

Commercial swine model: an alternative for direct pulp capping studies

Modelo de suíno comercial: uma alternativa para estudos de capeamento pulpar direto

Abstract

Purpose: To test the use of commercial swine as an *in vivo* model for studying pulp healing in response to direct capping agents.

Methods: Six swine *sus scrofa domesticus* (Landrace X Large White) were used in the experiment. Under anesthesia, class V cavities were prepared with a pulp exposure, which was directly capped with hard-setting calcium hydroxide (Dycal®, Dentsply, Petrópolis, Rio de Janeiro, Brazil). Restorations were performed with resin-modified glass ionomer cement (Vitremmer® 3M Espe St Paul, MN, USA). After 7 or 70 days (n = 6), the animals were died; the teeth were extracted, formalin-fixed and prepared for histological evaluation. The biological response was categorized using the following criteria: inflammatory response, soft tissue organization, reactionary and, reparative dentin formations.

Results: After seven days pulp in most specimens inflammatory response was present and after 70 days reparative dentin was formed at the pulp exposure area in almost all the animals. These responses were similar to those previously reported in human in similar periods of evaluation. In addition, the swine model showed another advantages: easy handling and economical feasibility compared to other animal models.

Conclusion: The commercial swine model seems to be an adequate animal model to test pulp biocompatibility.

Key words: Animals; swine; primary dentition; dental pulp capping

Resumo

Objetivo: O objetivo do estudo foi analisar o uso de suínos industriais como modelo para a avaliação da resposta pulpar a procedimentos de capeamento direto.

Metodologia: Em 6 suínos *sus scrofa domesticus* foram realizados preparos cavitários classe V até expor o tecido pulpar. Após a hemorragia ter sido controlada, o local exposto foi capeado com cimento de hidróxido de cálcio (Dycal®, Dentsply, Petrópolis, Rio de Janeiro, Brasil) e na sequência os dentes foram restaurados com cimento de ionômero de vidro (Vitremmer® 3M Espe St Paul, MN, USA). As respostas teciduais foram analisadas após 7 (n = 6) e 70 (n = 6) dias. Os animais foram abatidos, os dentes extraídos e, posteriormente, processados para análise morfológica descritiva quanto aos seguintes critérios: resposta inflamatória, organização do tecido pulpar, dentina reacional e reparativa.

Resultados: Após 7 dias observou-se a presença de infiltrado inflamatório e decorridos 70 dias, dentina reparativa foi formada na área de exposição em quase todos os espécimes, sendo estes achados similares aos achados previamente reportados em capeamentos realizados em humanos. Além disso, o modelo demonstrou outras vantagens, tais como a viabilidade econômica (baixo custo comparado a outros modelos animais) e facilidade de manuseio.

Conclusão: O uso de suínos de raças comerciais pode ser considerado como alternativa para os testes de biocompatibilidade dentino-pulpar.

Palavras-chave: Animais; dentição decídua; capeamento da polpa dentária

Antônio César Leites^a
Adriana Fernandes da Silva^b
Sandra Beatriz Chaves Tarquinio^b
Flávio Fernando Demarco^b
Thomas Normanton Guim^c
Evandro Piva^b

^a Instituto de Biologia, UFPel, Pelotas, RS, Brasil

^b Faculdade de Odontologia de Pelotas, UFPel, Pelotas, RS, Brasil

^c Programa de Pós-graduação da Faculdade de Medicina Veterinária, UFPel, Pelotas, RS, Brasil

Correspondence:

Adriana Fernandes da Silva
Rua Anchieta, 1533/702
Pelotas, RS – Brasil
96015-420
E-mail: adrisilvapiva@gmail.com

Received: April 20, 2009

Accepted: September 8, 2009

Introduction

A sequence of tests has been recommended to evaluate the biocompatibility of the restorative and/or liner materials regularly used in clinical Dentistry. There are different test categories and between them, preclinical application tests are considered to be of remarkable importance, involving the direct and indirect pulp capping, pulpotomy and application test in endodontic (1,2). The preclinical application tests have been used in animal labs, like guinea pigs, to assess the biocompatibility of new dentistry materials or new techniques (2). Biocompatibility can be defined as the capacity of one material to cause an expected outcome in an organism or life tissue (1). A favorable outcome of a direct pulp capping comprehends a stimulation of the pulp tissue to form reparative tertiary dentin, without alter the sound aspect of remaining vital pulp tissue (1).

As a biological model, there are several similarities between swine and human being: height, structure and internal organs, feeding pattern, gastric enzymes, endocrine system and dental characteristics (3,4). In a macroscopic study of the anatomic characteristics of the temporomandibular joint of dogs, cats, rats, rabbits, sheep, goats, cows, and swines, it was observed that only swine model could be selected as an optimal animals joint model (5). In addition, like human beings, the swines are omnivore, presenting similar masticatory system, adapted to process large variability of foods (6,7). Due to the similar phylogeny, the swine model has been largely used as study model in medical area, with potentiality to be used in dentistry research. However, when analyzing the scientific literature regarding preclinical tests of biocompatibility, mainly in cases of direct pulp capping (8,9), only miniaturized pigs have been used. Despite the favorable handling compared to normal swines, they have a high cost and lack of availability in the Brazilian market.

Otherwise, in the Brazilian market, and mainly, in the Rio Grande do Sul State, there is high availability of the commercial swine breed, which would present potential to be used as model for preclinical tests, considering especially its low cost compared to others animal models (beagle dogs, monkeys and minipigs).

Therefore, the aim of this study was to evaluate the use of commercial swine (*Sus scrofa domesticus*) as alternative model for direct pulp capping test, using calcium hydroxide as dressing material, and following the International Organization for Standardization 7405 guidelines (2).

Methodology

Experimental design

After local Ethics Committee in Animal Experimentation (Federal University of Pelotas) approval (Document 03/2005), animals were selected from and university farm (Federal University of Pelotas). Animals should be aged

around 105 days (± 10 days), weighting around 40 kg (± 5 kg). The swines used in this study were *Sus scrofa domesticus* species from of the commercial crossbreed conventionally used in the agricultural production system (Landrace X Large White).

Lower primary teeth were used only from the right hemi arch: 1st and 2nd incisors and 2nd and 3rd premolars (Fig. 1A and 1B) of 6 swines, which were divided in two periods of post-operative analysis: 7 and 70 days (according to ISO 7405:1997 guidelines). Two groups were analyzed with n=12 for period: HC_7d and HC_70d.

Clinical Procedures

Initially, animals underwent anesthesia, with an association of acepromazine (0.1 mg/kg), midazolam (0.3 mg/kg) and cetamin (4 mg/kg), which was injected intramuscle. After 15 minutes of application, anesthetic induction was carried out with sodium thiopental (12 mg/kg) by a catheter 22G fixed to the marginal vein of the ear. The animals were intubated with endotracheal tube number 8 for anesthesia maintenance connected to a closed system of inhalation anesthesia for administration of halothane in universal vaporizer. After the intervention, the swine were extubed in the moment that it was observed the return of laryngotracheal reflex. After that, animals were maintained in contention cage in a peaceful environment to avoid anesthesia complications. The post operative analgesia was administered with cetoprofen (2 mg/kg) by intramuscle route. The anesthetic procedure was carried out and supervised during the pulp capping by a veterinarian in the surgery room of a Veterinary Clinical Hospital (Federal University of Pelotas).

Class V cavity preparations on buccal surface without rubber dam isolation, with diamond bur #1013 in high speed, under refrigeration for enamel removal (Fig. 1C), and after this, a carbide bur #330 was used for dentin removal and to obtain the pulp exposition (Fig. 1D), which remained restricted to the bur diameter. To obtain haemostasia, the cavity was gently cleaned with saline solution. Hard-setting calcium hydroxide cement was applied (Dycal®, Dentsply, Petrópolis, Rio de Janeiro, Brazil) on the exposition. The cavities were restored with resin-modified glass ionomer cement (Vitremmer® 3M Espe St Paul, MN, USA) (Table 1), and a light-curing unit XL2500 (3M Espe St Paul, MN, USA) was used during the experiment. All of these clinical procedures took approximately 2 hours per animal and the anesthetic pos-operative recovering another 2 hours.

During the experiment, the animals were kept under confinement in single cages measuring approximated 4 m². The diet was constituted by normal food to grow swine. Water was available to drink *at libitum*, and the cage cleaning was daily performed.

Animal deaths and histological processing

The slaughter of the animals was carried out in a conventional manner as happens in the meat processing plant, under the regulation of inspection services, where electric shock desensitized the animal, and then bleeding was performed.

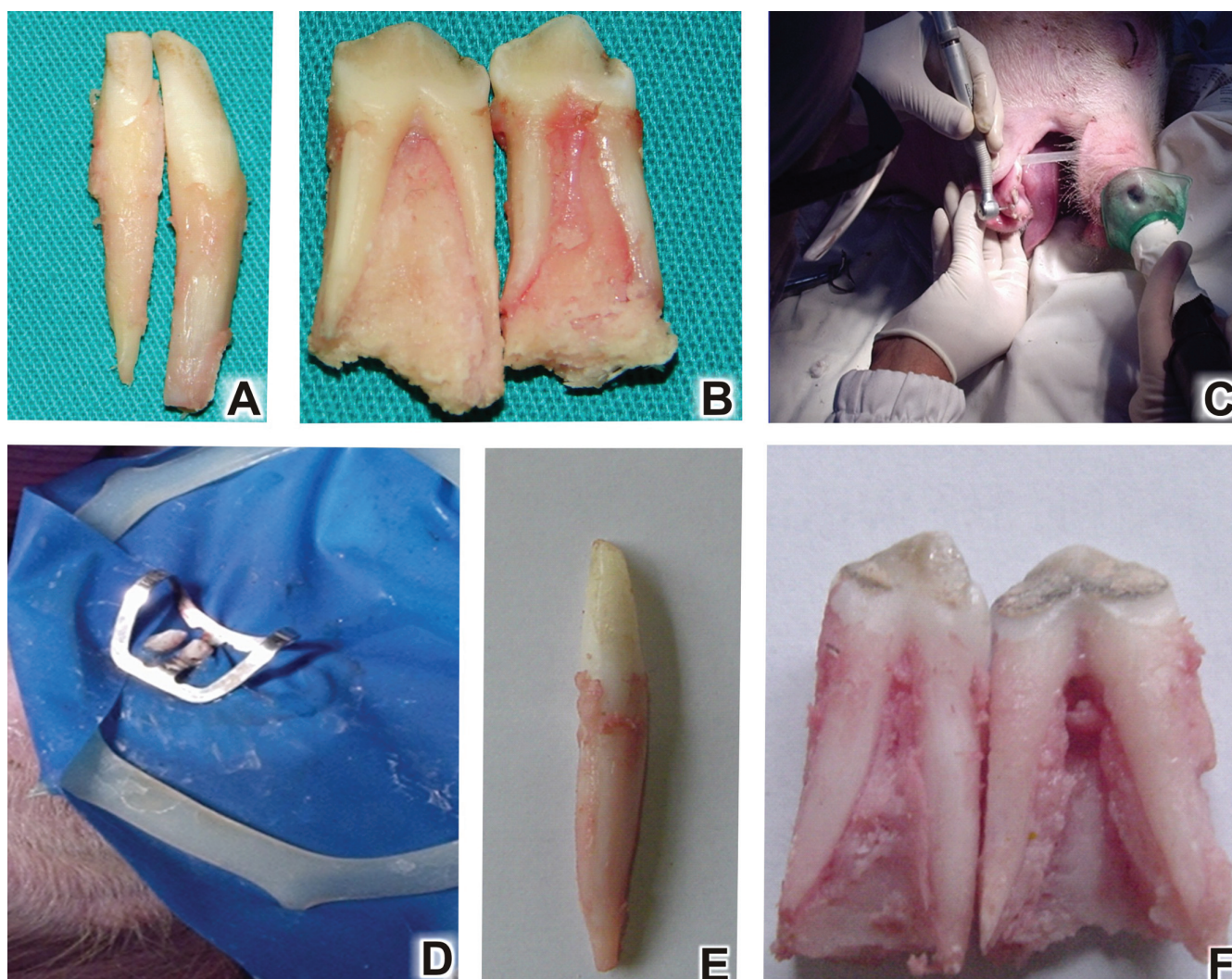


Fig. 1. Picture showing anatomic shape of lower primary teeth and many clinical procedures performed: **(A)** The anatomic shape of the 1st and 2nd incisors of slaughtered swine at 4 months of age. **(B)** This figure highlights the anatomical shape of the 2nd and 3rd swines premolars in the same period in "A". **(C)** Notice the easy access achieved to perform the class V preparation. **(D)** It is observed the easy access for rubber dam isolation before the imminent exposition. **(E)** Swine tooth at 6 months in the moment of the slaughter. There is no signal of root resorption. **(F)** This image represents the same period of "E", showing that the 2nd e 3rd premolars also do not present indication of root resorption.

Table 1. Chemistry composition, manufacturer and batch number of the materials used.

Groups	Capping material/ Manufacturer and batc	Restorative material/ Manufacturer and batch	Period of valuation
HC7d or HC_70d	Dycal® Dentsply*, Petrópolis, RJ, Brazil. 20905	Vitremer® 3M Espe†, St Paul, MN, USA. Powder: 4JH, Liquid: 4CN and Primer: 4AU	7 or 70 days

* Composition according to manufacturer. Base paste: glycolsalicylate ester, calcium phosphate, calcium tungstate, zinc oxide and mineral stains. Catalytic Past: n-ethyl-4-toluenesulfonamide, calcium hydroxide, zinc oxide, titanium dioxide, zinc estereate and mineral stains.

† Composition according to manufacturer. Powder: aluminiumsilicate fluor crystals, potassium persulfate, ascorbic acid and stains. Liquid: polyalkenoic acid, methacrylates groups, water, 2-hydroxyethylmethacrylate, canforoquinone. Primer: copolymer of the polyalkenoic modified acid, methacrylates groups, ethanol, and canforoquinone.

Exodontias were carried out immediately. The primary teeth were extracted observing the pre-established evaluation time (7 or 70 days). The extraction of each treated tooth was performed together with hard and soft tissues removal in a single block, using hand saw. These procedures should be carried out up to 10 minutes after the animal death to provide a quick immersion in formaldehyde in order to facilitate the tissue fixation. Two-thirds of the teeth root were sectioned with a diamond bur, to facilitate fixation agent penetration inside the pulp chamber for 48 hours. Later, the teeth were demineralized in 20% formic acid, during 3 to 4 weeks. Posteriorly, the teeth were transversally sectioned longitudinally through the center of the class V cavity. Pulp crown portion could be histologically analyzed in buccolingual direction and the radicular pulp of the specimens was hemi sectioned parallel to the frontal axis (coronoradicular direction) (Fig. 2). Subsequent to dehydration in ascending chain of ethanol and diaphanized in xylene, the teeth were embedded in paraffin and 5 micrometers serial sections were performed. Then, sections were stained alternatively with hematoxylin-eosin (HE) technique (histological evaluation) or with Brown & Hoops technique (10), in order to observe the presence of Gram positive and Gram negative bacteria.

Histological analysis

The histological sections were examined in light microscopic by 2 trained examiners, based on previously established criteria (ISO 7405 (2) and Akimoto et al. (11).

Results

Morphological analysis at 7 days (HC_7d): in most of the specimens it was observed the presence of inflammatory infiltrate, predominantly polymorphonuclear in the site of the exposition, under the superficial necrotic layer, ranging from mild to moderate intensity (Fig. 3A and B). There was also variation in relation to the extension of the inflammation within the pulp tissue. Edema, vasodilatation, hyperemia, angiogenesis and hemorrhage focuses were also frequently findings in the coronal pulp. A localized tissue loss was observed in almost all specimens, restricted to site of the exposition, under the superficial necrosis, which ranged from narrow to moderate. The beginning of reparative dentin formation was occasionally detected (2 specimens). Morphological analysis at 70 days (HC_70d): in almost all the specimens there was no signal of inflammation, and when present this inflammatory response was mild. It was also observed pulp retraction at the site of the exposition. In many specimens it could be observed the presence of necrotic superficial zone and also accidental angiogenesis, vasodilatation and hyperemia. All specimens showed extensive reparative dentin deposition (Fig. 3E). Eventually, the presence of reparative dentin was observed in the cervical portion of the radicular pulp (Fig. 3G). The reparative dentin had a mixed morphological pattern, sometimes osteodentin and in another irregular tubular dentin (Fig. 3F). The reparative dentin showed, frequently, continuous with the reational dentin. Odontoblast-like cells were seen underlining the dentin barrier.

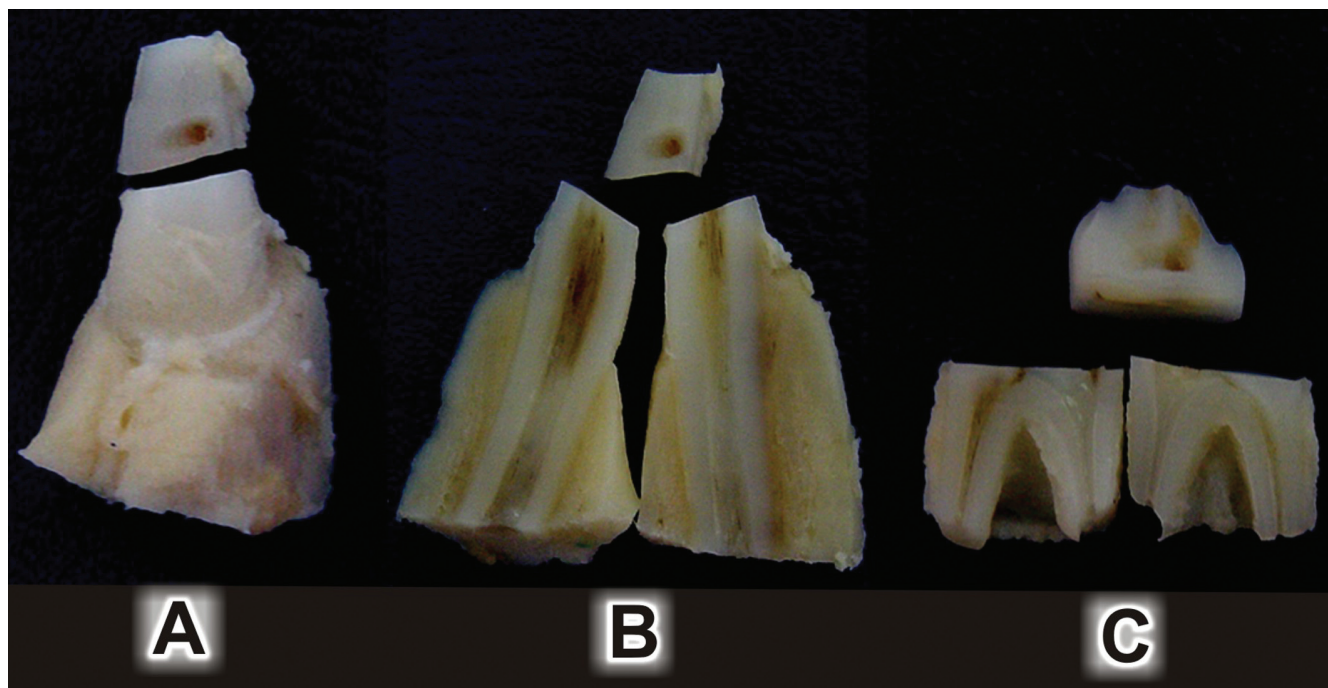


Fig. 2. It demonstrates the sections done along the tooth axis. (A) Lower incisor. In this analysis it is possible to observe the transversal section performed on class V cavity, separating the crown from the root. (B) Lateral incisor. Section performed in the root to analyze pulp: there was a section parallel to the frontal axis in all root extension. (C) The 2nd lower premolar with a section done in a similar manner than in "2B".

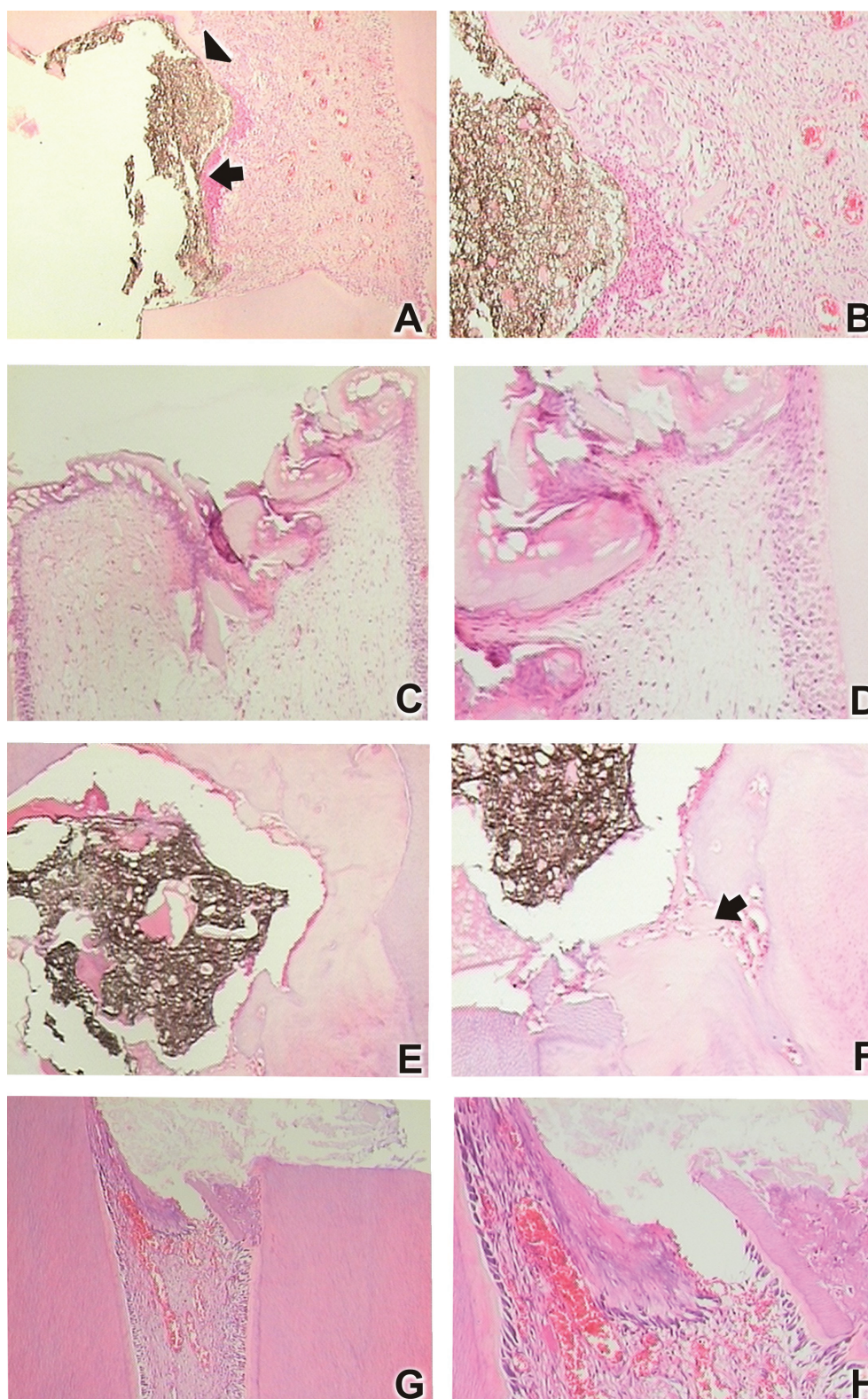


Fig. 3. Image of different histological sections at 7-day (A-D) and 70-day intervals (E-H) after direct pulp capping with CH. (A) Coronal pulp demonstrating the area of the exposition, that exhibits focal polymorphonuclear inflammatory infiltrate, which is superficially located (arrow), close to a focus of dentin barrier in initial phase of formation (head of arrow). Hyperemia is also noted (40X). (B) Higher magnification of "3A" (100X). (C) Same specimen of 3A, but demonstrating the radicular pulp. It is observed the absence of inflammatory infiltration in this location (40X). (D) Higher magnification from "3C" (100X). (E) Visualization of pulp crown at the 70 days. It is possible to observe the deposited reparative dentin region in the area of pulp exposition (40X). (F) Higher magnification of E (100X), being possible to observe a tunnel in the dentin barrier (arrow). (G) Radicular pulp at 70-day interval. Notice that the vitality is maintained (40X). (H) Higher magnification from "G" (100X). All sections were stained by Hematoxylin-eosin technique.

The final analysis was carried out with 6 specimens, due to problems during histological procedures, which will be elucidated in the discussion section.

Discussion

The overall analysis of histological findings revealed a pulp response for swine teeth similar to the findings observed in human teeth, in direct pulp capping studies using calcium hydroxide as dressing material (12,13). An initial inflammatory response was observed in the exposure site, which was resolved after 70 days, with the formation of dentin barrier.

Animals as monkeys, ferrets and minipigs have been recognized for use in pulp capping tests according to ISO 7405 (2); however, in Brazil these animal models have high cost, since they need special cares and they are not so available, been frequently imported (14). In the present study, animals from the commercial crossbreed were used, which are usually found in small farms and agricultural schools with commercial purpose, as well as in bioterium for scientific researches.

Although ISO 7405 (2) recommended the use of permanent teeth in the evaluation tests, the choice for primary teeth in this experiment was due to the fact that first permanent teeth erupt from 4 to 6 months, which would coincide with the second period of evaluation (after 70 days) and the swine would be with excessive height and weight, becoming difficult their maintenance and the handling, with we have chosen permanent teeth. In addition, some studies have demonstrated that more significant pulp alterations in the primary teeth are related to advanced stages of the root resorption (15,16). It is important to point out that primary teeth used in this experiment had complete apex and no evidence of any resorption (Fig. 1E and 1F).

In 2004, a survey was done on online databases (Medline, Lilacs, SciELO and Cochrane) (17) evaluating the use of different animals models. In Lilacs and SciELO scientific bases, in the period from 1998 to 2002, the largest number of studies employed dogs, followed by rabbits, swines and primates, and these results were probably due to the large availability of dogs in Latin America. Nevertheless, dogs, erroneously, are still seen as an animal model more similar to humans, but when taken in consideration the phylogeny, anatomy and physiology; dogs present smaller similarities when compared to swine (17). Moreover, other advantage of the swine model adopted is that it does not need periods of time larger than those required for meat processing plants in commercial swineculture (18), in order to use the animal for consumption. The only recommendation, in this case, would be the necessity of waiting for up to 96 hours after the administration of auxiliary anesthetics and drugs before the slaughter, providing enough time for the elimination of these drugs (19).

In the present research, there was not interference in the necessary minimum periods for the slaughter. According to

Standards of Ethical Principles for Animal Experimentation of COBEA (*Colégio Brasileiro de Experimentação Animal – Lei Federal nº 6638 (08/05/1979)*), there is not mention of contraindication for consumption of animal meat used in research. Nevertheless, it is very important that the researcher common sense about the use or not of the swine meat, depending the methodology used and the type of material that would be tested. Therefore, the administration of drugs and materials or even not well-known procedures, it would be recommended to avoid the human consumption of the animal meat.

Other important factor to be mentioned is about the care with utensils that, sometimes, are used to decrease the stress of the animal, as iron chains, which are placed in the cages. After long periods of monitoring (70 ± 5 days) the anterior teeth presented accentuate incisal wear, impairing their use to evaluate the histological response in this study. It was observed, during the experiment, that removing the chains, the wear did not happen, been this technical procedure important to be carried out in order to avoid loss of incisors for further analysis. Furthermore, since was an initial experimental protocol for our team, other teeth were also lost due to the root fracture during the exodontics. These are the reasons for the reduction of 'n' from 12 to 6 for each period of time.

According to swine protocol adopted, it is important to point out the facility of access to make the preparation in the teeth used in relation to mouth opening after the general anesthesia. The choice of the teeth (lower incisor and the premolar) used in this study was also for the similarities with the human clinical crown. On the other hand, the upper incisors were almost entirely non-erupted in the cavity, with a very small clinical crown (20,21), impairing their use for experimental procedures. Canines were not used in this study, because they were not still erupted.

Direct pulp capping, indirect pulp capping or pulpotomy tests are used to assess the biocompatibility of dental materials (2). This kind of studies precedes the use of new materials and drugs in human before usage in clinical practice. Many of these studies are carried out in animal primary teeth, mainly in dogs (22-24). The lack of references using the biological model presented in our study of biocompatibility perhaps is due to the difficulties that can be found as handling and maintenance of a big animal.

Other advantages, beyond the acceptable pulp-dentin response, were the economic availability due to low cost of acquisition of this species when compared to models as minipigs, beagle and monkeys; lower cost in the maintenance during the period of the research and, also, the use of meat for human consumption.

The challenge in exploring new methods using animal models provides basic information of swine breeding, anatomic aspects, and eruption chronology that could be useful for several multidisciplinary investigations. Other interesting fields could be also examined with this new model here presented: organogenesis studies, which can

be performed firstly in teeth, and then in other organs; restorative materials with desirable properties to be used in veterinary application and others.

Finally, as in other animal models, the comparison with humans should be carefully analyzed, since each animal model has its particular physiology and that may accelerate or reduce the repair responses, for example.

Conclusions

The overall analysis of our findings demonstrate that the alternative model using commercial swine for the evaluation of pulp capping repair seems to be a viable model, presenting a similar pattern response to the response obtained in human beings with calcium hydroxide.

References

- Browne RM. Animal tests for biocompatibility of dental materials – relevance, advantages and limitations. *J Dent* 1994; 22:S21-4.
- ISO-7405. Dentistry: Preclinical evaluation of biocompatibility of medical devices used in dentistry-test methods for dental materials. International Organization for Standardization; 1997:1-18.
- Malavasi LNG. Anestesia e analgesia em suínos utilizados como modelo experimental. *Rev Cons Fed Med Vet* 2004;31:23-7.
- Pond WG, Houpt KA. The biology of the pig. In: Miller ER, Ullrey DE. *The pig as a model for human nutrition*. New York: Comstock; 1987. p.693-706.
- Bermejo AO, Gonzalez O, Gonzalez JM. The pig as an animal model for experimentation on the temporomandibular articular complex. *Oral Surg Oral Med Oral Pathol* 1993;75:18-23.
- Weaver ME, Sorenson FM, Jump EB. The miniature pig as an experimental animal in dental research. *Arch Oral Biol* 1962; 7:17-24.
- Herring SW. The dynamics of mastication in pigs. *Arch Oral Biol* 1976;21:473-80.
- Oguntebi BR, Heaven T, Clark AE, Pink FE. Quantitative assessment of dentin bridge formation following pulp-capping in miniature swine. *J Endod* 1995;21:79-82.
- Nakamura Y, Hammarstrom L, Matsumoto K, Lyngstadaas SP. The induction of reparative dentine by enamel proteins. *Int Endod J* 2002;35:407-17.
- Brown RC, Hopps HC. Staining of bacteria in tissue sections: a reliable gram stain method. *Am J Clin Pathol* 1973;60:234-40.
- Akimoto N, Momoi Y, Kohno A, et al. Biocompatibility of Clearfil Liner Bond 2 and Clearfil AP-X system on nonexposed and exposed primate teeth. *Quintessence Int* 1998;29:177-88.
- Silva AF, Tarquinio SB, Demarco FF, Piva E, Rivero ER. The influence of haemostatic agents on healing of healthy human dental pulp tissue capped with calcium hydroxide. *Int Endod J* 2006;39: 309-16.
- Piva E, Tarquinio SB, Demarco FF, Silva AF, de Araujo VC. Immunohistochemical expression of fibronectin and tenascin after direct pulp capping with calcium hydroxide. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2006;102:e66-71.
- Junqueira JL, Ubatuba FB. Espécies de animais empregados em experimentação laboratorial; 2006. [Acessado em 28 de abril de 2006]. Viável em <http://www.unb.br/fs/clm/labcor/Animalab/Animalab.htm>.
- Ruschel HC, Fossati AC. Bases biológicas. In: Kramer PF, Feldens CA. *Traumatismos na dentição decídua-prevenção, diagnóstico e tratamento*. São Paulo:Santos; 2005. p.9-50.
- Sari S, Aras S, Gunhan O. The effect of physiological root resorption on the histological structure of primary tooth pulp. *J Clin Pediatr Dent*, 1999;23:221-5.
- Fagundes DT, Taha MO. Modelo animal de doença: critérios de escolha e espécies de animais de uso correntes. *Acta Cir Bras* 2004;19:59-65.
- Roppa L. Carne suína: Mitos e Verdades; 2006 [acessado em 08 de abril de 2006]. Viável em <http://www.abipecs.org.br/mitosverdades.pdf>.
- Papich MG. Drug residue considerations for anesthetics and adjunctive drugs in food-producing animals. *Vet Clin North Am Food Anim Pract* 1996;12: 693-706.
- Sisson S. Sistema Digestivo do Suíno. In: Sisson S, Grossman JD. *Anatomia dos animais Domésticos*. Rio de Janeiro: Guanabara Koogan; 1981. p.1188-202.
- Dyce KM, Sack WO, Wensing CJG. *Tratado de anatomia veterinária*, 2. ed. Rio de Janeiro: Guanabara Koogan; 1997.
- Oztas N, Ulusu T, Oygur T, Cokpekin F. Comparison of electrosurgery and formocresol as pulpotomy techniques in dog primary teeth. *J Clin Pediatr Dent* 1994;18:285-9.
- El-Meligy O, Abdalla M, El-Baraway S, El-Tekya M, Dean JA. Histological evaluation of electrosurgery and formocresol pulpotomy techniques in primary teeth in dogs. *J Clin Pediatr Dent* 2001;26:81-5.
- Ribeiro RA, Myaki SI, Gioso MA. Resposta pulpar de dentes decíduos de cães a um sistema adesivo ou ao cimento de hidróxido de cálcio. *Pesq Odont Bras* 2000;14:47-52.
- Stanley HR. Pulp capping: conserving the dental pulp – can it be done? Is it worth it? *Oral Surg Oral Med Oral Pathol* 1989;68: 628-39.