

## **Chronothanatognosis, utilization of dental pulp as a tool to determine the death time: a review**

**Cronotanatognose, utilização da polpa dentária como ferramenta para determinar o tempo de morte: uma revisão**

**Cronotanatognosis, uso de la pulpa dental como herramienta para determinar el momento de la muerte: una revisión**

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### **Abstract**

The post-mortem interval is the period of time that has passed since the death occurred until the moment when the body and /or human remnants are studied. The estimation of this interval is a matter of great relevance in the forensic sphere due to its important role in the resolution of criminal cases. Teeth are fundamental structures in a forensic context due to their high strength and specificity. Therefore, this article aims to present how the dental pulp is used to determine the time of death of the corpse. A systematic review of the scientific literature was carried out with a descriptive and qualitative character, allowing the inclusion of experimental and non-experimental studies for a complete coverage of the analyzed phenomenon. For data collection, online searches were carried out on the bases: SciElo, LILACS and Elsevier. All journal papers that used the selected terms were included in this review. The degradation of dental pulp after death consists of a complex process that has not been fully studied, but with the techniques and changes shown in the text, reliable post-mortem interval results are possible.

**Keywords:** Forensic Dentistry; Post-mortem; Thanatology.

### **Resumo**

O intervalo post-mortem é o período de tempo decorrido desde a ocorrência da morte até o momento em que o corpo e / ou restos humanos são estudados. A estimativa desse intervalo é de grande relevância na esfera forense devido ao seu importante papel na resolução de processos criminais. Os dentes são estruturas fundamentais no contexto forense devido à sua alta resistência e especificidade. Portanto, este artigo tem como objetivo apresentar como a polpa dentária é utilizada para determinar o momento da morte do cadáver. Foi realizada uma revisão sistemática da literatura científica de caráter descritivo e qualitativo, permitindo a inclusão de estudos experimentais e não experimentais para uma cobertura completa do fenômeno analisado. Para a coleta de dados, foram realizadas buscas online nas bases: SciElo, LILACS e Elsevier. Todos os artigos de periódicos que usaram os termos selecionados foram incluídos nesta revisão. A degradação da polpa dentária após a morte consiste em um processo complexo que não foi totalmente estudado, mas com as técnicas e mudanças apresentadas no texto, resultados confiáveis do intervalo post-mortem são possíveis.

**Palavras-chave:** Odontologia Forense; Post-mortem; Tanatologia.

### **Resumen**

El intervalo post-mortem es el período de tiempo que ha transcurrido desde que ocurrió la muerte hasta el momento en que se estudia el cuerpo y / o los restos humanos. La estimación de este intervalo es un asunto de gran relevancia en el ámbito forense por su importante papel en la resolución de causas penales. Los dientes son estructuras fundamentales

en un contexto forense por su alta resistencia y especificidad. Por tanto, este artículo tiene como objetivo presentar cómo se utiliza la pulpa dental para determinar el momento de la muerte del cadáver. Se realizó una revisión sistemática de la literatura científica con carácter descriptivo y cualitativo, permitiendo la inclusión de estudios experimentales y no experimentales para una cobertura completa del fenómeno analizado. Para la recogida de datos se realizaron búsquedas online en las bases: SciElo, LILACS y Elsevier. Se seleccionaron todos los artículos de revistas que utilizaron los términos seleccionados. La degradación de la pulpa dental después de la muerte consiste en un proceso complejo que no ha sido completamente estudiado, pero con las técnicas y los cambios que se muestran en el texto, es posible obtener resultados confiables del intervalo post-mortem.

**Palabras clave:** Odontología forense; Post-mortem; Tanatología.

## 1. Introduction

Forensic dentistry is defined as the branch of dentistry that, for clarification of justice, deals with dental evidence and facts (Plourd, 2010). This branch plays an important role in the assessment of body damage, more specifically in the assessment of post-traumatic orofacial damage. The dental examination may contribute data regarding the place, the circumstances of death and considering the data of the necroscopic examination, it seeks to establish the identification of the corpse, the mechanism, the time, the cause and the diagnosis of death, be it by accident, homicide, suicide or death by natural causes (Gomes, 1980).

When the corpse is found, a factor that effects the quality of an investigation of a case is knowing at what time the episode occurred (Garrido & Naia, 2014). In most cases, when corpses are discovered, the timing of the fatal event is not known, which makes it impossible to start a comprehensive investigation. This timing is often variable and dependent on several factors, such as the manner and place of death and the environmental conditions where the body was found. Thus, one of the main problems that still exist in criminal and forensic expertise is the determination of the post-mortem interval.

The post-mortem interval (PMI) is the period of time from the occurrence of death to the moment when the analysis of the body and/or its remnants begins (Szelecz, et., 2018). The estimation of this interval is a matter of great relevance in the resolution of criminal cases, since it is a crucial clue in obtaining information about the circumstances of death. This information can assist in the validation of alibis associated with the crime as well as in its resolution and allowing the distinction between a recent death and an older death.

There are several techniques to estimate this interval. In forensic thanatology, chronathanatognosis seeks to diagnose this time interval based on the study and analysis of the deformative and putrefactive processes that occurred after death (Garrido & Naia, 2014; Menon, et al., 2011; Vass, et al., 2002). Dental structures are considered the most resistant of the human body and are able to withstand the most varied conditions of the environment, such as high temperature, humidity, post-mortem degradation and could be used in situations where other organs and tissues are degraded (Malaver & Yunis, 2003; Silveira, 2006; Krishan, 2015).

Through exploratory and descriptive research, developed from indirect documentation from secondary sources such as books and scientific papers published on digital bases, this present study aims to present how dental pulp is used as an accessory tool to determine the time of death of the corpse.

## 2. Methodology

A systematic review of the scientific literature was carried out with a descriptive and qualitative character, allowing the inclusion of experimental and non-experimental studies for a complete coverage of the analyzed phenomenon. For data collection, searches were carried out in the electronic databases: SciElo, LILACS and Elsevier. All scientific papers that used the terms "Legal dentistry", "Post-mortem interval", "Time of death", "Chronathanatognosis" in the title, keywords or abstracts of the articles, available in Portuguese, English and Spanish languages only were selected. The publication date was not specified. Scientific papers that did not specifically address the time of death of cadavers from teeth were also excluded.

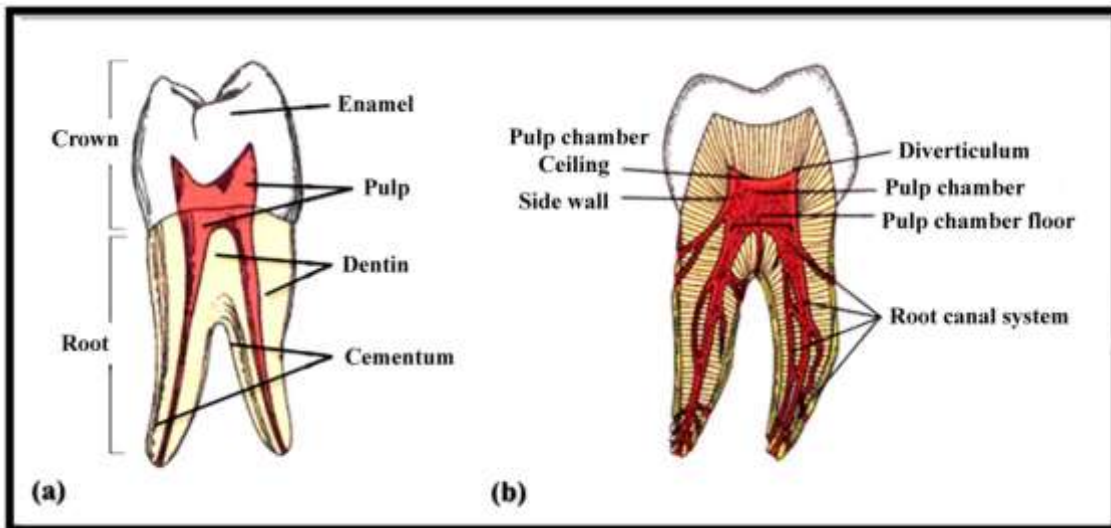
### 3. Results and Discussion

#### 3.1 Internal dental anatomy

The human tooth can be divided between crown and root (Figure 1a), consisting of mineralized tissues, enamel and mostly dentin, which circumscribes the pulp cavity. Dentin is covered in the crown, by the enamel, and in the root, by the cementum. The pulp cavity (Figure 1b) is the space located in the center of the crown and root of the tooth, limited almost exclusively by dentin and dental pulp (Madeira, 1997). The cavity can be divided into the coronary portion (pulp chamber) and root portion (root canal system), this is formed according to the external anatomy of the tooth (Cortês & Bastos, 2008).

The pulp is the most important tissue of the tooth; it is unique, soft of mesodermal origin, composed of specialized cells, odontoblasts, which are organized in a peripheral way and in direct contact with the dentin matrix. The pulp reacts to physical, chemical and bacteriological attacks, seeking to protect the tooth (Cohen & Hargreaves, 2006).

**Figure 1** - Histo-structural division of the tooth (A), and components of the pulp cavity (B).



Source: Cortês and Bastos (2008) modified by the authors.

The pulp chamber is located in the region of the dental crown, following its external shape, assuming a general characteristic common to the elements of each group of teeth. According to each group, they may have walls (buccal, lingual, mesial, distal, occlusal and cervical), which correspond to the coronary sides. The occlusal wall is related to the occlusal surface, being called the roof of the chamber. The wall related to the cervical surface, on the other hand, is called floor of the chamber, generally parallel to the roof. In the roof (Figure 1b), there are pulp horns that are indentations underlying cusps, tubers and other protrusions in the crown (Cortês & Bastos, 2008).

#### 3.2 Methods for identifying dental pulp changes

The determination of the exact moment of death of a body is a major factor in investigations and in solving crimes. Despite having great resistance, the teeth undergo changes due to physical-chemical factors related to the putrefaction process or to the action of biological agents through the infiltration of fungi and bacteria (Ferreira, et al., 2008; Menon, et al., 2011).

Caballin et al. (2010) performed the evaluation of the histological changes of the dental pulp to estimate the post-mortem interval. Their results showed three stages in the gradual loss of the pulp parenchyma and its organization until the seventh day. Carrasco et al. (2017) present the first histological study of the dental pulp to estimate the post-mortem interval up

to 6 months. The authors described changes in the pulp from 26 teeth of individuals with a known death time of 24 h, 1 month, 3 months and 6 months.

The pulp tissues obtained were fixed in formalin for the subsequent generation of histological sections, stained with Hematoxylin-Eosin and Masson's Trichrome. Microscopic analysis of the samples showed a progressive transformation of the cellular components and fibers of the dental pulp over the interval. With the data, they created qualitative and quantitative parameters to be used in the estimation of the interval based on the microscopic degradation of the dental pulp (Table 1).

**Table 1** - Histological transformations of the human dental pulp in the *post-mortem* interval of: 24 h., 1 month, 3 months and 6 months.

<i>Post-mortem</i> Interval	Hematoxylin-Eosin Stain	Masson's Trichrome Stain
24 hours	<ul style="list-style-type: none"> <li>• Large number of nuclei with homogeneous distribution in connective tissue</li> <li>• Circular, ovoid and extended form nuclei that suggest different types of cells: macrophages, fibroblasts</li> <li>• Well-maintained endothelial, blood vessel cells</li> <li>• Homogeneous distribution of extracellular matrix without vacuolization</li> </ul>	<ul style="list-style-type: none"> <li>• Relaxed connective tissue</li> <li>• Large number of nucleated cells homogeneously distributed</li> <li>• Higher proportion of collagen</li> <li>• Large number of nuclei with conserved nuclear morphology</li> <li>• Blood and lymphatic vessels.</li> <li>• Thin and wavy collagen fibers, with no defined pattern, distributed homogeneously in the tissue</li> <li>• Conserved nuclear morphology</li> <li>• Dense wavy collagen fibers</li> <li>• Partial loss of homogeneity of the extracellular matrix</li> </ul>
1 month	<ul style="list-style-type: none"> <li>• Redistribution of nuclei from the center to the periphery of the tissue</li> <li>• Conserved nuclear morphology</li> <li>• Absence of blood and lymphatic vessels</li> <li>• Partial loss of homogeneity of the extracellular matrix</li> <li>• Areas with vacuolization</li> </ul>	
3 months	<ul style="list-style-type: none"> <li>• Nucleus morphology not defined, with irregular chromatin</li> <li>• Extracellular matrix with disaggregated areas and vacuolization area</li> </ul>	<ul style="list-style-type: none"> <li>• Nuclei at the periphery of the tissue</li> <li>• Absence of blood and lymphatic vessels</li> <li>• Collagen fibers with homogeneous distribution</li> <li>• Vacuolization of connective tissue</li> </ul>
6 months	<ul style="list-style-type: none"> <li>• Tissue with a fibrous appearance</li> <li>• Low density of cores</li> <li>• Nuclei distributed randomly</li> <li>• Disaggregated extracellular matrix and with total loss of homogeneity</li> </ul>	<ul style="list-style-type: none"> <li>• Low cell density</li> <li>• Many collagen fibers</li> <li>• Disaggregated extracellular matrix</li> </ul>

Source: Carrasco et al. (2017), and changed by the authors.

Physiologically, Menon et al, have observed that up to 180 days after death there is a good conservation of the mineralized tissues of the teeth, which maintains their histological aspects stable; however, the soft tissues, such as the pulp and the periodontal ligament, may be fragmented or absent<sup>13</sup>. The dental pulp is made up of richly vascularized connective tissue and is therefore the first dental tissue to degrade (Corte-Real, et al., 2012). Since, it has a low cell composition, presenting a low oxygen consumption rate compared to most tissues. In the pulp, the highest metabolic rate is found in the region of odontoblasts, and the lowest, in the central area of the pulp, where most of the nerves and blood vessels are located (Vavpotic, et al., 2009).

Also, according to Vavpotic et al. (2009) and Duffy et al. (1991), the pulp is still capable of producing energy by carbohydrate metabolism, allowing it to function in different degrees of ischemia, which explains its post-mortem survival between 4 days to 2 weeks. Depending on environmental changes, the number of odontoblasts decreases after death, until the pulp tissue completely disappears, five days after death. About 130 odontoblasts are destroyed per mm<sup>2</sup> per hour, this rate of disintegration does not show regression with cooling (Menon, et al., 2011; Vavpotic, et al., 2009). Twenty-four hours after death 41% of the pulp tissue cells are still viable, and 6 days after death, DNA degradation is still minimal and there are no morphological changes due to autolysis (Vavpotic, et al., 2009). Therefore, extraction of DNA from the pulp after a certain time of death is, in some cases, possible Oliveira et al. (2004) observed that three items interfere with the conservation of the dental pulp. They are the degradation conditions of the post-mortem DNA, conditions in which the burial was carried out and cause of death.

Higgins et al. (2015) showed in their study that there are different percentages of nuclear and mitochondrial DNA in the different tissues that make up the tooth after the death of an individual. Histological and molecular studies of decaying teeth during small and medium postmortem time intervals reveal that the nuclear DNA in the pulp decreases dramatically with the reduction of the structural integrity of the tooth.

The PCR technique is applied in cases where the dental pulp serves as genetic material, being compared later with hair or cells found in the individual's belongings (Silva, 1997). Gaytmenn and Sweet (2003) claim that teeth are a useful source of DNA, especially in mass accidents, where identification by other routes becomes impossible.

For genetic analysis, mitochondrial DNA has the advantage of a high number of copies per cell. When the amount of extracted DNA is very small or severely degraded, such as DNA obtained from bodies reduced to their skeleton, the probability of obtaining a DNA profile from mitochondrial DNA is much higher than from DNA nuclear (Sweet & Sweet, 1995). The same authors isolated high molecular weight DNA from the dental pulp of a third molar, derived from the remains of a murder victim, who had an almost completely carbonized body. Therefore, while teeth that have not been decomposed yet have sufficient amounts of either nuclear DNA or mitochondrial DNA for further analysis, the same is not true when the teeth are already highly decomposed.

Another advantage of mitochondrial DNA is that it is resistant to enzymatic digestion due to its circular structure. The analysis of this type of DNA is utilized in the study of ancient and even archaeological tissues (Vieira, et., 2010). Malaver and Yunis (2003) evaluated different dental tissues as a source of DNA for forensic analysis; the teeth buried for 5 years were exhumed in the year 2000. When comparing the pulp, dentin and cement, they identified that the pulp produced the strongest signs of PCR amplification.

It is worth emphasizing, regarding the quantification of DNA present in the different dental tissues, that the individual's age influences the tooth decomposition. According to the study by Higgins et al.<sup>21</sup> there is a greater resistance to the decomposition of older teeth, as more mature teeth have apices completely closed and are usually more mineralized and less porous than younger teeth, thus the DNA is better preserved in older people (Higgins, et.al., 2015).

Schwartz et al. (1991) carried out tests in conditions of varying pH (3, 7 and 10), temperature (4°C, 25°C, 37°C and

tooth incineration), humidity (20%, 66% and 98%) and various types of soil (sand, potting soil, garden soil), sea water with teeth buried outdoors. The authors found that environmental conditions did not affect the collection of high molecular weight DNA from dental pulp tissue.

However, Remualdo et al. (2005) observed that the 2°C variation in soil temperature can affect the preservation of both nuclear and mitochondrial DNA. Higgins et al. (2015) showed that when the temperature is lower, the DNA degradation is less and therefore it will be easier to analyze it later.

Tsuchimochi et al. (2002) verified the possibility of extraction and amplification of DNA from dental pulp of teeth subjected to temperatures up to 300°C. However, they observed that teeth subjected to temperatures above 300°C did not allow any amplification of DNA. Menon, Prado and Silva (2011) observed that the inhumation of teeth for periods of 7, 30, 60 and 180 days demonstrated good conservation of mineralized tissues of the teeth and fragmentation of the dental pulp. In cases where the body is soaked, room temperature is a decisive factor in obtaining a DNA sample from dental tissue, due DNA degradation (Silva, 2012).

Since RNA degrades more quickly than DNA, its ability to possess genetic information is minimally explored in post-mortem research (Fordyce). Also, factors such as temperature, environmental, chemical conditions, body storage location of remnants, microbiological contamination, and tissue pH are some variables with great relevance that might influence the behavior of the RNA molecule immediately after death (Catts, et al., 2005; Sampaio-Silva, et al., 2013). Another factor to consider, is that the RNA molecule is also fragile and has variable availability. Its levels can fluctuate from person to person due to factors such as diseases, medical treatments or genetic variability (Young, et al., 2013).

Young et al. (2013) conducted a study to test the post-mortem interval estimate using pig teeth and making an association between RNA degradation and pulp color changes. Two teeth of each of eight pigs, previously buried in a shallow grave were randomly sampled at 0 hours, 7 days (d), 14 d, 21 d, 28 d, 42 d, 56 d, 70 d, 84 d, 98 d, 112 d, 126 out of 140 d. Although the authors obtained favorable results from this association with an effective method for estimation in a period of up to 84 days, they demonstrate that, although possible, using the levels of RNA extracted from the dental pulp for the estimate would be difficult. As the dental pulp is a highly vascularized connective tissue that is located inside the complex and is protected by the layers of dentin and enamel, the pulp presents itself as a place of possible favorable conditions for the RNA extraction to be carried out for analysis.

The observation of a previous study on the use of RNA from dental pulp has shown that this molecule has a relatively stable behavior and is more resistant to the action of temperature in this tissue when compared to the behavior of other tissues of the body (Conde, et al., 2012). Poór et al. (2016) also demonstrated that estimating PMI through an RNA molecule extracted from the pulp can be an effective methodology.

Poór et al. (2016) analyzed RNA extracted from pulps of premolars and third molars to show whether the degradation of RNA from these samples could be used to estimate PMI. The authors ended the degradation as RNA integrity number (RIN) by PCR after reverse transcription. They concluded that the method, despite its limitations, is promising with regard to the PMI estimate, allowing determination of the post-mortem interval with a high level of confidence in the first 21 days.

Another physiological post-mortem change that occurs in teeth is the so-called “pink teeth” phenomenon, first described by Thomas Bell, which consists of a change in the color of the intraosseous part of the tooth, without changing the enamel (Mansilla, et al., 2003; Soriano, et al., 2009; Thapar, et al., 2013). Pink coloration is a natural event caused by the infiltration of fluid containing hemoglobin, or products derived from its degradation in dentin through dentinal tubules, due to decomposition or liquefaction of the pulp.

After death, and with the appropriate conditions, the pulp tissue undergoes autolysis and the hemoglobin is released from the erythrocytes, but kept in solution, which diffuses into the tubules. The change in the color of the teeth occurs only

after hemolysis, because the diameter of the erythrocytes is larger than that of the tubules and therefore it is necessary that they undergo cell lysis for the pigments to infiltrate. Thus, the staining only becomes visible macroscopically after 6 days, and this period without visible staining can continue up to weeks (Soriano, et al., 2009; Thapar, et al., 2013).

According to Rai and Kaur (2011), pink pigmentation is a chromatic alteration of the teeth, which may be related to sudden and violent deaths, resulting from blood leakage from the dental pulp, with the penetration of hemoglobin or its by-products inside the teeth dentinal tubules. These authors stated that factors such as humidity, temperature and the position in which bodies are found are supporting factors in the development of the phenomenon (Rai & Kaur, 2011).

#### 4. Conclusion

The determination of the interval after the death of an individual is essential for police investigation. It contributes to the differential diagnosis of the event and to the reconstruction of the crime scene and dynamics, which could oppose or validate suspects' alibis.

The degradation of dental pulp after death is a complex process that has not been fully studied, but with the techniques shown in the text, reliable post-mortem interval results are already possible through this analysis. However, further studies are still needed for a more precise use of pulp tissue as a tool for determining PMI.

The authors are realizing new researches to improve the use of dental pulp in Chronothanatognosis.

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