-Blackwell Author Services for further information on the preparation and submission of articles and figures.

2. ETHICAL GUIDELINES

Dental Traumatology adheres to the following ethical guidelines for publication and research.

2.1. Authorship and Acknowledgements

Authors submitting a paper to the journal do so on the understanding that the manuscript has been read and approved by all authors and that all authors have agreed to submit the manuscript to the Journal. ALL authors MUST have made an active and significant contribution to the development of the concept and/or design of the study, and/or analysis and interpretation of the data and/or the writing of the paper. ALL authors must have

critically reviewed its content and must have approved the final version that is submitted to the journal for consideration for publication. Participation solely in the acquisition of funding or the collection of data does not justify authorship.

Dental Traumatology adheres to the definition of authorship set up by The International Committee of Medical Journal Editors (ICMJE). According to the ICMJE, the criteria for authorship should be based on: 1) substantial contributions to the concept and design of, or acquisition of data or analysis and interpretation of data, 2) drafting the article or revising it critically for important intellectual content, and 3) final approval of the version to be published. Authors should meet conditions 1, 2 and 3.

It is a requirement that all authors have been declared as appropriate upon submission of the manuscript. Contributors who do not qualify as authors should be mentioned under the Acknowledgements section on the title page.

Acknowledgements: In the Acknowledgements section, you can specify contributors to the article other than the authors. The acknowledgements should be placed on the title page, and not in the main document, in order to allow blinded review.

2.2. Ethical Approvals

Human Studies and Subjects

For manuscripts reporting medical studies that involve human participants, a statement identifying the ethics committee that approved the study and confirmation that the study conforms to recognized standards is required, for example: Declaration of Helsinki; US Federal Policy for the Protection of Human Subjects; or European Medicines Agency Guidelines for Good Clinical Practice. It should also state clearly in the text that all persons gave their informed consent prior to their inclusion in the study.

Patient anonymity should be preserved. When detailed descriptions, photographs, or videos of faces or identifiable body parts are used that may allow identification, authors should obtain the individual's free prior informed consent. Authors do not need to provide a copy of the consent form to the publisher; however, in signing the author license to publish, authors are required to confirm that consent has been obtained. Wiley has a standard patient consent form available for use.

Animal Studies

A statement indicating that the protocol and procedures employed were ethically reviewed and approved, as well as the name of the body giving approval, must be included in the Methods section of the manuscript. Authors are encouraged to adhere to animal research reporting standards, for example the ARRIVE guidelines for reporting study design and statistical analysis; experimental procedures; experimental animals and housing and husbandry. Authors should also state whether experiments were performed in accordance with relevant institutional and national guidelines for the care and use of laboratory animals:

- US authors should cite compliance with the US National Research Council's Guide for the Care and Use of Laboratory Animals, the US Public Health Service's Policy on Humane Care and Use of Laboratory Animals, and Guide for the Care and Use of Laboratory Animals.
- UK authors should conform to UK legislation under the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039).
- European authors outside the UK should conform to Directive 2010/63/EU.

2.3 Clinical Trials

Clinical trials should be reported using the CONSORT guidelines available at www.consort-statement.org. A [CONSORT checklist](#) should also be included in the submission material.

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Do not use sub-headings in the Discussion section, The Discussion should flow from one paragraph to the next in a cohesive and logical manner.

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Lam R, Abbott PV, Lloyd C, Lloyd CA, Kruger E, Tennant M. Dental trauma in an Australian Rural Centre. *Dent Traumatol* 2008; 24: 663-70.

Text book chapters:

Andreasen J, Andreasen F. Classification, etiology and epidemiology. IN: Andreasen JO, Andreasen FM, eds. *Textbook and Color Atlas of Traumatic Injuries to the Teeth*. 3rdEdn. Munksgaard, Copenhagen. 1994;151-80.

Thesis or Dissertation:

Lauridsen, E. Dental trauma – combination injuries. Injury pattern and pulp prognosis for permanent incisors with luxation injuries and concomitant crown fractures. Denmark: The University of Copenhagen. 2011. PhD Thesis.

Corporate Author:

European Society of Endodontology. Quality guidelines for endodontic treatment: consensus report of the European Society of Endodontology. *IntEndod J* 2006;39:921-30.

American Association of Endodontists. The treatment of traumatic dental injuries. Available at: URL:

'http://www.aae.org/uploadedfiles/publications_and_research/newsletters/endodontics_colleagues_for_excellence_newsletter/ecfe_summer2014%20final.pdf'. Accessed September 2015.

6. AFTER ACCEPTANCE

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APÊNDICES

APÊNDICE A

ARTIGO APRESENTADO NA QUALIFICAÇÃO E SUBMETIDO AO PERIÓDICO DENTAL TRAUMATOLOGY

MANUSCRIPT TYPE: Original Research Article

CALCIFIC METAMORPHOSIS OF PULP AFTER EXTRUSIVE LUXATION

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Running title: Luxation & calcific metamorphosis of pulp

Keywords: Tooth luxation, Dentin, Dental pulp, Calcific Metamorphosis

Conflicts of Interest

There are no conflicts of interest.

Suggested reviewer: Prof. Dr. Antonio Renato Lenzi, Email:
dr.rlenzi@gmail.com

CALCIFIC METAMORPHOSIS OF PULP AFTER EXTRUSIVE LUXATION

ABSTRACT

Background/Aim: The existent literature about the pathogenesis of extrusive dental luxation has been based on the reactions of periodontal tissues, without describing what specifically happens to the pulp tissue. The aim of this study was to perform microscopic evaluation of dental pulp response of rats submitted to extrusive luxation. **Material and Methods:** The maxillary right central incisors of 30 rats were luxated and repositioned after 5min. The animals were euthanized after 7, 14, or 30 days, respectively, comprising Groups I, II and III. Longitudinal histological sections were HE stained for histomorphometric analysis of the odontoblast layer, tertiary dentin deposition, Hertwig's epithelial root sheath kept together with the tooth, necrosis, and periapical inflammatory infiltrate. **Results:** In the majority of cases, concomitantly with vascular neoformation, tertiary dentin deposition occurred on the pulp walls and within the pulp. Over the course of time, in some cases this dentin occupied the entire pulpal space, and no other types of non-odontogenic hard tissues were observed. In the minority of cases, necrosis with the presence of periapical inflammatory infiltrate developed. No statistical differences were observed during the studied time intervals. **Conclusions:** In extrusive luxation, there is great possibility of the occurrence of calcific metamorphosis of the pulp. **Keywords:** Tooth luxation, Dentin, Dental pulp, Calcific Metamorphosis.

INTRODUCTION

Luxation commonly occurs in cases of dental traumatism, particularly in children and teenagers where loss or damage of a tooth has lifetime consequences. Extrusive luxation must be characterized by complete rupture of the neurovascular supply to the pulp and severance of periodontal ligament fibers¹. There is consensus that root development is decisive for the maintenance of pulp vitality by vascular neoformation, or the occurrence of

necrosis. Immature root development has shown the highest level of healing potential when compared with more mature root development^{1,2}. However, explanations about the pathogenesis of extrusive luxation, especially with reference to teeth with incomplete rhizogenesis, have been based on experiments, clinical reports, and studies about avulsion. There are no manuscripts about what specifically happens to the pulp tissue, although Miyashin et al.³ have cited the reactions caused by extrusive luxations in periodontal tissues, with brief citations relative to pulp tissue.

This gap perhaps exists because Andreasen⁴ described extrusive luxation as partial dental avulsion and Lee et al.⁵ considered that the pulpal outcome of severe extrusion may be comparable with that of a replanted tooth. In both avulsion and extrusive luxation, with rupture of the blood supply, hypoxia represented a stimulus to reparative phenomena in the face of trauma. Skoglund et al.⁶ and Fiane et al.⁷, speculated about the consequent revascularization, affirming that anastomoses could occur between the ruptured apical vascular supply terminals, which would initiate in the apical portion of the pulp, with the growth of vessels in the internal direction 4 days after immediate autotransplantation observed in only one dog tooth with incomplete rhizogenesis. Okiji⁸ affirmed that the dentin-pulp complex had singular type of defense and repair reaction that was not observed in the majority of the other connective tissues, which involved the formation of new mineralized tissue. This tissue might be formed by pulp cells and host mesenchymal cells in the pulp cavity⁹⁻¹³.

This study is justified since the pathogenic response to extrusive luxation remains uncertain, with the possibility of occurrence of revascularization (neovascular formation) and hard tissue formation has been described. To date, the literature does not specifically describe the pulp reaction to hypoxia resulting from extrusive luxation.

The aim of this study was to perform a microscopic evaluation of the dental pulp response of rats submitted to extrusive luxation.

MATERIAL AND METHODS

The Project received approval from the Ethics Committee on Animal Use of the School of Dentistry of Araçatuba – UNESP (Process 001876 2/2). Thirty male Wistar rats, ten weeks old, weighing between 280g and 300g were used. The animals were kept at the bioterium of the School of Dentistry of Araçatuba for the duration of the experiment, fed with solid food and water *ad libitum*, except during the 12-hour period preceding the surgery. For three days after surgery, the rats were fed on crushed rations.

For the surgery, the animals were anesthetized via intramuscular injection, using 0.6mg of xylazine chloride (Dopaser, HertapeCalierSaúde Animal Ltd., Juatuba, MG, Brazil) for every 100g body weight to induce muscular relaxation, followed by 0.7mg/100g of ketamine hydrochloride (Dopalen®, Agribrands Ltd., Paulinia, SP, Brazil). After this, antisepsis of the anterior portion of the maxilla was performed with Polyvinylpyrrolidone-iodine (PVP-I, Riodeine®, Ind. Farmaceutica Rioquímica Ltd., São José do Rio Preto, SP, Brazil), followed by syndesmotomy, luxation and displacement of 3mm in the axial direction of the maxillary right central incisor of each animal. After 5min, antisepsis of the traumatized area was performed once again, and the tooth was repositioned in its alveolus. There was no need to place a flexible splint as recommended by the IADT Guidelines² due to the anatomy of the rat tooth. Following this Guidelines², antibiotics were not administered.

The animals were separated into three groups according to the time elapsed after surgery:

Group I: 7 days

Group II: 14 days

Group III: 30 days

Two animals died during the experimental period (one from Group I and the other from Group II). After these time intervals, the animals were euthanized by anesthetic overdose. The right maxillae, containing the luxated tooth, was separated along the median line using a scalpel (Embramac, Campinas, SP, Brazil). A cut with straight scissors in the distal portion of the third molar made it

possible to obtain the specimen, which was then fixed in 10% formalin solution for 24h and decalcified with EDTA solution (10%, pH 7.0).

After decalcification, the pieces were processed and included in paraffin. Three histological semi-serial sections 6µm wide, were then sectioned longitudinally and HE stained for histomorphometric analysis.

Microscopic analysis was performed with an optic microscope (Axiolab, Carl Zeiss, Germany). Each specimen was examined in all segments of the pulp: coronal, middle and apical, as well as the periapical region (Fig. 1).

Figure 1

To analyze the histomorphological occurrences in each of the three groups, ordinal scores adapted from Nishioka et al.¹⁴ were attributed, ranging from absent to present in full. Five different events, according to the observations described by Fianeet et al.⁷ and Nishioka et al.¹⁴, were analyzed and listed below:

- A. Odontoblast layer
- B. Tertiary dentin deposition at: 1) coronal third; 2) middle third; 3) apical third
- C. Hertwig's epithelial root sheath maintained together with the tooth
- D. Necrosis
- E. Periapical inflammatory infiltrate

The images were captured with a digital camera (Leica DFC soofx, Leica microsystems, Heerbrug, Switzerland) coupled to the Carl Zeiss microscope (Axiolab) and connected to a computer equipped with digital image analyzing software (Leica Camera Software Box, Leica Imaging Manager - IM50 Demo software).

After collection, statistical analyses were performed using R software. Differences between groups were analyzed using nonparametric Kruskal-Wallis rank sum test and logistic regression. For both analyses, the level of significance of 5% was used.

RESULTS

The histomorphological occurrences in the quantity of the teeth of each of the three groups are presented in the Table 1. The statistical analyses showed no differences over the course of the studied time.

Table 1

Seven days after extrusive luxation. The odontoblast layer was present in six teeth, except for the three that already presented necrosis. Necrosis was considered when cariolysis was observed and it was not possible to observe the nucleus of cells. Intense inflammatory infiltrate was observed in the periapical region of pulps that suffered necrosis. Tertiary dentin deposition was observed in all the teeth, even those with necrosis, following the same direction of secondary dentin deposition; that is, in the coronal portion of the pulp with greater intensity and diminishing gradually up to the apical third. The pulp space was occupied by odontoblast cells permeated with a recently deposited matrix and with already mineralized areas (Fig. 2A). All the teeth showed tertiary dentin deposition in the coronal third, and in some of them, dentin deposition in the apical third was verified, apparently obstructing the foramen. In some areas, especially in the coronal and middle thirds, there was intense dentin formation, leading to the pulp cells being included in the neoformed tissue in an osteoid or osteodentinal pattern, giving the impression of concerning these tissues (Fig. 2A, and B). Islands of dentin with a layer of osteoblasts around them were seen in the midst of pulp tissue in some cases, and in others, cells with odontoblast characteristics could be identified distant from the habitual site (Fig. 2B). Some blood vessels were shown to be congested or with plasma (Fig. 2C). Hertwig's epithelial root sheath was preserved in the apical region in 3 teeth, isolated from the luxated tooth. In these cases, no necrosis was observed, or presence of periapical inflammatory infiltrate, but the formation of mineralized tissue consolidating the bond between the luxated root and the apical fragment containing the sheath (Fig. 2D).

Figure 2

Fifteen days after extrusive luxation. Dysplastic tertiary dentin was observed in a large part of the pulp space (Fig. 1 and 3C, and D). Columnar or polyhedral-shaped pulp cells were present, with polarized nuclei, morphologically suggesting a metaplasia for odontoblasts in the central zone of the pulp. In the samples in which the odontoblast layer was present, these cells appeared in larger numbers, even to the point of occupying the entire pulp space. Many of them presented hydrophobic change represented by plasmatic vacuolization (Fig. 3A, and B). There was greater dentin deposition on some canal walls than on others, and calcospherites and demarcating lines of reversion could be observed. Islands with randomized pattern of dentin deposition, demonstrated by the entangled basophilic coils, were visualized within a large portion of the pulp tissue (Fig. 3C, and D). Hertwig's epithelial root sheath was present in only one tooth that showed no necrosis or any periapical inflammatory infiltrate.

Figure 3

Thirty days after extrusive luxation. The odontoblast layer was present in half of the tooth samples. In the majority of cases, there was tertiary dentin deposition with almost complete obliteration of the pulp space (Fig. 2) and in continuity with the dentin wall (Fig. 2A, and C). In some of the teeth the dentin deposited was disorganized, with inclusion of cells and eventual islands of live pulp tissue (Fig. 2A, and B). In other there were no included cells (Fig. 2C e D).

Figure 4

DISCUSSION

The maxillary right central incisor of rats was used in this study, because it simulates human teeth with incomplete rhizogenesis and open apex. The use of this tooth involved a difficulty in performing the movement of luxation, due to its great curvature, which was overcome by means of careful manipulation. The

experimental extrusive luxation was performed manually, differently from the use of a device in rat molars³, whose induced luxation resembled that of the lateral type, due to the direction of force applied. By detaching the tooth 3mm manually, there was rupture of the neurovascular bundle. The experimental evaluation time intervals were determined, based on studies existent in the literature, about tooth luxation³ and reimplantation^{9,10,14} in rats, in which the dentin deposition began on the seventh day^{3,9,10} or after 14 days¹⁴.

In the literature about tooth luxation, there are epidemiological/review studies^{4,5,13}, of indications of treatment^{1,2,15}, and about the events that occur in the tissues involved³, however, no studies were found reporting the dental pulp response to the hypoxia resulting from ischemia as a consequence of rupture of the neurovascular bundle. What happens to the pulp while revascularization does not occur?

The repair process will begin in the apical region and advance towards the coronal region, depending on the pulp-periodontal interface, and may result in the maintenance of pulp vitality with vascular neoformation, or in the development of necrosis¹. After vascular neoformation, there will be mineralized tissue formation^{1,11}. Anastomoses may occur between the ruptured apical vascular supply terminals^{6,7}, with the growth of vessels in the internal direction after 4 days of autotransplantation, and after 10 days, vessels would be visible in the apical half of the canal⁶. Therefore, tertiary dentin deposition would only begin after 10 days, and in the apical portion of the pulp, where the so-called revascularization would begin. In this study, using rat incisor teeth, it was observed that at 7 days there had been tertiary dentin deposition in the coronal, middle and apical root thirds studied. Vascular neoformation and dentin deposition would occur almost concomitantly.

Tertiary dentin deposition followed the direction of secondary dentin deposition, with the coronal third presenting a larger quantity of hard tissue. The pattern of tertiary dentin deposition was always random or irregular, and thus, it could - microscopically - be called dysplastic dentin. While there were nutritional reserves, the pulp response involved the formation of new mineralized tissue in response to aggression^{17,18}. In some areas of the teeth of Groups I and II and

almost the entire canal in Group III, tertiary dentin deposition was observed in an intense and irregular manner, including pulp cells in the neoformed tissue with the microscopic morphology of osteodentinal tissue¹⁹, that is, an osteodentinal pattern. The explanation for this accelerated production of dentin would, presumably, be the loss of autonomous nerve and/or sensory control of the odontoblasts¹ or the attempt by cells to defend themselves from the aggression caused by the deficiency of oxygenation²⁰ resulting from the luxation suffered. In cellular biology, in the majority of times, an aggression could be considered a stimulus.

When specifically analyzing the pulp response to the hypoxia caused by the ischemia resulting from the extrusive luxation to which the rat incisor teeth were subjected, it was possible to observe that the mineralized tissue deposited was dentin. This was produced by both the odontoblasts that formed part of the pre-existent odontoblast layer, and those that were differentiated from the undifferentiated mesenchymal cells existent in the pulp, as has previously been described⁹⁻¹². Aggression caused to pulp cells by hypoxia leads to signaling for the production and release of growth factors that act by stimulating different types of cells^{16,20-22}. Consequently, the pulp cells undergo metaplasia, leading to all of them producing dentin in a disordered and random manner, distant from the pulp walls. In the literature the metaplasia suffered in the pulp is also commonly referred to as calcific metamorphosis of the pulp^{23, 24}, and this is more frequently associated with traumatism with a lower degree of repercussion, such as concussion or subluxation²³.

Studies have discussed the nature of neoformed tissue in pulp after trauma^{1,3,6,11,12}. By immunohistochemistry, Zhao et al.¹⁰ demonstrated the presence of osteopontin (OPN), bone sialoprotein (BSP) and dentinal sialoprotein (DSP) within the pulp of reimplanted or transplanted teeth, in patterns that differentiate them into bone-like and dentin-like tissues. However, studies have suggested that tertiary dentin has both dentinogenic and osteogenic characteristics that are mediated by TGF-beta¹²⁵, since BSP and OPN were consistently and clearly observed^{25,26}, and DSP in lower expression²⁶ or expressed by odontoblasts and odontoblast-like cells²⁵.

Even if the pulp as a whole were to be disorganized by the aggression suffered, in many regions of various teeth it was possible to observe organized odontoblast layers. They preserved the characteristics of a pseudo stratified epithelium and cells with intense secretory activity; that is, they were high, columnar and polyhedric, with polarized and basic nuclei. Many of them could be seen in the radicular region of the tooth, indicating that the secretory activity was shown to be increased. In many teeth, cells such as odontoblasts were seen producing dentin in the midst of pulp tissue, as islands, distant from their habitual site, and that were juxtaposed to the dentin wall.

It was possible to observe that blood vessels were shown to be densely congested. No inflammatory infiltrate was observed in the pulp, which was compatible with the absence of microbial contamination in the procedure. In these time intervals of observation, the inflammation was represented by the reparative phenomena in progress.

Hertwig's epithelial root sheath, even with all the care taken to perform the experimental luxation in the least traumatic manner possible, was shown to be nonexistent in the majority of the teeth in the sections examined. Possibly, in these cases, it was luxated together with the tooth, and compressed when the tooth was repositioned in the alveolus. However, no necrosis or associated periapical inflammatory infiltrate were observed. In some cases of folding of Hertwig's epithelial root sheath, an increment in dysplastic tertiary dentin deposition was observed. Odontoblast differentiation and the production of dentin are stimulated by mediators released by Hertwig's epithelial root sheath during normal odontogenesis¹⁹.

As shown by Andreasen; Andreasen¹, in the present study, in the majority of cases anastomosis of the vessels associated with vascular neof ormation occurred with concomitant tertiary dentin deposition, and no other types of hard tissues were observed. In the minority of cases, the result was development of necrosis resulting from the lower capacity of pulp tissue to respond to rupture of the neurovascular bundle, and bacterial contamination. This contamination would have occurred while the experimental procedure was

being performed, as suggested by Nishioka et al.¹⁴. When necrosis occurred, it was always accompanied by intense periapical inflammatory infiltrate.

Based on the histomorphological findings of the pulp response to hypoxia resulting from rupture of the neurovascular bundle caused by the extrusive luxation in rat teeth, there is great possibility of the occurrence of calcific metamorphosis of the pulp.

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LEGEND TO TABLE

Table 1. Quantity of teeth that presented the events evaluated in the histomorphological analysis in relation to the total number of the group teeth, according to the attributed ordinal scores (Kruskal-Wallis rank sum test and logistic regression analysis at 5% level of significance showed no differences).

LEGENDS TO FIGURES

Figure 1. Morphological aspects of longitudinal section of the rat maxillary right central incisor of Group II. H. E.

Figure 2. Main histomorphological occurrences in Group I (7 days after extrusive luxation). H. E. A) Tertiary dentin deposition (blue arrow) and pulp space occupied by odontoblast cells permeated with recently deposited matrix (orange color, white arrow) and other previously mineralized areas (basophilic, black arrow). B) Dentin island surrounded by odontoblasts (black arrow) or included in neoformed tissue (white arrow), with osteoid or osteodentinal pattern. C) Congested vessel (white arrow), with plasma (black arrow) and slightly disorganized but preserved odontoblast layer (blue arrow). D) Presence of Hertwig's epithelial root sheath (black arrow), with areas of tertiary dentin deposition with osteodentinal pattern (white arrow).

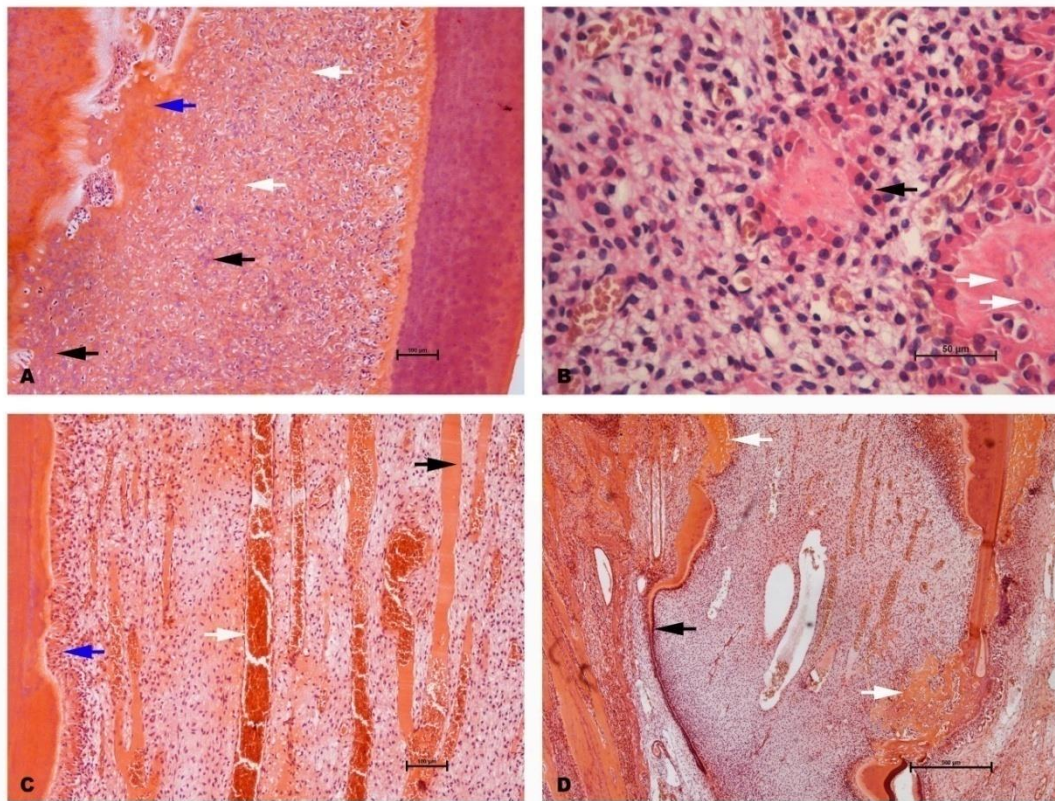
Figure 3. Main histomorphological occurrences at the Group II (15 days after extrusive luxation), with highlighted area. H. E. A and B) Osteoblast-like pulp cells. Congested vessel and hydrophic change in some cells, represented by plasmatic vacuolization. C and D) Dentin islands with odontoblasts in their peripheral regions, and some included within them. Demarcation line of reversion (black arrow) with calcopherites on the dentin pulp wall (white arrow). In D, the randomized dentinal deposition pattern was observed by the entangled basophilic coils that characterized the coloration of mineralized tissue.

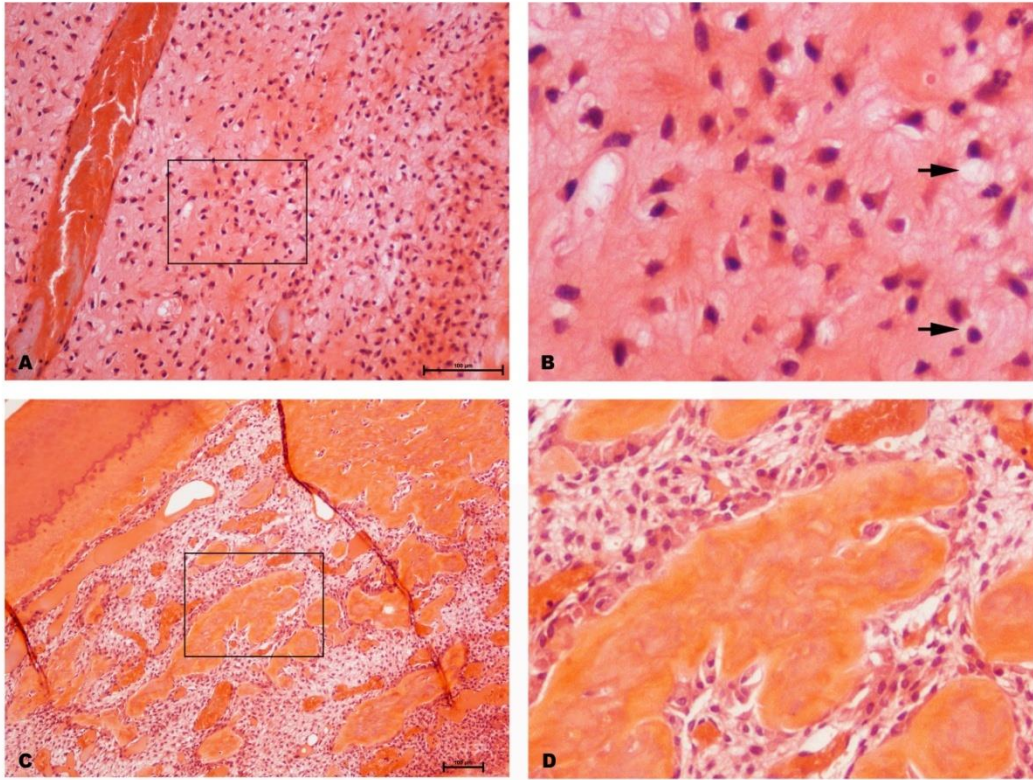
Figure 4. Main histomorphological occurrence at the Group III (30 days after extrusive luxation), with area highlighted. H. E. Dysplastic tertiary dentin almost completely occupying the pulp space in continuity with the dentin wall. A and B) Their disorganization is pointed out, with inclusion of cells (black arrow) and eventual islands of live pulp tissue (white arrow). C and D) Here without inclusion of cells.

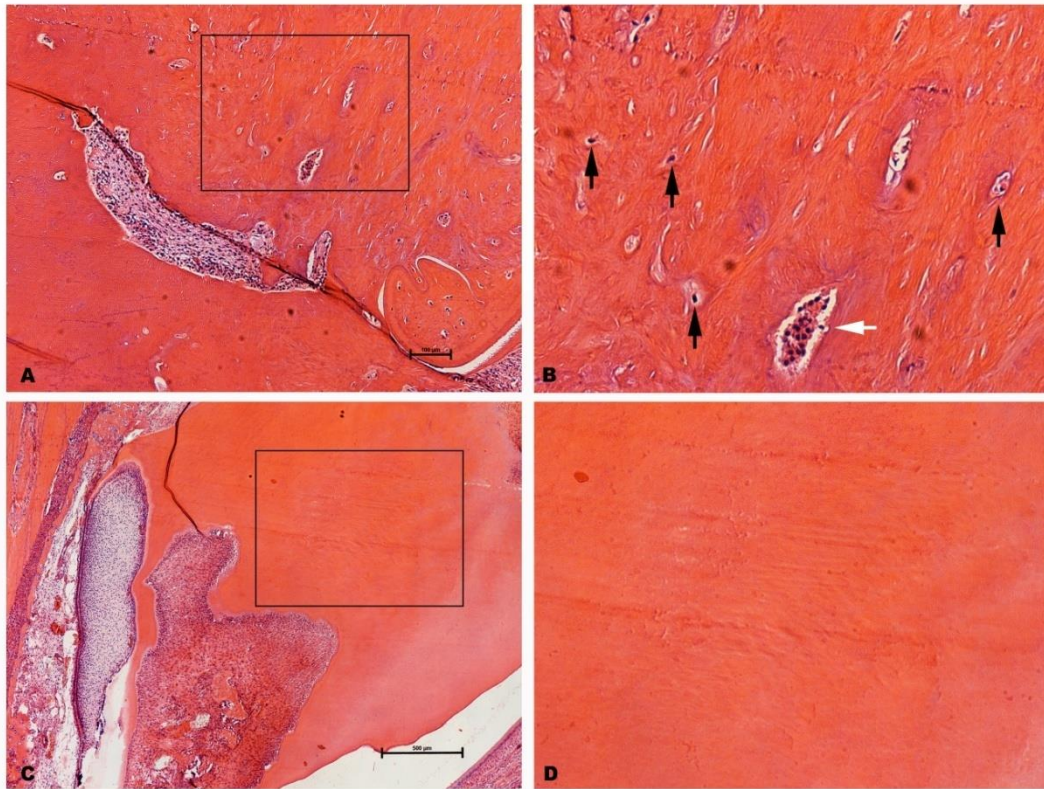
Time elapsed after extrusive luxation	Ordinal scores	Odontoblast layer	Tertiary dentin deposition in the root thirds			Hertwig's epithelial root sheath maintained together with the tooth	Necrosis	Periapical inflammatory infiltrate
			coronal	middle	apical			
7 days (n=9)	Absent	3/9	0/9	0/9	0/9	6/9	6/9	6/9
	Present in restricted area	0/9	0/9	2/9	8/9	na	0/9	0/9
	Partially present	4/9	7/9	6/9	1/9	na	1/9	0/9
	Fully present	2/9	2/9	1/9	0/9	3/9	2/9	3/9
14 days (n=9)	Absent	2/9	0/9	0/9	0/9	8/9	6/9	5/9
	Present in restricted area	2/9	0/9	2/9	6/9	na	0/9	1/9
	Partially present	3/9	5/9	6/9	3/9	na	0/9	0/9
	Fully present	2/9	4/9	1/9	0/9	1/9	3/9	3/9
30 days (n=10)	Absent	5/10	0/10	0/10	0/10	6/10	6/10	6/10
	Present in restricted area	1/10	1/10	2/10	6/10	na	0/10	0/10
	Partially present	0/10	4/10	5/10	2/10	na	1/10	0/10
	Fully present	4/10	5/10	3/10	2/10	4/10	3/10	4/10

na = not applicable

Faltou inserir a figura 1 que eu não tenho. A do corte longitudinal do dente







APÊNDICE B

ARTIGO APRESENTADO NA QUALIFICAÇÃO EM FASE FINAL DE PREPARAÇÃO PARA SER SUBMETIDO AO PERIÓDICO JOURNAL OF APPLIED ORAL SCIENCE

Luxação extrusiva e avulsão dentária: reações pulpares semelhantes?

Abstract

Luxação extrusiva e avulsão dentária têm em comum a ruptura do feixe vâsculo-nervoso. O reparo pós-luxação extrusiva seria semelhante aos estágios iniciais do reparo pós-avulsão? Objetivo: Analisar, comparativamente por meio de microscopia, a reação da polpa dental de ratos nos quais se promoveu a luxação extrusiva/reposição e a avulsão/reimplante. Material e métodos: Os incisivos centrais maxilares superiores de ratos foram luxados e reposicionados após 5min (n=30) ou extraídos, imersos em soro fisiológico por 5min e reimplantados (n=30). Os animais foram eutanasiados após 7, 15 ou 30 dias. Cortes histológicos longitudinais foram corados com HE para análise histomorfométrica da camada de odontoblastos, deposição terciária de dentina, bainha epitelial de Hertwig mantida junto com o dente, necrose e infiltrado inflamatório periapical. Conclusão: Na luxação extrusiva e na avulsão dentária houve a deposição de dentina terciária no interior do canal radicular desde que a polpa ou parte dela estivesse vital. A gravidade do trauma sofrido na avulsão dentária e a contaminação microbiana decorrente levaram à necrose pulpar, que determinou a estagnação da deposição de dentina terciária e a presença de infiltrado inflamatório periapical.

Keywords: Polpa dentária. Luxação dentária. Avulsãodontária.

Introduction

Explanations about the pathogenesis of extrusive luxation have been based in epidemiologic studies¹⁻⁴, or clinical reports^{5,6}. Authors had presented the findings of pulpal and periapical tissues after extrusive luxations⁷⁻⁹, and pulp healing after replantation¹⁰⁻¹⁶.

But are the pulp reactions to these two types of trauma similar? Do they differ only in intensity? Andreasen¹ described extrusive luxation as partial dental avulsion and Lee et al.⁴ considered that the pulpal outcome of severe extrusion is comparable with that of a replanted tooth.

In both, complete rupture of the neurovascular supply to the pulp causes an ischemia that affects all pulp cells. The hypoxia represents a stimulus to reparative phenomena in the face of trauma and to the maintenance of pulp vitality by vascular neoformation, or a partial or total necrosis may occur. In cases of incomplete rhizogenesis, the possibility of vascular neoformation through the primary apical foramen should be theoretically the same throughout root development, since the width of the primary apical foramen as well as the number of vessels entering it appears to be relatively constant until the final root length is reached¹⁷.

If there is a common rupture of the neurovascular supply in both avulsion and extrusive luxation, however, due to the complexity of avulsion trauma, there were several factors that influence the success of the repair procedure. Extra-alveolar time¹⁸, the storage medium of avulsed tooth¹⁸⁻²², root development stage²³, ligament damage degree^{24,25}, and the pulp condition¹⁷. It is also added that the presence of bacterial infection may be one of the main causes of complications in the repair process, resulting in pulp necrosis and inflammatory resorptions¹¹. Differently, in the extrusive luxation, the damage to the pulp and the root formation stage are the factors that may influence the repair².

Thus, the purpose of this work was to analyze, comparatively by microscopy, the reactions of the rat pulps in which the extrusive luxation/replacement or the avulsion/replantation was promoted. The

hypothesis tested is that the extrusive luxation causes a pulp reaction similar to that caused by avulsion in its early stages, in rat teeth.

Material and Methods

The Project received approval from the Ethics Committee on Animal Use of the School of Dentistry of Araçatuba – UNESP (Protocols 0763/2011 and 001876 2/2).

Sixty male Wistar rats (*Rattus norvegicus*, albinus), weighing between 150g and 300g were used. The animals were kept at the bioterium of the School of Dentistry of Araçatuba for the duration of the experiment, fed with solid food and water *ad libitum*, except during the 12-hour period preceding the surgery. For three days after surgery, the rats were fed on crushed rations.

For the surgery, the animals were anesthetized via intramuscular injection, using 0.6mg of xylazine chloride (Dopaser, HertapeCalierSaúde Animal Ltd., Juatuba, MG, Brazil) for every 100g body weight to induce muscular relaxation, followed by 0.7mg/100g of ketamine hydrochloride (Dopalen®, Agribrands Ltd., Paulinia, SP, Brazil). After this, antisepsis of the anterior portion of the maxilla was performed with Polyvinylpyrrolidone-iodine (PVP-I, Riodeine®, Ind. FarmaceuticaRioquímicaLtd., São José do Rio Preto, SP, Brazil). For extrusive luxation group, syndesmotomy, luxation, and displacement of 3mm in the axial direction of the maxillary right central incisor of each animal were done. After 5min, antisepsis of the traumatized area was performed once again, and the tooth was repositioned in its alveolus. For avulsion group, nontraumatic extraction of the maxillary right central incisor of all animals was performed. The tooth was immersed in saline for 5min and replanted. There was no need to place a flexible splint as recommended by the IADT Guidelines^{26,27} for both groups due to the anatomy of the rat tooth. Following these Guidelines, antibiotics were not administered for luxation group and intragastric tetracycline (Doxy® suspension; LaboratórioCepav Pharma Ltda, São Paulo, SP, Brazil) 2.5 mg/kg was administered 12/12h, for 7 days after tooth replantation.

The animals of both groups, extrusive luxation and avulsion, were separated according to the time elapsed after surgery: 7, 15, and 30 days. Four

animals died during the experimental period (extrusive luxation group 7 days and 15 days, and avulsion group 7 days and 15 days). After these time intervals past from surgery, the animals were euthanized by anesthetic overdose. The right maxillae, containing the luxated or replanted tooth, was separated along the median line using a scalpel (Embramac, Campinas, SP, Brazil). A cut with straight scissors in the distal portion of the third molar made it possible to obtain the specimen, which was then fixed in 10% formalin solution for 24h and decalcified with EDTA solution (10%, pH 7.0).

After decalcification, the pieces were processed and included in paraffin. Three histological semi-serial sections 6µm wide, were then sectioned longitudinally and HE stained for histomorphometric analysis.

Microscopic analysis was performed with an optic microscope (Axiolab, Carl Zeiss, Germany). Each specimen was examined in all segments of the pulp: coronal, middle and apical, as well as the periapical region.

To analyze the histomorphological occurrences in each group, ordinal scores adapted from Nishioka et al. were attributed, ranging from absent to present in full. Five different events, according to the observations described by Fianeet al.²⁸ and Nishioka et al.¹¹, were analyzed and listed below:

- A. Odontoblast layer
- B. Tertiary dentin deposition at: 1) coronal third; 2) middle third; 3) apical third
- C. Hertwig's epithelial root sheath maintained together with the tooth
- D. Necrosis
- E. Periapical inflammatory infiltrate

The images were captured using a high-resolution camera (Nikon, DS-File, Shimjuku, Japan) coupled to the optical microscope (Nikon, Eclipse80i, Shimjuku, Japan) and connected to a computer equipped with digital image analyzing software (Imaging Software Nis Elements, version 4.0).

After collection, statistical analyses were performed using R software. Differences between groups were analyzed using One-way ANOVA and Two-way ANOVA, level of significance of 5%.

Results

The histomorphological occurrences in the quantity of the teeth of each group are presented in the Table 1. The extrusive luxations group was statistically different from avulsed group to all studied parameters. On the other hand, no statistically significant differences were observed between the times analyzed.

Table 1- Quantity of teeth that presented the events evaluated in the histomorphological analysis in relation to the total teeth of each group, according to the attributed ordinal scores (One-way ANOVA and Two-way ANOVA at 5% level of significance showed no differences)

Time elapsed after extrusive luxation or avulsion	Ordinal scores	Odontoblast layer		Tertiary dentin deposition in the root thirds						Hertwig's epithelial root sheath maintained together with the tooth		Necrosis		Periapical inflammatory infiltrate		
				Coronal		Middle		Apical								
		EL	A	EL	A	EL	A	EL	A	EL	A	EL	A	EL	A	
7 days (n=9, each group)	Absent	3/9	8/9	0/9	0/9	0/9	0/9	0/9	0/9	0/9	6/9	9/9	6/9	1/9	6/9	0/9
	Present in restricted area	0/9	0/9	0/9	0/9	2/9	4/9	8/9	9/9	na	na	0/9	0/9	0/9	1/9	
	Partially present	4/9	1/9	7/9	9/9	6/9	5/9	1/9	0/9	na	na	1/9	0/9	0/9	0/9	
	Fully present	2/9	0/9	2/9	0/9	1/9	0/9	0/9	0/9	3/9	na	2/9	8/9	3/9	8/9	
15 days (n=9, each group)	Absent	2/9	9/9	0/9	0/9	0/9	0/9	0/9	0/9	8/9	9/9	6/9	0/9	5/9	0/9	
	Present in restricted area	2/9	0/9	0/9	0/9	2/9	4/9	6/9	9/9	na	na	0/9	0/9	1/9	0/9	
	Partially present	3/9	0/9	5/9	7/9	6/9	5/9	3/9	0/9	na	na	0/9	0/9	0/9	0/9	
	Fully present	2/9	0/9	4/9	2/9	1/9	0/9	0/9	0/9	1/9	na	3/9	9/9	3/9	9/9	
	Absent	5/10	9/10	0/10	0/10	0/10	0/10	0/10	0/10	6/10	10/10	6/10	0/10	6/10	0/10	

30 days (n=10, each group)	Present in restricted area	1/10	0/10	1/10	0/10	2/10	8/10	6/10	10/10	na	na	0/10	0/10	0/10	0/10
	Partially present	0/10	0/10	4/10	8/10	5/10	2/10	2/10	0/10	na	na	1/10	0/10	0/10	0/10
	Fully present	4/10	0/10	5/10	2/10	3/10	0/10	2/10	0/10	4/10	na	3/10	10/10	4/10	10/10

EL= extrusive luxation, A= avulsion, na = not applicable

Figure 1 highlights some findings comparatively between extrusive luxation and avulsion, presenting in a visually facilitated way.

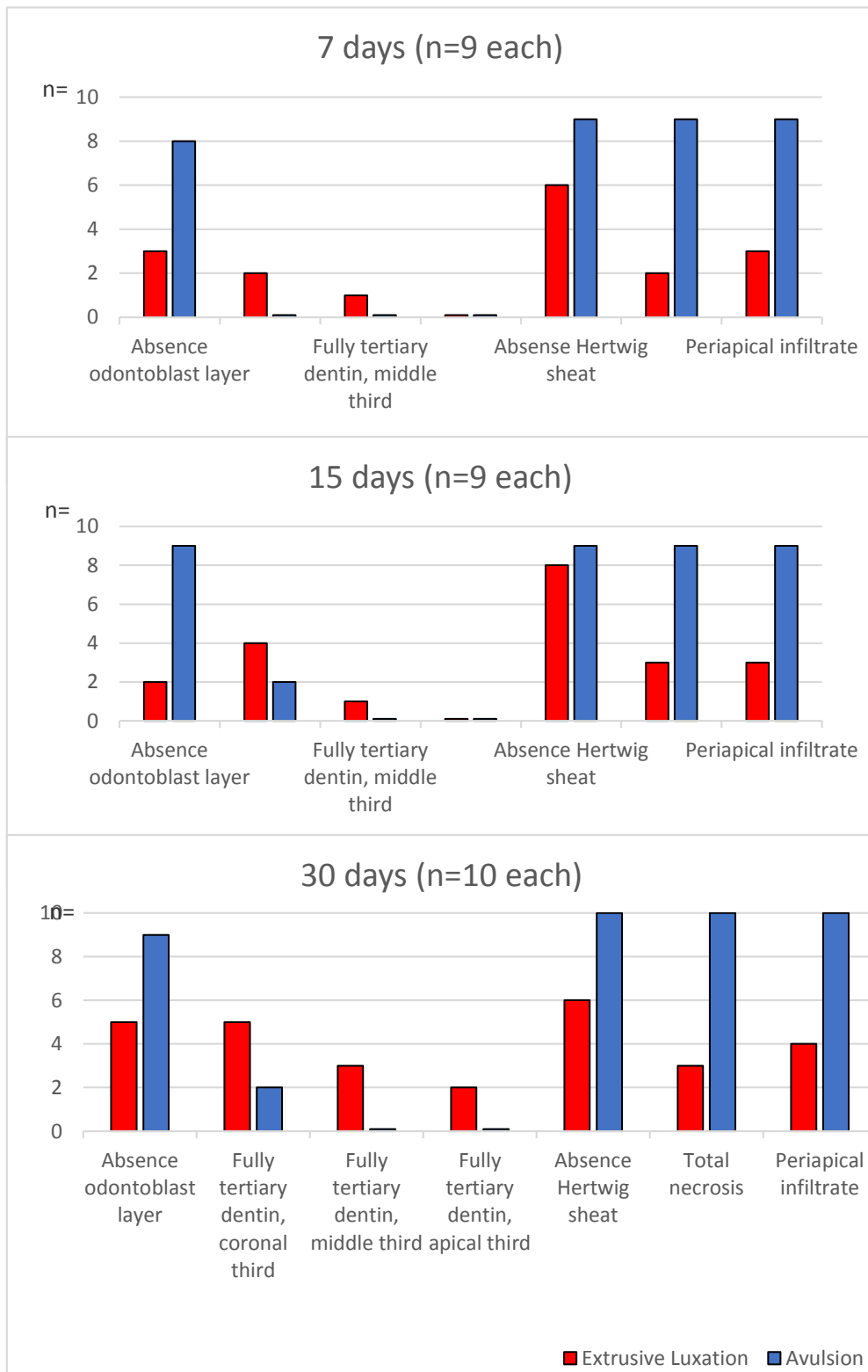


Figure 1- Comparative findings of extrusive luxation and avulsion group.

Seven days after extrusive luxation or avulsion procedures. Na Fig.2A é possível observar o aspecto geral do que ocorreu em todos os dentes do grupo, nos quais aproximadamente 2/3 do canal apresenta-se calcificado e que esta calcificação se iniciou na coroa dirigindo-se apicalmente. O tecido pulpar restante se encontra aparentemente normal, sem reação inflamatória periapical. No grupo Avulsão (Fig.2B), observa-se que houve uma tentativa de formação de tecido duro principalmente no terço coronário do dente e diminuição gradativa nos terços médio e apical. Nota-se uma reação inflamatória periapical, que existiu em todos os dentes desse grupo. A camada de odontoblastos aparenta estar próxima da normalidade na maioria dos dentes do grupo Luxação (setas brancas das Fig.2A1 e A2), o mesmo valendo para a polpa como um todo, com muitas células sugerindo ser, morfológicamente, odontoblastos e com vasos sanguíneos congestos e alguns com soro (setas pretas). Na região periapical (Fig.2A2), fragmento da Bainha Epitelial de Hertwig (seta azul) se encontra deslocada da raiz e foi responsável pela formação de um tecido mineralizado (seta amarela) consolidando a união entre a raiz e o fragmento. No grupo Avulsão (Fig.2B1), a camada de odontoblastos estava ausente na maioria dos dentes ou se mostrando completamente alterada, com grande número de células apresentando núcleos com cariorrexis, com a cromatina adquirindo uma distribuição irregular, e outras com cariólise, perda da coloração do núcleo, sinais de necrose (seta branca). Na Fig.2B2 é possível observar a ocorrência de picnose na maioria das células do tecido pulpar e também a ausência de vasos sanguíneos (setas brancas).

7 dias

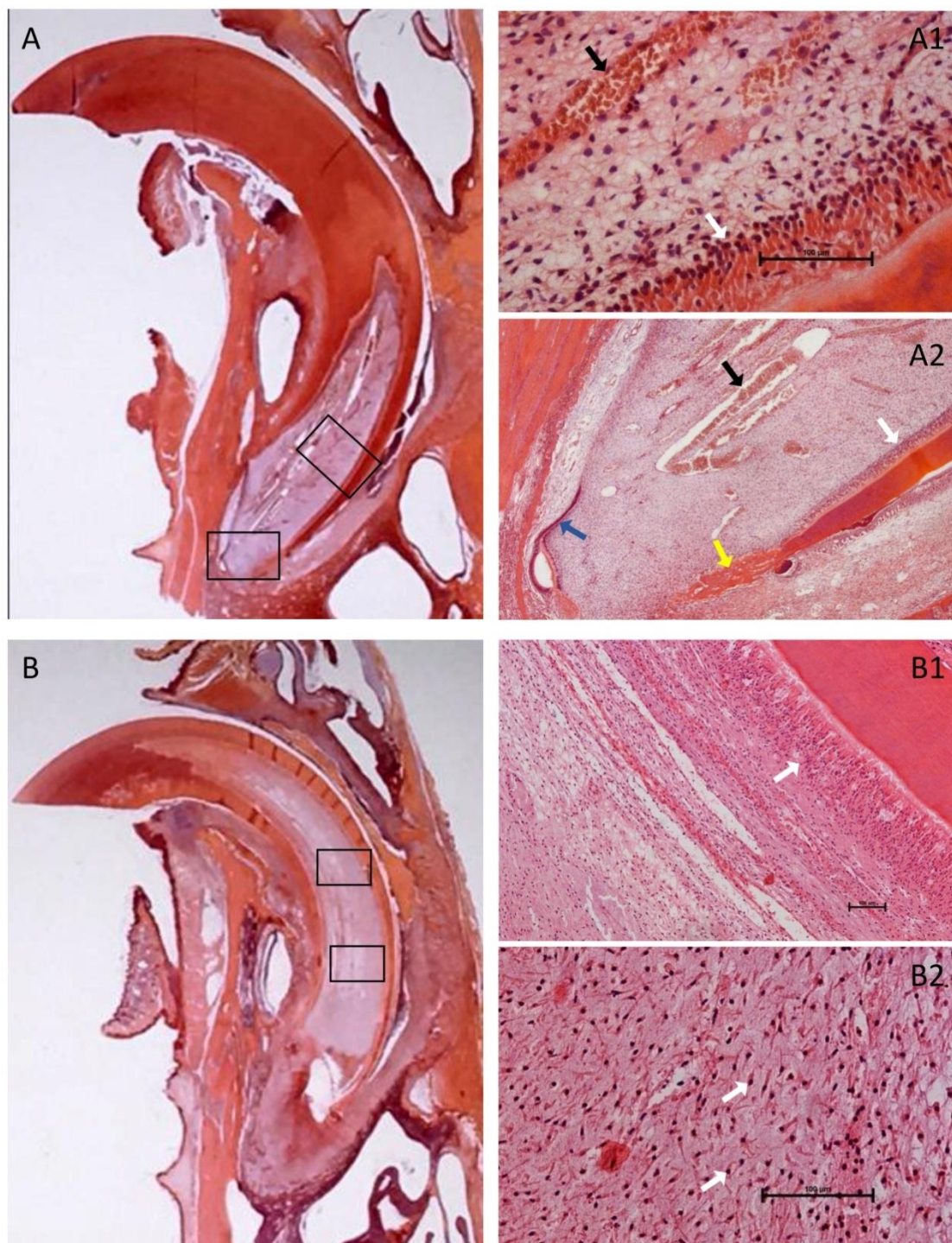


Figure 2-Aspectos gerais e destaques dos dentes reposicionado (A, A1 e A2) e reimplantado (B, B1 e B2) após 7 dias.

Fifteen days after extrusive luxation or avulsion procedures. Observa-se calcificação de aproximadamente 2/3 do canal no grupo Luxação (Fig.3A), com ilha de tecido degenerado incluso na calcificação no 1/3 coronário e a deposição ocorreu com maior intensidade no sentido coroa ápice. No grupo Avulsão (Fig.3B), o espaço pulpar estava preenchido no terço coronário por tecido calcificado e com tecido necrosado nos terços médio e apical, com presença de reação inflamatória periapical. A Fig.3A1 destaca a camada de odontoblastos com grande número de células (seta branca), tecido pulpar aparentemente normal, mas mostrando grande número de vasos congestos (setas pretas) no grupo Luxação, enquanto no grupo Avulsão, o tecido pulpar se encontrava completamente necrosado com todas as células com cariólise (Fig.3B1, seta vermelha) e intensa reação inflamatória (seta lilás) apical com presença de abscesso agudo (Fig.3B2, seta azul). A Bainha Eitelial de Hertwig preservada em fragmento apical da raiz principalmente quando ocorria fratura apical pode ser observada na Fig.3A. Neste caso ponto de consolidação entre raiz e fragmento apical pode ser visto na fig.3A2 (seta verde). Ainda pode ser visto grande número de vasos sanguíneos congestos no tecido pulpar correspondente a raiz (setas pretas) e ausência de vasos congestos na região apical. Há ilha de dentina com odontoblastos ao entorno e a camada de odontoblastos apresentava-se preservada (seta amarela).

15 dias

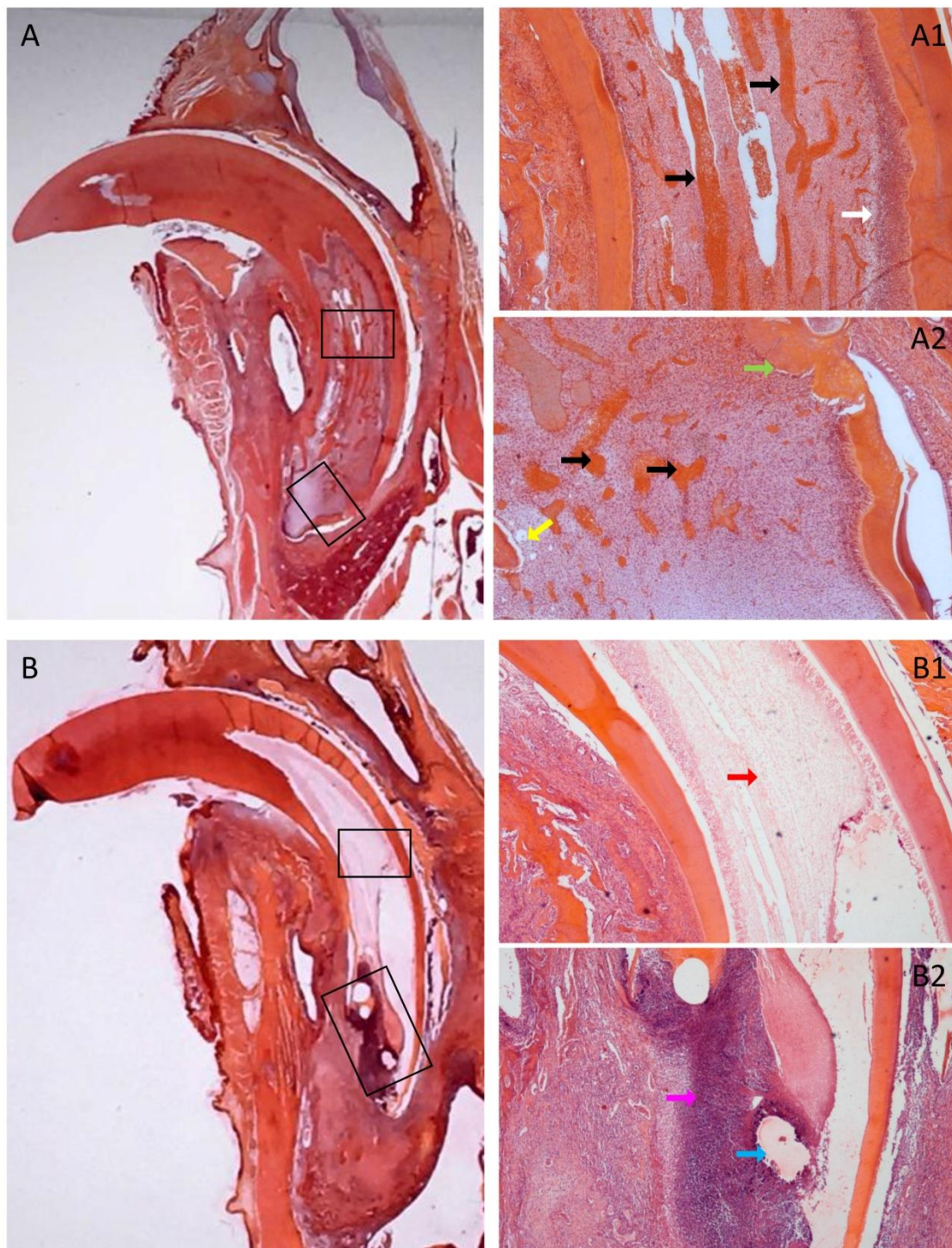


Figure 3 - Aspectos gerais e destaques dos dentes reposicionado (A, A1 e A2) e reimplantado (B, B1 e B2) após 15 dias.

Thirty days after extrusive luxation or avulsion procedures. No grupo Luxação (Fig. 4A), na maioria dos casos, o canal estava quase que completamente calcificado, com exceção de pequena ilha de tecido degenerado no terço coronário e ilhas de tecido vivo (seta branca) no terço apical com odontoblastos na margem da dentina (Fig. 4A2,). Esse tecido aparenta normalidade (Fig. 4A1), com a presença da camada de odontoblastos e de grande número de células, deposição de dentina de forma irregular com formação de ilhas de dentina no tecido pulpar (seta lilás), vasos sanguíneos congestos (setas pretas). No grupo Avulsão (Fig. 4B), estava mantida a observação dos 15 dias, com o terço coronário calcificado limitado pelo avanço da necrose pulpar presente nos terços médio e apical (seta vermelha) (Fig. 4B1), com presença de reação inflamatória periapical (Fig. 4B2, seta lilás),

30 dias

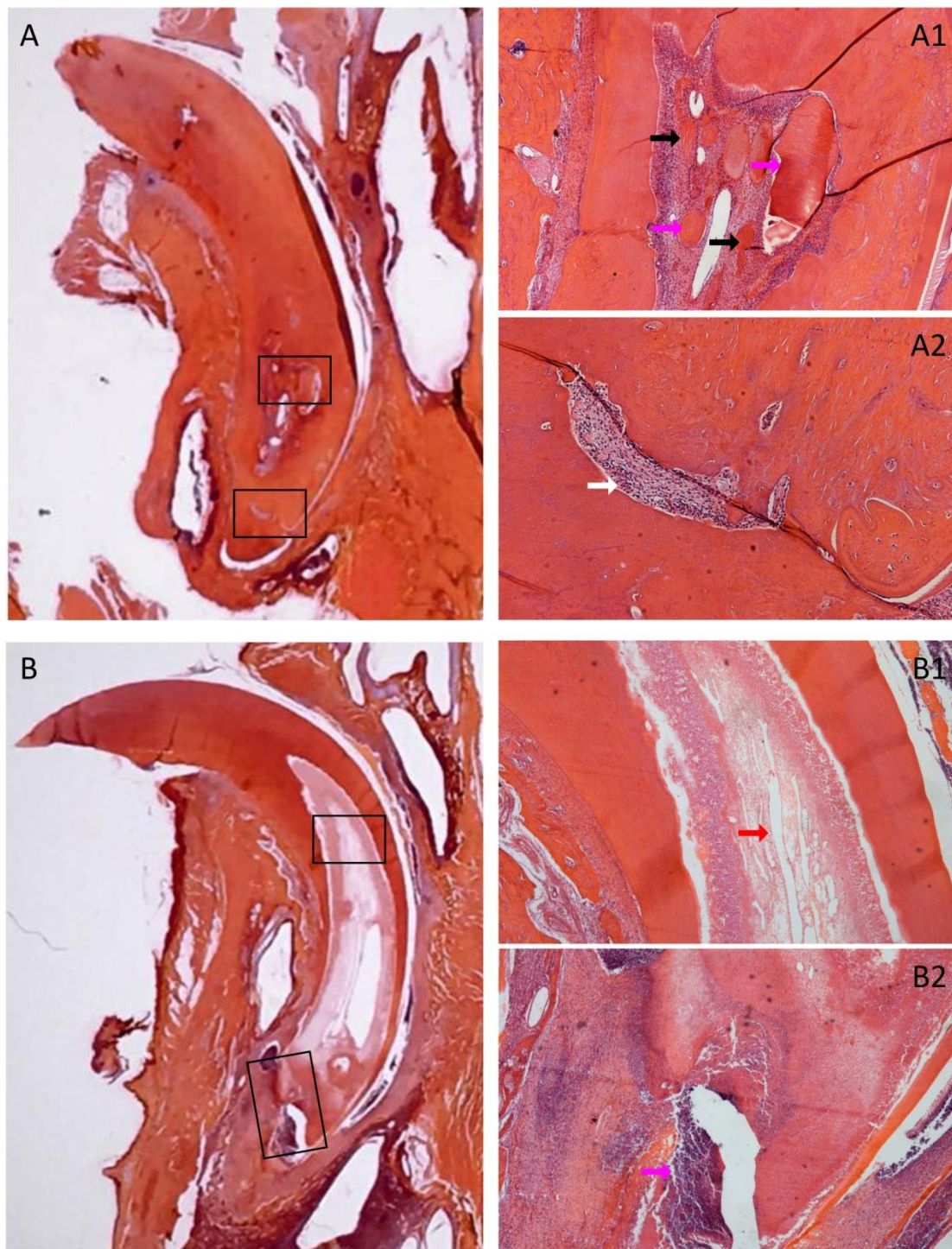


Figura 4 - Aspectos gerais e destaques dos dentes reposicionado (A, A1 e A2) e reimplantado (B, B1 e B2) após 30 dias.

Discussion

A escassez de manuscritos descrevendo especificamente o que acontece na luxação extrusiva, possivelmente se deve à baixa complexidade do procedimento de reposicionamento do dente, realizado pelo cirurgião dentista clínico, sem necessidade de um centro especializado. No entanto, sabe-se da dificuldade encontrada para o estabelecimento de um plano tratamento e os procedimentos a serem adotados nessas lesões traumáticas menos graves²⁹. Já o reimplante dental subsequente a uma avulsão de dentes permanentes é considerado um procedimento complexo, sendo o prognóstico muito dependente das ações realizadas imediatamente após a avulsão^{17,22,27}. A gravidade da lesão e a complexidade do procedimento a ser adotado proporcionam intensa pesquisa e publicação a respeito. Muitas vezes, a partir dela, explica-se e extrapola-se o que sucederia na luxação extrusiva, já que em ambas acontece o rompimento do feixe vículo-nervoso.

Estas lesões traumáticas de deslocamento, seja parcial ou total para fora do alvéolo, ocorrendo em dentes que apresentem rizogênese incompleta, podem levar à necrose pulpar ou à uma resposta frente ao trauma na forma de deposição de tecido mineralizado no interior do canal radicular^{1,9,15}. Os resultados encontrados corroboram essas duas vias. Foi possível observar a deposição de dentina já aos sete dias após a realização dos procedimentos, quando a polpa se mantinha vital, com maior intensidade no terço coronário e diminuindo progressivamente em direção ao terço apical nos dois grupos. A necrose foi observada em alguns casos de luxação extrusiva e na quase totalidade dos casos de avulsão, subsequentemente.

A deposição de dentina no interior do canal radicular é considerada um sinal de cura da polpa dentária nos casos de ruptura do feixe vículo-nervoso^{13,31}. Entre os fatores que influenciam para que ou ocorra a deposição de tecido duro ou a necrose da polpa, cita-se o tipo da lesão^{4,14}. Quanto maior o dano à polpa, menor a chance de cura, de resultar em calcificação pulpar. Foi possível observar que a polpa se manteve vital na maioria dos casos de luxação extrusiva, sendo a deposição de dentina muito mais intensa do que nos casos de avulsão em que ela ocorreu sempre parcialmente. Assim foi possível observar que nos grupos de luxação extrusiva houve maior número de calcificações pulpares do que nos grupos de avulsão onde a necrose foi preponderante.

A infecção é uma das principais causas de complicações pós-reimplante dental, como pode ser a necrose pulpar¹⁷. O ligamento periodontal de um dente avulsionado pode tornar-se contaminado por microrganismos da cavidade bucal, meio de armazenamento ou por qualquer outra coisa a que tenha sido exposto durante o período extra-alveolar³¹. Tal fato foi observado nesta pesquisa, na qual a maioria dos

dentes do grupo avulsão, de todos os tempos estudados apresentou necrose e infiltrado inflamatório na região periapical. Já na luxação extrusiva, verificou-se um número bem reduzido de contaminação microbiana e de infiltrado inflamatório.

A formação radicular é determinada pela atividade da bainha epitelial de Hertwig e durante a luxação extrusiva, a avulsão ou no reposicionamento/reimplante pode ocorrer dano a ela²³. Neste trabalho foi possível observar que, em poucos casos do grupo da luxação extrusiva, a bainha epitelial de Hertwig permaneceu no ápice radicular de forma normal. Nos demais, pode ter havido o seu deslocamento do restante do dente³⁰ e quando o dente foi reposicionado ocorreu uma consolidação entre a raiz e o fragmento apical. Diferentemente, em todos os casos do grupo da avulsão, não foi possível observar a bainha epitelial de Hertwig junto ao ápice, possivelmente em decorrência do trauma ou de dano no momento do reimplante dentário e somente fragmentos da bainha foram observados em alguns dentes.

Existe controvérsia no que diz respeito ao tipo de tecido mineralizado formado na obliteração do espaço pulpar. Segundo Andreasen¹⁷, na maioria dos casos, forma-se um tecido semelhante a cimento, osso ou osteodentina sobre as paredes do canal radicular. Shimizu et al.¹², Tsukamoto-Tanaka et al.¹³ e Zhao et al.¹⁴ demonstraram por imuno-histoquímica que matrizes semelhantes a osso e a dentina são formadas na cavidade pulpar após o reimplante. Já nos casos de luxação extrusiva, Miyashin, Kato e Takagi⁷ descreveram que uma dentina irregular foi depositada no interior do canal radicular, porém, na maioria dos trabalhos há somente a descrição da obliteração do canal radicular, sem determinar que tipo de tecido é formado¹⁻³. De acordo com a análise microscópica realizada e com base no aspecto morfológico celular, não foi possível observar outro tecido que não fosse dentina⁹. De maneira evidente, no grupo da luxação extrusiva no qual a polpa se manteve predominantemente vital, ocorreu em maior número de casos a deposição de dentina terciária e conseqüentemente, maior obliteração do canal. No grupo da avulsão, onde ocorreu maior número de necrose da polpa, houve deposição de dentina terciária até determinado ponto, ou seja, enquanto ainda havia parte de tecido vital. A partir do momento da instalação da necrose, cessou a deposição de dentina terciária.

Verificou-se que os achados observados aos 15 dias após a realização dos procedimentos são muito semelhantes daqueles verificados aos 7 dias. Com isso, sugere-se ser desnecessário a inclusão de tal tempo de análise nos próximos estudos.

Conclusion

Foi possível concluir que, tanto na luxação extrusiva como na avulsão dentária, a reação da polpa dental de ratos se deu da mesma forma, ou seja, com a deposição de dentina terciária no interior do canal radicular desde que a polpa ou parte dela estivesse vital. A gravidade do trauma sofrido na avulsão dentária e a contaminação microbiana decorrente levaram à necrose pulpar, que determinou a estagnação da deposição de dentina terciária e a presença de infiltrado inflamatório periapical. A hipótese nula testada, que a luxação extrusiva provoca uma reação pulpar semelhante daquela provocada pela avulsão nos seus estágios iniciais, em dentes de ratos, não pode ser rejeitada.

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