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SUCCESSFUL DIRECT PULP CAPPING DURING GESTATION IN DOGS: HISTOLOGICAL STUDY

Dental Science

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ABSTRACT

Pulp capping is a technique used in dental restorations to prevent the dental pulp from necrosis, after having been exposed during cavity preparation, following traumatic injury or a deep cavity that penetrates the center of the tooth and provokes pulp death. The success of endodontic treatment depends primarily on the availability of dental stem cells, as well as growth factors and hormones present in the pulp environment. In addition, the physico- chemical conditions required for differentiation and cell division, followed by tissue growth of the newly-formed structures, are essential. The aim of this study is to identify the different tissues neo-formed after pulp capping treatment. Although it is difficult to study the success of this therapy in women on a cellular and molecular scale, we used dogs as an animal model to perform the experiments needed to evaluate the success of this endodontic treatment. We used one pregnant dog and a second non-pregnant one to compare the tissue formed in both cases. Histologically, we note that the response of the dentin-pulpal system to these treatments is better in pregnant than in non-pregnant dogs, witch open the door once again to biological therapeutics by taking advantage of the regenerative capacity associated with pregnancy as a special physiological state in a woman's life, thus considering pregnancy an opportunity and not a limit for endodontic interventions.

KEYWORDS

Odontostomatological regeneration, pulp capping, pregnancy, histology

INTRODUCTION

Direct pulp capping is an endodontic treatment used when the pulp is visibly exposed due to caries, trauma or an iatrogenic factor such as accidental exposure during tooth preparation or caries removal (1). The procedure generally involves stopping any pulp hemorrhage, then covering and sealing the exposed pulp tissue to preserve its health, function and viability (2). The aim of this treatment is to induce the formation of a mineralization bridge at the exposed pulp site, using a bioactive material. It also eliminates all bacteria. In 1965, Kakehashi and al. demonstrated that rat pulp exposed without bacterial contamination showed no necrosis after 7 days and a dentinal bridge after 32 days (3). In contrast, exposed pulp with bacterial contamination showed necrotic spots on day 8 and complete necrosis on day 14 (4).

Some biomaterials, such as calcium hydroxide or MTA, stimulate pulpal repair and regeneration potential in the case of moderate pulpal exposure. (5). Successful direct pulp capping leads to the development of a mineralized dentine bridge by the pulp tissue. However, the mechanisms involved in pulp healing during direct capping are complex (6). Events following pulpal exposure can be divided into phases of inflammation, proliferation and remodeling. But since pulp wound healing is a continuous process, the beginning and end of each phase cannot be clearly determined, and the phases overlap (7). Exposure of pulp tissue induces transient inflammation. Initially, pulp tissue adjacent to the exposure is characterized by variable quantities of necrotic elements, defense cells and extravasated blood cells. Inflammatory edema is then resorbed by drainage into the lymphatic system. Macrophages phagocytose necrotic residues and inflammatory cells that havecompleted their function (8). Old inflammatory site then becomes the site of a proliferation of new fibroblasts. These cellssynthesize collagen and fibronectin (9). The collagen fibers then attract the mineral salts needed to mineralize this extracellular matrix, forming a mineralized tissue layer with an irregular structure. This mineralized matrix, known as fibro dentin, appears to be necessary for the differentiation of replacement odontoblasts and the formation of reparative dentin (10). A fibronectin-rich matrix could serve as a reservoir for growth factors such as TGF- and other bioactive dentin molecules. These act as signal molecules for odontoblast differentiation (11). Thus, fibronectin modulates odontoblast-like cell differentiation during repair dentin formation.Pulpal effraction leads to the destruction of primary odontoblasts. Healing of the odontoblastic palisade therefore requires the attraction of new cells. One day after capping, a proliferation of pulp cells is observed, linked to the existence of a lesion. After two weeks, these cells migrate towards the pulp lesion. They differentiate, giving rise to a new population of odontoblast-like cells capable of generating reparative dentin in the pulp zone beneath the capping material (12, 13). Following the arrival of these "neo-odontoblasts", multiple mineralized foci appear and develop in the form of osteo-dentin. The latter is named for its bone-like structure, it contains cellular and vascular inclusions. The cells sequestered within these foci have been shown to be progenitor cells. This osteo-dentin therefore consists of a reparative dentin-like matrix secreted by cells expressing markers specific to secretory odontoblasts (DSP, nestin). These foci of mineralization within the pulp subsequently fuse to form a dentin bridge (14). Restorative dentin is synthesized late and slowly. It appears after neo-odontoblast differentiation, which takes around 20 days in humans, and is secreted at a rate of 1.5 µm per day. The dentin bridge is thus formed within a few weeks after direct pulp capping. 3 months later, the dentin bridge is visible on X-ray (15). However, pulp repair is only possible in the absence of an acute, i.e. irreversible, inflammatory process, and in the presence of a pulp with sufficient blood supply to promote healing (16).The biomaterial to be used must not have any adverse effects on the pulp, but must stimulate thespecific functions of the target tissue (17). The qualities required of the ideal capping material are as follows; ***Biological properties*****:** the material used must be biocompatible with the pulp-dentin complex, non-toxic, non-allergenic, anti-inflammatory, and capable of inducing dentin-genesis: the product must trigger a cellular reaction to form tertiary dentin (18); ***Physico-chemical properties*****:** the material must not induce tooth discoloration, it must adhere to dentin surfaces, be watertight, have sufficient mechanical resistance to pressure, be compatible with coronal filling materials, be radio-opaque, and be easy to use and handle (19).

Glass ionomer cements are the leading family of materials. This long-standing and original family has evolved considerably. The latest development is that of high-viscosity glass ionomer cements (CVIHG) (20). A major advantage of CVI is their biological properties. They have excellent biocompatibility and some bioactivity (21). This good biocompatibility is due to the absence of resin. They are, in fact, water-setting cements. This results in anti-cariogenic effects and very good tolerance to moisture in the oral cavity. In addition, there are no potential toxic effects on either the pulp or the oral environment (22).

Another relatively new capping material is MTA (mineral trioxide aggregate), a material that can replace some of the endodontic materials normally used to induce calcified barrierformation, notably calcium hydroxide. MTA is a bioactive material that was developed in

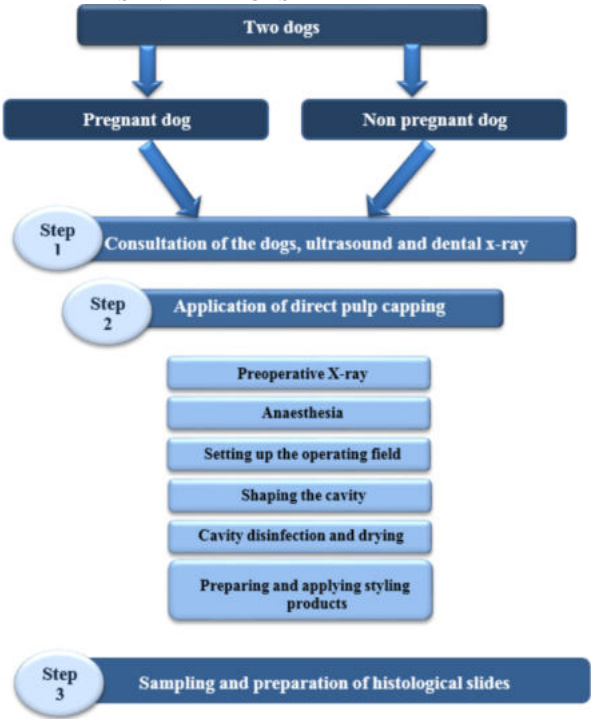
the early 1990s (23). It is essentially a refined preparation of Portland cement, a mixture of dicalcium silicate, tricalcium silicate, tricalcium aluminate, gypsum and tetracalcium aluminoferrite. Generally speaking, in vitro studies indicate that MTA is a biocompatible material with the ability to stimulate osteo/odontogenic differentiation (24). The ability of MTA to induce reparative dentinogenesis or mineralized bridge formation has been demonstrated in in vivo studies in which direct pulp capping and/or pulpotomy have been performed (25-26).

The major drawback of this material is its setting time, which is around two hours.During pregnancy, a woman changes. She prepares to welcome a new being and provide it with all the elements necessary for its development and growth (27). From the moment of implantation, the hormonal profile of the pregnant woman is altered, enabling the embryo and then the fetus to grow. The key organ at the origin of this upheaval is the placenta, which is considered an incomplete endocrine organ, with a great capacity for synthesis, but requiring precursors from the fetus or the mother (28). It also has numerous hormone receptors. It also produces steroid hormones, cytokines, glycoproteins and several growth factors (29). Among the growth factors produced during pregnancy are several secreted by the decidua, which play a key role in the growth of placental fibroblasts and are considered to be local immunosuppressive factors (30). In endodontics, these growth factors and hormones are involved in cell proliferation, extracellular matrix synthesis, odontoblastic differentiation and dentinogenesis.

Pregnancy is an exceptional time for a woman, during which the body undergoes many physiological and hormonal changes that can have beneficial effects on the healing process of certain medical conditions (32). Some studies have suggested that pregnant women have a better ability to heal wounds. This is partly due to an increase in blood flow, as well as an increase in hormones (estrogen and progesterone), which can promote cell regeneration and tissue growth.

The aim of this present study is the success of odontomatological regeneration during coarsening, using the dog as an animal model, and applying direct pulp capping to teeth whose pulp we have previously intentionally necrotized.

MATERIALS AND METHODS



Two dogs, one pregnant and the other not, were selected for this study. To carry out this experiment, this work was in close collaboration with a veterinarian to provide anesthesia and tomonitor the dogs during the operation. The operating protocol consisted of 3 essential stages; The first step consists of consulting the animals to assess the state of health of the two dogs. Dental X-rays of the teeth that will be treating were taken (the last premolar and first molar), and performed a pelvic ultrasound to confirm the dog's pregnancy. The second one consists of applying the direct pulp capping protocol. During this stage, the treatment to two teeth was applied (the last pre-molar and the first molar) and two different capping materials were used (MTA and CVI), to assess the response to treatment in both cases. During the last step an osteotomy specimens are taken under anesthesia, and the blocks are then placed in vials containing 10% formalin. These specimens are sent to the pathology laboratory for histological evaluation. The following figure shows the different steps of the protocol

Histological Study Protocol

The aim of the histological study of the dental block is to visualize the tissues, cells or extra- cellular matrix (ECM) after application of the pulpal revascularization procedure, with the aim of identifying the various tissues and cells neo-formed after this treatment by determining their location and configuration. Histology thus enables us to describe cellular and tissue morphology in terms of architecture and molecular interactions. Once the dental specimens have been removed, they are fixed in 10% formalin. The aim of fixation is not only to immobilize the cellular and tissue components, but also to prevent cell autolysis following physico-chemical changes in the preservation medium, which are totally different from the animal's physiological conditions. After fixation, the samples placed in a decalcifying solution, as the hardness of the tooth makes it impossible to cut with a microtome without a decalcification treatment, which softens the mineralized tissues sufficiently to obtain sections fine enough to be interpreted under the microscope. The decalcification process eliminates the mineral matter contained in hard tissues (bone, cartilage, teeth), making them more supple. It is therefore also essential for cutting alveolar bone.

This is followed by the kerosene embedding stage, the aim of which is to produce fine, regular sections. This is followed by microtome slicing, where the kerosene block is cut with a microtome to produce sectional slices (sections) 2 to 5µm thick. The sections are collected on glass slides. The stain used in our case is hematoxylin-eosin as a base dye.

Finally, after dehydration, the stained sections are mounted between slide and coverslip using a synthetic resin with a refractive index close to that of glass. The result is a "microscopic preparation" ready for observation under the light microscope.

RESULTS

The histological study was carried out on two teeth, the first using MTA as capping material, the second using glass ionomer cement (GIC).

The slides are ready for light microscopy to identify the various tissues and cells present in the treatment environment.

Microscopic examination of the slides of both teeth of the pregnant dog, shows that they are composed essentially of dentin, covered externally by enamel and internally by cement. Both teeth have the same appearance, with a richly vascularized hypertrophic pulp and active odontoblasts producing a dense fibrous tissue rich in collagen, giving stability to the dental structures, with a minimal inflammatory reaction with lymphocytes. We note a very solid enamel with a consistent fibrous tissue that is not loose (solid appearance), a very significant fibrosis reaction with many blood vessels filled with blood.

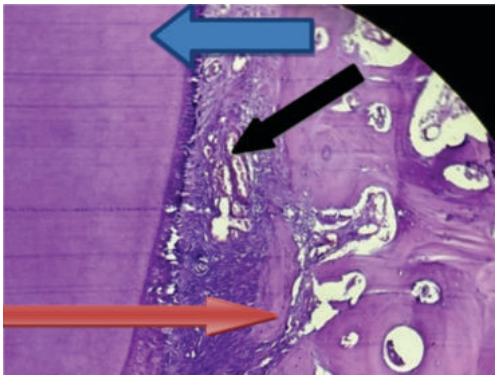


Figure 2: *Cross-section of part of the pulp chamber at coronal level, blue arrow indicates dentin, red arrow indicates enamel and black arrow shows blood vessels.*
(40x magnification) (Pregnant Dog)

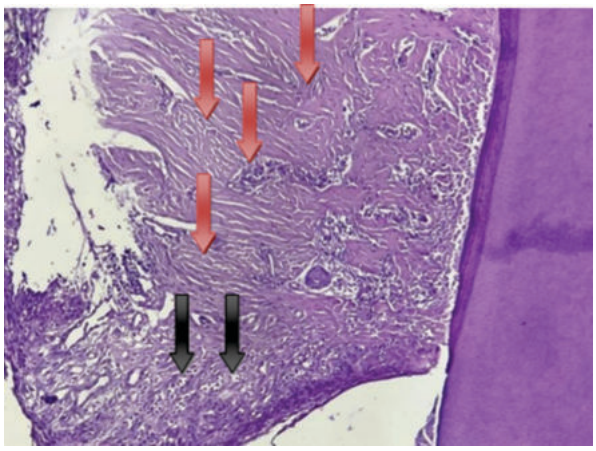


Figure 3: *Cross-section of part of the pulp chamber at coronal level on the enamel side Black arrows indicate blood vessels with red blood cells (small dots inside), and red arrows indicate fibers. 40x magnification* (Pregnant Dog)

Very strong enamel with many fibers and blood vessels

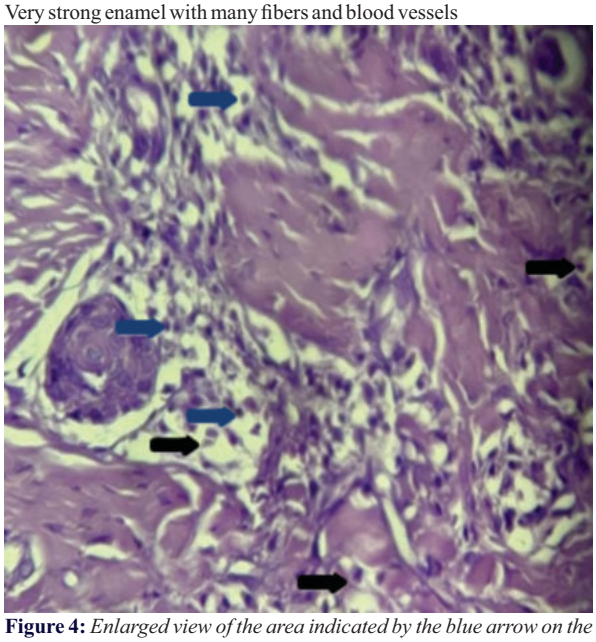


Figure 4: *Enlarged view of the area indicated by the blue arrow on the previous figure Presence of highly vascularized pulp treatment space tissue at the coronal level of the removed tooth (enlarged view of previous figure) Magnification x100* (Pregnant Dog)

This figure shows a cross-section of the coronal part of the pulp treated by direct pulp cappingof the extracted tooth from the pregnant dog, showing highly vascularized tissue. Blue arrows show blood vessels and black arrows show lymphocytes.

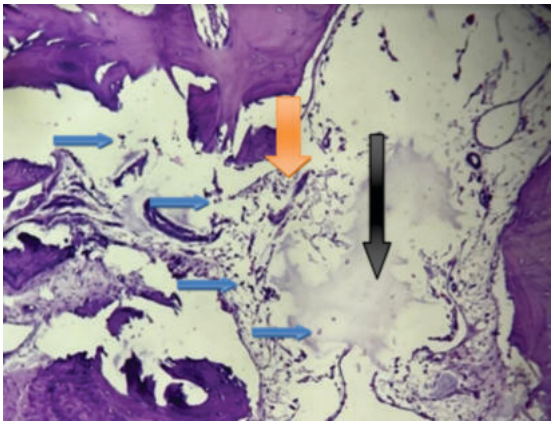


Figure 5: *Cross-section of part of the pulp chamber at the coronal level of the first tooth, showing an edematous appearance with very loose enamel; the blue arrows show various inflammatory cells (lymphocytes and plasma cells).*

The black arrow shows the loose edematous appearance of the enamel (magnification x40).

(non-pregnant dog)

The histological study was carried out on two teeth (of the non-pregnant dog), composed essentially of dentin, covered externally by enamel and internally by cement. The pulp of the first tooth shows edematous and congestive changes, giving it a loose appearance rich in polymorphous inflammatory elements, mainly lymphocytes and plasma cells. The pulp of the second tooth shows congestive changes without edema, with minimal fibrosis and a few inflammatory plasma cells. It has the same loose appearance as the first tooth, but is more congestible (many blood-filled vessels inside).

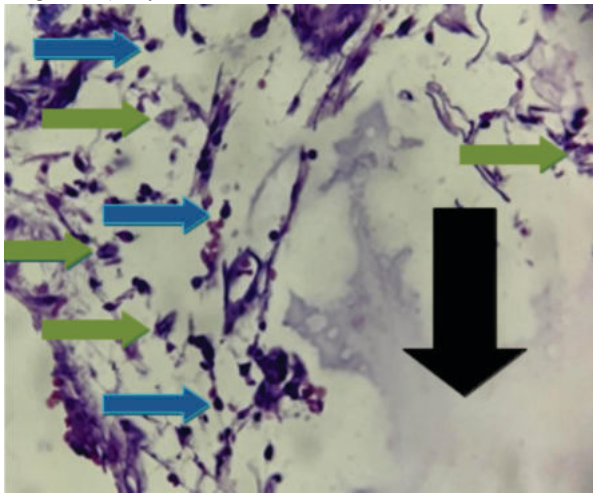


Figure 6: enlarged view of the area indicated by the orange arrow on the previous figure The blue arrows show the presence of red blood cells, the green arrows indicate the presence of lymphocytes and the large black arrow shows the edematous appearance of the enamel. (x100 magnification) **(non-pregnant dog)**

DISCUSSION

The histological results of samples from teeth treated with direct pulp capping from pregnant dogs are essentially composed of dentin, covered externally by enamel and internally by cement. The two teeth treated with two different capping materials (MTA and CVI) have the same appearance, with a richly vascularized hypertrophic pulp with active odontoblasts producing a dense fibrous tissue rich in collagen, giving stability to the dental structures, with a minimal inflammatory reaction with lymphocytes, very solid enamel, consistent fibrous tissue that is not loose, and a very important fibrous reaction with many vessels. In the case of the non-pregnant dog, it is clear from the histological analysis of the two teeth that they are essentially composed of dentine, covered on the outside by enamel and on the inside by cement. The pulp of the first tooth shows edematous and congestive changes, giving it a loose appearance rich in polymorphous inflammatory elements, mainly lymphocytes and plasma cells. The pulp of the second tooth shows congestive changes without edema, with minimal fibrosis and a few inflammatory plasma cells. Pregnant dog shows a strong fibrosis reaction with many vessels, in contrast to non- pregnant dog, which shows a very loose, edematous appearance. In both cases, there was no complete regeneration as such; this was due to the fact that the time between treatment and removal was only three weeks. With time, however, new tissue or complete regeneration of the necrotic pulp may be observed. In addition to the time factor, several other factors can directly affect the results of the pulp capping we have applied:

Bacterial Infection, Bacterial infection is a factor that can interfere with the healing process of exposed dental pulp. Bacteria have the potential to penetrate the exposed pulp through caries, cracks or fractures in the tooth and, as a result, marginal degradation occurs at the interface between the restoration and the tooth. At the time of direct pulp capping treatment, the exposed pulp area is at risk of bacterial

contamination. When caries is excavated, bacterial- containing shards of dentin can be displaced into the exposed pulp tissue, causing chronic inflammation. Bacteria can also enter the pulp wound through leakage aroundthe restoration and the direct pulp capping agent. Studies have shown that bacterial-induced pulp inflammation is mainly caused by *Streptococcus mutans* , but other different bacterial species are also responsible for inducing pulp inflammation. Bacterial infection of the exposed pulp can cause odontoblast cell death, and odontoblast cell processes dislodge from dentinal tubules and transform into dead pathways. These pathways are highly permeable and therefore represent apotential threat to pulp integrity. Pulpal inflammation is caused by bacteria that impede the repair process, which can be prevented after appropriate resolution of the inflammation, which probably occurs afterdisinfection of the inflamed area (33, 34).

The success of direct capping depends on preventing bacterial penetration of the cavity preparation. This will increase the longevity of cavity restorations. Complications of bacterial infection include postoperative sensitivity, marginal discoloration, inflammation, recurrent caries and ultimately the need for further endodontic treatment (35). One of the main factors that can affect the success of direct pulp capping is the extent of pulp bleeding after pulp exposure and before capping material placement. Excessive bleeding after pulp exposure is generally associated with increased inflammation, which in turn reduces repair capacity at the exposure site. Bleeding at the exposure zone increases the moisture level of dentin surfaces, leading to increased contamination when an adequate seal is difficult to achieve, and the possibility of bacterial infection is greater. It has been reported thatpulpal bleeding should stop within a few minutes. Furthermore, the time required to stop pulpal bleeding has shown no effect on treatment results. In particular, depending on the type of exposure, the time taken to stop bleeding varies from 1 to 2 min to 10 min (36). A successful result after direct pulp capping depends on controlling bleeding and preventing the formation of blood clots between the material and the pulpal tissue. Although itmay be difficult to apply DPC material to a moist surface such as a blood clot, the presence of a blood clot can lead to an increased risk of postoperative infection. If the capping material could not adhere to the exposed pulp tissue due to uncontrolled bleeding, a gap could form between the placed material and the exposed pulp tissue. As a result, it was hypothesized that hyperplasia of the pulp tissue in the gap could lead to a polyp-like projection of the pulp tissue (37).

Pulpal inflammation, Inflammation after exposure of the dental pulp can be caused by a number of factors, including mechanical irritation during cavity preparation, penetration of micro-organisms through decay into the exposed pulp, and mechanical irritation caused by the capping material. All these factors are responsible for inducing inflammatory cellular infiltration, which then leads to pain and hard tissue remodeling. Different stages of pulp inflammation have been studied after direct pulp capping, including mild to moderate or severe inflammation that can lead to pulp necrosis (38). Although inflammation is a destructive process for pulp tissue, the immune response it triggers is necessary to accelerate the healing process. In fact, the initial stage of inflammation is the recovery of damaged pulp tissue (39).

And finally, operative debris includes dentin fragments from cavity preparation and foreign particles that are mixed with capping materials. When operative debris is present in the exposed pulp, it can interrupt the integrity of the dentin bridge, weakening the bridge structure and, as a result, odontoblast cells are unable to secrete dentin. The presence of surgical debrishas also been associated with increased pulpal inflammatory cell activity. Surgical debris may be contaminated with bacteria. These bacteria can penetrate the pulp tissue and provoke an immunological reaction. In severe cases, this can lead to pulp necrosis and inhibition of dentinal bridge formation. Frequently, surgical debris migrates deep into the pulp tissue from the exposure zone (40). After pulp exposure, the presence of operative debris, such as dentin shavings, can be considered an advantage as they can stimulate dentin bridge formation. Although it has been indicated that there may be a direct correlation between the area of operative debris and dentinal bridge formation, where operative debris appears to have no advantage, only healing complications. As mentioned in the introduction, during pregnancy, the body undergoes a number of physiological changes that promote the regeneration and repair process, including pulp capping, which applied to the teeth of both pregnant and non-pregnant dogs. In the pregnant dog, a very significant fibrosis reaction was observed, with many vessels, in contrast to the non- pregnant dog, where a very loose, edematous appearance was observed, This is due to the physiological state of the pregnant dog, which favoured the fibrosis observed. These results need to be compared with other molecular studies aimed at understanding the mechanism of regeneration during pregnancy in the presence of estrogens in large quantities, as well as hormones and growth factors. In summary, we note that the histological response of the dentin-pulpary system to these treatments was better in the pregnant dog than in the non-pregnant dog (the reactions were more intense in the pregnant dog).

These results open the door once again to biological therapeutics, taking advantage of the regenerative capacity associated with pregnancy as a special physiological state in a woman's life. The oral care of pregnant women, and more specifically endodontics, can be reviewed in a different light, considering pregnancy not as a limitation but rather as an opportunity for endodontic, biological and regenerative care.

CONCLUSION

Pulp capping is considered a better option for treating immature teeth with non-vital pulp. The advantage of this therapeutic approach is twofold: it takes only two to three visits over a few weeks, and it helps regain pulp vitality. The success of pulp capping depends on two essential elements: the presence of stem cells and growth factors to direct the differentiation of these same cells towards a well- defined lineage that will subsequently lead to the formation of new homo or heterogeneous tissue structures. Cell differentiation, growth and survival require the presence of several elements, suchas sufficient oxygen for metabolism, nutrients, and an aseptic support in the form of the disinfected pulp canal.

Pregnancy is a rather special period, characterized by physiological changes that will contribute to the success of direct pulp capping treatment, as there is hypersecretion of growth factors and hormones by the placenta and various other endocrine glands, an increase in stem cell stocks, and a high demand for oxygen to ensure proper featal development. We took advantage of all these changes to apply direct pulp capping to teeth with artificially necrotic pulp, and the histological results obtained validate our hypothesis.

In the case of the pregnant dog, we observed the presence of primary inflammatory foci (which may, over time, lead to complete regeneration of the previously destroyed tissue).

With this Study, we open the door once again to biological therapeutics, taking advantage of the regenerative capacity associated with the state of gestation. The oral care of pregnant women, more specifically in endodontics, can be reviewed in a different way, considering management not as a limitation but rather as an opportunity for biological and regenerative endodontic care.

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