

ORIGINAL ARTICLE

Histological and histochemical aspects of the dentin-pulp complex in offsprings of rats treated with fluoxetine during pregnancy and lactation

ABSTRACT

Aim: This study aimed to analyze histological aspects of the dentin-pulp complex in rats neonates treated with fluoxetine during pregnancy and lactation.

Methodology: For this purpose were used first molars of 24 Wistar rat pups, 25 days old-aged, divided into four groups. PCG and PCGL whose mothers positive controls received 0.9% NaCl (oral) during gestation and gestation/lactation, respectively; FG and FGL both mothers received fluoxetine at 20 mg/kg (oral) during pregnancy and gestation/lactation, respectively. The animals were anesthetized, maxillaries removed and fixed in 4% formaldehyde, decalcified in EDTA and processed conventionally for light microscopy. Paraffin sections were stained by Hematoxylin and Eosin, Masson's Trichromatic and Pricosirius Red.

Results: There was no evidence of structural and histochemical changes in the pulp-dentin complex in the groups studied.

Conclusions: Therefore, we could conclude that the fluoxetine in 20 mg/kg dosage, administrated during pregnancy and lactation, did not interfered in the dentin-pulp complex development in offsprings of rats when analyzed in the age of 25 days.

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Received 2020, March 30

Accepted 2020, July 28

KEYWORDS dental development, depression, fluoxetine, pregnancy, rats

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Peer review under responsibility of Società Italiana di Endodonzia

[10.32067/GIE.2020.34.02.10](https://doi.org/10.32067/GIE.2020.34.02.10)

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Introduction

Depression is a mental disorder characterized by persistent sadness and loss of interest in normally pleasant activities, accompanied by an inability to carry out daily activities for at least two weeks. Women are considered to be a risk group for this kind of disorder, whether in pregnancy or in the postpartum period. During pregnancy, the female organism produces placenta hormones that can bring about significant organic and behavioral changes (3). Currently, 10-13% of pregnant women suffer from depressive episodes associated with pregnancy (2).

The depression treatment during the gestational and postpartum period is important to prevent obstetric complications, such as the birth of low weight preterm infants (1). The advance of pharmacology has promoted the emergence of highly selective chemicals that act specifically on the neurotransmitter systems. For the pharmacological treatment of mood and anxiety disorders, the most widely prescribed antidepressives worldwide are SSRIs such as fluoxetine (4, 5).

Fluoxetine is the antidepressive of choice for administration in women who are gestating and breast fed. Its metabolite, norfluoxetine, is able to cross the placental barrier and be excreted in breast milk, resulting in high therapeutic levels in plasma. Fluoxetine is able to induce long-term changes in the serotonin system. However, the precise effects are not yet clear. Details, including underlying neural mechanisms, are still being outlined (6). There is a shortage of data on the direct action of SSRIs in differentiating human bone cells; however, some studies have shown that they have a deleterious effect on the formation and proliferation of osteoblasts from the defective signaling of the 5-HT receptors, causing their apoptosis.

The tooth and periodontium development process has been the object of a number of studies, which attempt to clarify the phenomenon of the histogenesis, induc-

tion and histodifferentiation of the tissues before its functional activity in the post-birth life. However, very little has been described in the literature about the influence of SSRIs on odontogenesis and the development of periodontal tissues and the basal bone of the jaw.

Based on the evidence that the serotonergic system is associated with developing different human body tissues, mediating important epithelium mesenchymal interactions, this research was justified in order to evaluate fluoxetine's influence in developing and growing the dentin-pulp complex during gestation and lactation.

Materials and Methods

This research was approved by the Ethics Committee on the Use of Animals at the Federal University of Pernambuco (CEUA-UFPE), process n° 23076.017680/2011-83. All measures have been taken to minimize the pain or discomfort of mothers and offsprings. Were used 24 Wistar rats (*Rattus norvegicus albinus*, Rodentia, Mammalia) with 25 days of life, coming from the Bioterium of the Department of Nutrition of the UFPE. Six albino rats of the Wistar strain were used, kept in a room with a temperature of 23 ± 2 °C and a light and dark cycle of 12/12 hours (light from 6 am to 6 pm and dark from 6 pm to 6 am), receiving the standard diet of vivarium (Presença Ratos e Camudongos - Presença, Paulínia, SP, Brazil) and water ad libitum. Mating between adult animals and vaginal smear tests were performed to prove pregnancy. The day of proof of pregnancy was the first day of pregnancy and the beginning of the animals' procedures (10).

Two groups comprised the research: the control group (Group C) and the group treated with fluoxetine (Group F). The groups were further divided into two subgroups: animals that were treated only during pregnancy (PCG and FG) and animals that were treated during pregnancy and lactation (PCGL and FGL). One pregnant rat was used for each subgroup of the control groups (PCG and PCGL) and, from each litter, 6 offsprings were

used, of both sexes (n=6). For the group treated with fluoxetine, two pregnant rats were used for each subgroup and from each litter three offsprings were used, of both sexes, totaling six (n=6) animals per subgroup, totaling a sample of 24 offsprings. In the PCG group, the animals came from rats that received 0.9% sodium chloride at a dose of 10 µl/g orally (gavage) throughout the gestation period; in the PCGL group the animals came from rats that received 0.9% sodium chloride at a dose of 10 µl/g orally (gavage) during pregnancy and lactation and in the FG and FL groups the animals came from rats that were treated with fluoxetine hydrochloride (Pharma Nostra, Rio de Janeiro, Rio de Janeiro, Brazil) at a dose of 20 mg/kg of animal weight orally (gavage) administered until the end of pregnancy (group FG) and in group FGL the animals were treated with fluoxetine during pregnancy until the end of breastfeeding, that is, 21 days after birth (table 1).

When they reach 25 days, the animals were anesthetized by intraperitoneal injection with an association of ketamine (CEVA, Paulínia, São Paulo, Brasil) at a dose of 50 mg/kg animal weight and xylazine (CEVA, Paulínia, São Paulo, Brasil) at a dose of 20 mg/kg animal weight. The offsprings were perfused intracardially with 4% paraformaldehyde (Sigma Aldrich, São Paulo, Brasil) solution in sodium phosphate buffer. The heads were sectioned frontally so as to obtain transverse cuts of the maxilla mesially to the

first molars and were immersed in solution of the same fixative for 24 hours at room temperature. The pieces were decalcified in a solution of 5% ethylenediaminetetraacetic acid (EDTA) (Vetec Química Fina Ltda, Duque de Caxias, Rio de Janeiro, Brasil) for 15 days.

After the decalcified, were inclusion in paraffin (Michalany, 1980) and sectioned in a hand microtome (LEICA RM 2125 RT, Leica Biosystems Nussloch GmbH, Nussloch, Alemanha), adjusted to 5 µm-thick each. Serial cuts were made of all specimens that were stained with Hematoxylin and Eosin (HE), Masson's Trichrome (MT) and Picrossirius Red, mounted with Entellan® (Merck KGaA, Darmstadt, Alemanha).

For histological analysis of the central zone of the coronary and root pulp, HE stained preparations were selected and a LEICA ICC50 HD light microscope (Leica Biosystems Nussloch GmbH, Nussloch, Germany) was used coupled to a microcamera, connected to a computer containing a plate image capture.

For the histochemical analysis of collagen, preparations stained by HE, TM and Picrossirius Red were used using a LEICA ICC50 HD light microscope (Leica Biosystems Nussloch GmbH, Nussloch, Germany), coupled to a microcamera, connected to a computer containing a plate image capture.

No statistical test was performed because the study design has a qualitative and histomorphological approach.

Table 1
Study groups

	Control (n=12)		Experimental (n=12)	
Mothers	PCG (n:1)	PCGL (n:1)	FG (n:2)	FGL (n:2)
Offsprings	n:6	n:6	n:3 n:3	n:3 n:3
Total offsprings				n:24

(*)Source: Own data

PCG: Positive control gestacion

PCGL: Positive control gestacion and lactacion

FG: Fluoxetine gestacion

FGL: Fluoxetine gestacion and lactacion

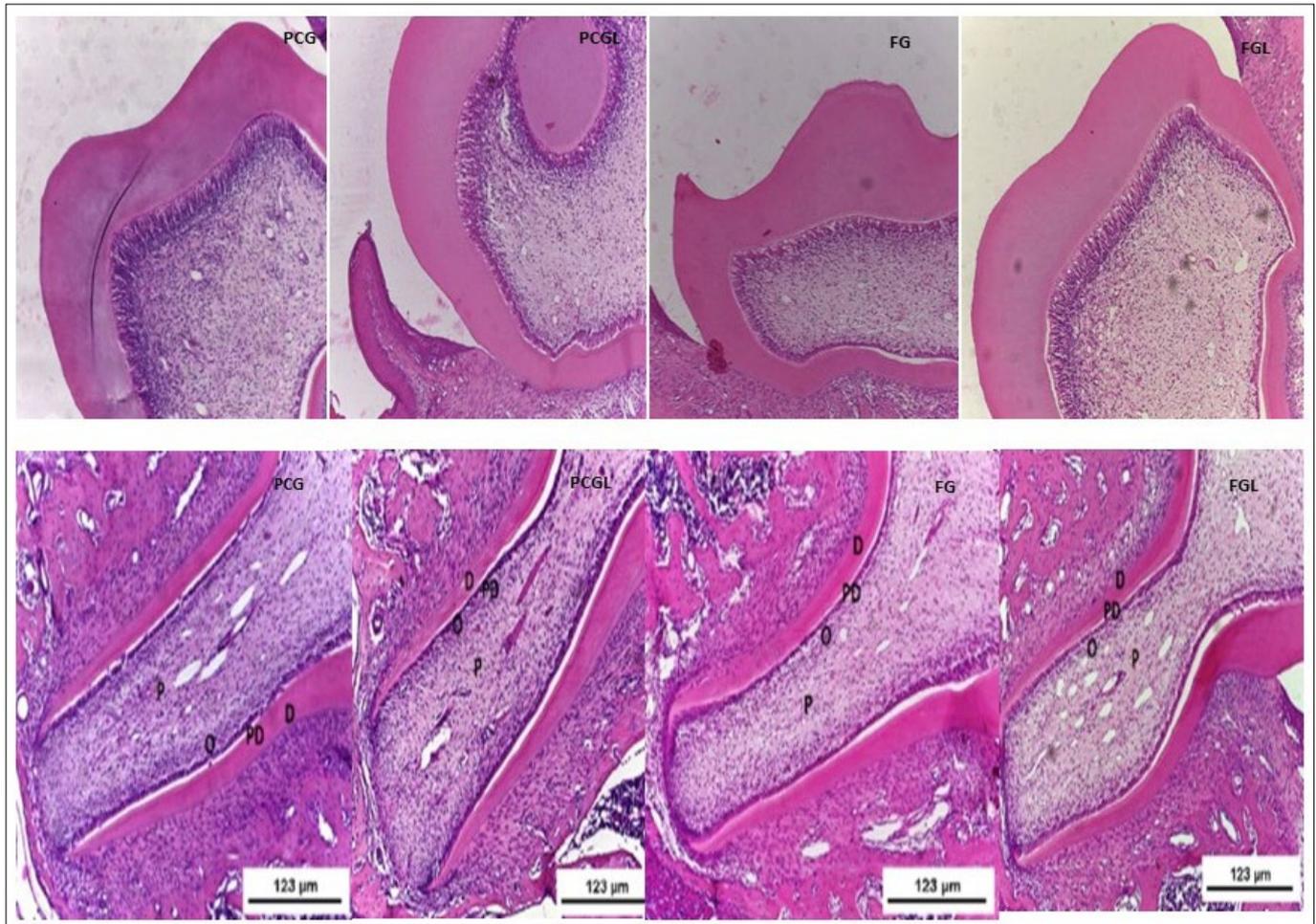


Figure 1.

Photomicrographs of part of the first upper molar crown and root of the rat with 25 days of life, corresponding to the Positive Control Gestation (PCG), Positive Control Gestation and Lactation (PCGL), Fluoxetine Gestation (FG) and Fluoxetine Gestation and Lactation (FGL). Observe the odontoblasts (O) in the region of dental crown with high prismatic aspect and with the nucleus polarized toward the dental pulp, dentin (D) thicker than the dentin (PD). P: Dental Pulp. VS: Blood Vessels. HE: coloration. In the root portion, the odontoblasts (O) present a low prismatic aspect and in a single layer with a polarized nucleus toward the dental pulp, dentin (D) thicker than pre-dentin (PD). P: Dental Pulp. VS: Blood Vessels. HE: coloration.

Results

It was observed that at 25 days of life the dental element of the first upper molars of rats was in phase of root development, and the apical portion was in formation, so the foremen was not closed. Comparing the coronary and radicular dentin-pulp structures between the groups NC, PCG, PCGL, FG e FGL, no differences were found in histological arrangement.

Analysis of the preparations stained with HE

Histologically, in all analyzed groups, the dentine presented thicker in the crown

than in the root containing homogeneous matrix, acidophilic, with evidence of the Tomes fibers in its interior. These fibrils were arranged perpendicular to the pulp surface, distributed in a centripetal way. Below the dentin, it was evidenced a thin layer of pre-dentin, lighter than the surrounding dentin, exhibiting less pronounced acidophilia than the first, stained in light pink. Inside of pre-dentin, it was observed the layer of odontoblasts, distributed in palisade, with high prismatic aspect in the crown and low prismatic in the regular region. In the cuspid portion, they exhibited a similar aspect to a pseudostratified epi-

thelium, which characteristic was not evident in the crown areas and radicular region. The odontoblasts showed an elongate nucleus, in the proximal region, with strongly acidophilous cytoplasm with basophilic tones in the basal region. The dental pulp showed a subodontoblast layer containing mesenchymal cells densely clustered below the odontoblastic layer, interspersed by small-caliber blood vessels. The central zone of the pulp showed less concentrated fibroblasts than in the peripheral region, with weakly acidophilic blood vessels and weakly acidophilic collagenous matrix being evidenced between them. The root pulp had a conical aspect, with a forming foramen. No accessory channels or collateral foramina were observed between the studied groups (Figure 1).

pre-dentin being less stained than dentin. The pulp exhibited odontoblasts and fibroblasts with nuclei dyed black. The intercellular matrix of the coronary and radicular pulp showed a weakly positive reaction to the collagen, staining in light blue, being slightly more concentrated in the root (Figure 2).

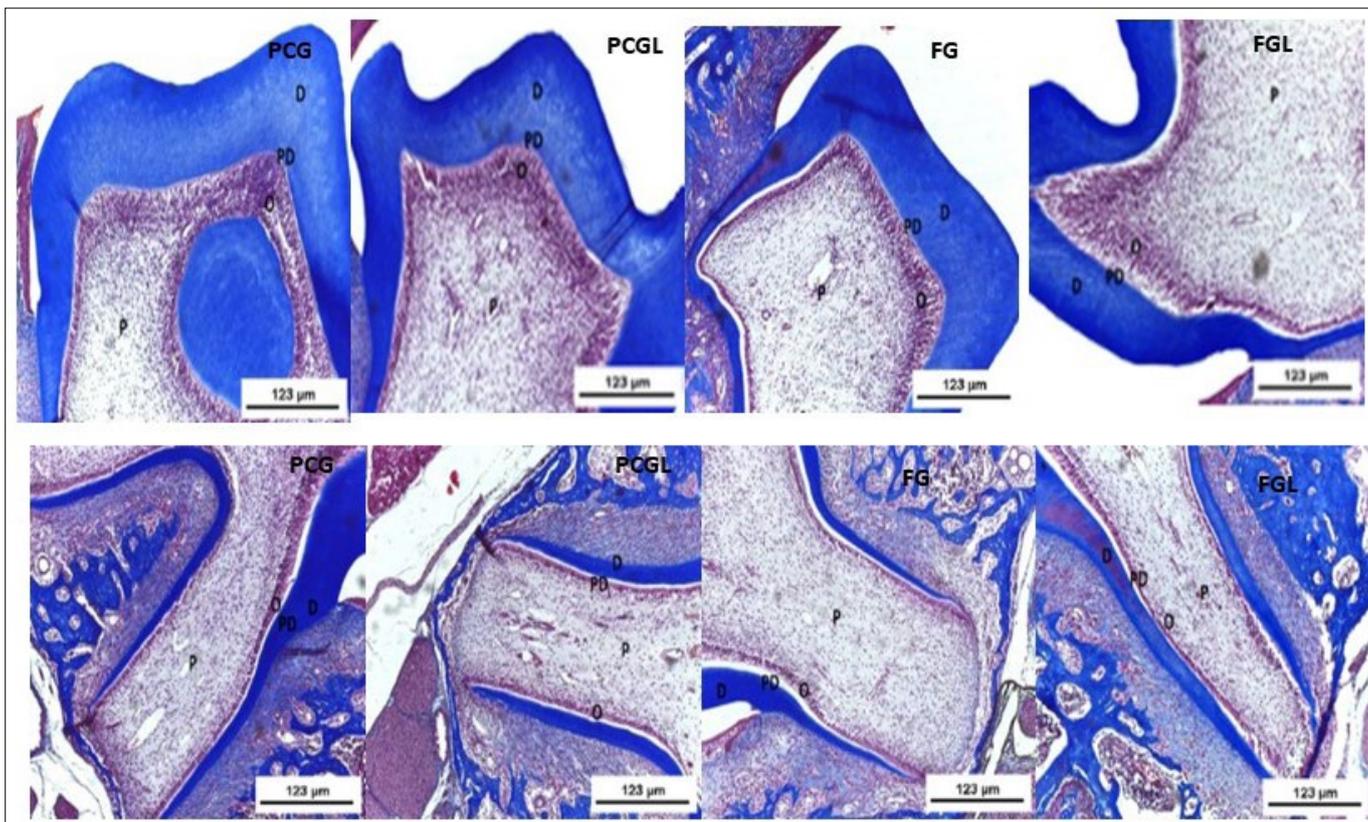
Histochemical analysis of preparations stained with Picrossirius Red

The analyzed samples showed no differences regarding the type and disposition of the collagen in the dentin, pre-dentin and dental pulp of the animals subjected or not to fluoxetine during its development. The preparations showed positive reaction to Picrossirius Red in the dentin and pre-dentin of all animals from the studied groups, with the dentin stained in intense red and the pre-dentin in light red. On the other hand, the pulp expressed weakly reactive acquiring the green or yellow shade in the odontogenic region and a light red in the central region, being more intense in the radiular portion than in the crown (Figure 3).

Histochemical analysis of preparations stained with TM

When treated with TM, the dentin and pre-dentin of all analyzed groups were stained in blue, presenting a positive reaction for the presence of collagen, with

Figure 2. Photomicrographs of part of the first upper molar crown and root of the rat with 25 days old, corresponding to the Positive Control Gestation (PCG), Positive Control Gestation and Lactation (PCGL), Fluoxetine Gestation (FG) and Fluoxetine Gestation and Lactation (FGL). Pay attention to the dentin and pre-dentin with a strongly positive reaction for Masson's trichrome. Odontoblasts (O); dental pulp (P). Observe a positive reaction to the Masson's trichrome showing collagen fibers with bluish coloration. P: Dental Pulp. VS: Blood Vessels.



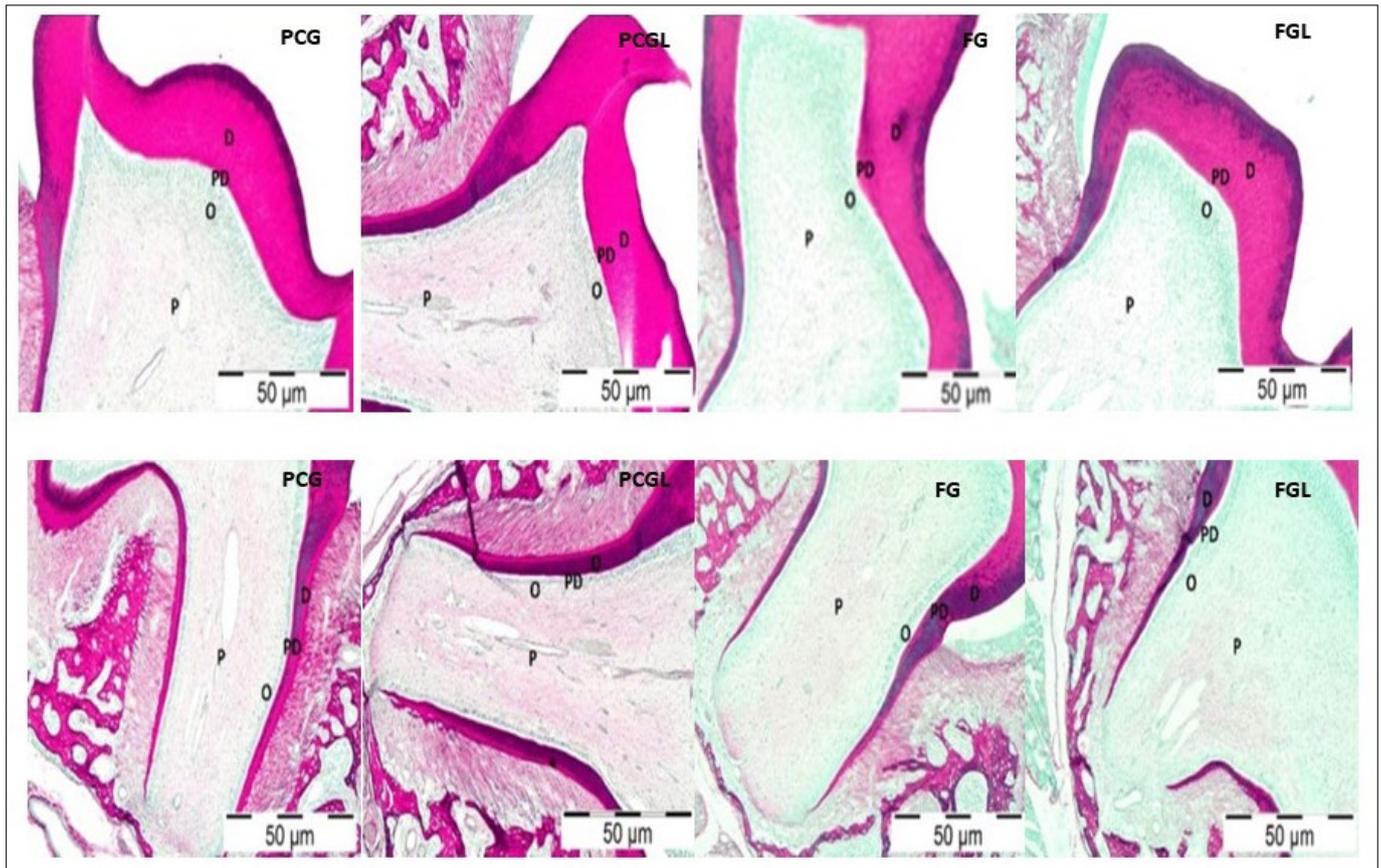


Figure 3.

Photomicrographs of the upper first molar crown and root of the rat with 25 days old, corresponding to the Positive Control Gestation (PCG), Positive Control Gestation and Lactation (PCGL), Fluoxetine Gestation (FG) and Fluoxetine Gestation and Lactation (FGL). Observe positive reaction for Picrosirius Red, showing dentin (D) and pre-dentin (PD) in reddish coloration. P: Dental Pulp. VS: Blood Vessels. Observe positive reaction for Picrosirius Red showing collagen "I" in reddish color. P: Dental Pulp. VS: Blood Vessels. Picrosirius Red coloration.

Discussion

In the present study, it was observed that the chronology of the development of the upper first molar of the animals of 25 days of life showed in a similar way in all groups, in other words, it was in the phase of rhizogenesis, with half of root grown and the apex not closed yet. The crown had already erupted and the odontoblasts were in juxtaposed position with the pre-dentin layer and in greater number by area in nearest portions of the cusps, corroborating with data already showed in the literature (11). Histologically, when stained with HE, it was not observed structural changes in the crown or radicular dentin-pulp complex in the experimental groups when compared with control groups following the same structural pattern written in publications (12). As dentinogenesis progressed, the odontoblasts were synthesizing the organic matrix of the dentin, retreating and leaving a cellular extension within the dentin. A narrow band of pre-dentin between the

odontoblasts and dentin was easily distinguishable in 25-day-old animals. These findings are in accordance with data reported in the literature where the presence of a richly sulfuric and less acidophilic matrix layer of the dentin organic matrix (pre-dentin) in the vicinity of the odontoblastic (13-15).

We observed that the dental pulp presented itself as a loose connective tissue still young and immature, with great number of cells, more evident in the root portions. These data also are reported in 2006 when they say that the presence of undifferentiated mesenchymal cells in dental pulp of the rat show that this tissue is capable of growth and reparation (16). The collagen fiber plays an important role keeping shape and structural role of the connective tissues. Changes in its molecular organization, amount and special distribution may provoke a series of pathologies (17). In the dentin-pulp complex, the collagen is the most abundant protein of the matrix and plays several functions as support, associ-

ation to the molecules of the amorphous substance and nucleation of hydroxyapatite crystals in dentin (18).

Two histochemical methods were used for detection of the collagen: Trichrome (TM) and Picrosirius Red. For the evidence of collagen fibers, the Masson's trichrome, is widely used to evidence collagen fibers and stained them in light green or blue-green (19). In an article from 2002, Kiernan describes the principle of the method characterizing a trichrome as a technique which uses one or more anionic dyes used in conjunction with phosphotungstic or phosphomolybdic acid. Its principle of action is not clear yet, but it is believed that these acids would bind to collagen as a mordant giving this molecule a negative charge which would attract the anionic dyes, in this case the aniline blue (20).

The TM technique allowed to observe the presence of collagen, which was abundantly distributed in a homogeneous way, independently of the growth phases of the tooth, corroborating with the findings (16). However, it was not possible to identify types of collagen present, since the technique does not have specify for differentiate types of collagen. Beyond that, the fibrils can be associated in bundles of different thickness, present different directions depending on the type of amino acid present and the function of the tissue. For this purpose, we use coloring with Picrosirius. The Picrosirius Red has been widely applied for detection of the collagen. It is a strongly acidic azo dye that presents six sulfonic groups, which allows detecting small amounts of collagen. This dyeing makes strong bonds between its acidic sulfonic groups and the basic groups of the collagen molecule, making it easily identifiable as collagen I, II or III by different colorations (21).

In the preparations stained with the Picrosirius Red under light microscope, there were thick reddish type I collagen fibers. However, the dental pulp showed weak reactivity to Picrosirius in the odontogenic zone, and the light green field tintorial characteristic indicates the presence of immature collagen fibers, which was also described in the literature [8]. The

central zone shows a smooth red reaction, which has a thicker fibrillar arrangement than in the previous layer.

A lot studies describe that the collagen I is the predominant component in the dentin matrix. Besides that, the presence of collagen type I in the dentin, pre-dentin and dentin pulps has been classically reported as fibers stained red or yellow by the Picrosirius Red method (12, 16).

Previous studies, observed neonatal repercussions on craniofacial development in in the offspring of the Wistar rats treated with fluoxetine only during gestation at a dosage of 10 mg/ kg. In these studies, possible morphological changes during dentinogenesis, amelogenesis and formation of the temporomandibular joints (TMJs) were analyzed, in which, similarly, no significant alterations were observed (22-25).

In our study, when we analyzed the group treated with the control group, we found that the administration of 20 mg/kg shows similarity in color nuances, with no morphological and structural development of dentin-pulp tissues in both groups. Thus, we observed that the signaling of serotonin receptors was not sufficient to generate reduced and morphological changes in the present study.

Conclusions

Thus, our results allow us to conclude that fluoxetine at a dosage of 20 mg/kg of animal weight, administrated during pregnancy and lactation, did not interfere with the tissue arrangement as well as the process of formation of the intercellular collagen matrix during the development of the dentin-pulp complex in the offspring of rats when analyzed at 25 days of age. Studies using long-term administration of fluoxetine are required to confirm the safety of drug use during pregnancy and lactation, especially regards to the formation of mineralized tissues and odontogenesis.

Ethic Statement

Research approved by the Ethics Committee on Animal Use (CEUA) of the Center for Biological Sciences of the Federal



University of Pernambuco (CCB-UFPE) under the number 23076.017680/2011-83.

Clinical Relevance

The present study is important because it adds information to what is still little described: about the role of serotonin in the development of mineralized and non-mineralized dental tissues.

Conflict of Interest

All authors declare no conflict of interest

Acknowledgements

Pernambuco Foundation for Science and Technology Support (FACEPE).

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