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# Prenatal ultrasound and postmortem histologic evaluation of tooth germs: an observational, transversal study

Mariana Seabra<sup>1,7\*</sup>, António Felino<sup>2</sup>, Rosete Nogueira<sup>3,8,9</sup>, Francisco Valente<sup>4</sup>, Ana Cristina Braga<sup>5</sup> and Paula Vaz<sup>6</sup>

## Abstract

**Introduction:** Hypodontia is the most frequent developmental anomaly of the orofacial complex, and its detection in prenatal ultrasound may indicate the presence of congenital malformations, genetic syndromes and chromosomal abnormalities.

To date, only a few studies have evaluated the histological relationship of human tooth germs identified by two-dimensional (2D) ultrasonography. In order to analyze whether two-dimensional ultrasonography of tooth germs may be successfully used for identifying genetic syndromes, prenatal ultrasound images of fetal tooth germs obtained from a Portuguese population sample were compared with histological images obtained from fetal autopsies.

**Methods:** Observational, descriptive, transversal study. The study protocol followed the ethical principles outlined by the Helsinki Declaration and was approved by the Ethics Committee of the School of Dental Medicine, University of Porto (FMDUP, Porto, Portugal) and of the Centro Hospitalar de Vila Nova de Gaia/Espinho (CHVNG/EPE, Porto, Portugal) as well as by the CGC Genetics Embryofetal Pathology Laboratory. Eighty-five fetuses examined by prenatal ultrasound screening from May 2011 to August 2012 had an indication for autopsy following spontaneous fetal death or medical termination of pregnancy. Of the 85 fetuses, 37 (43.5%) were randomly selected for tooth germ evaluation by routine histopathological analysis. Fetuses who were up to 30 weeks of gestation, and whose histological pieces were not representative of all maxillary tooth germs was excluded. Twenty four fetus between the 13<sup>th</sup> and 30<sup>th</sup> weeks of gestation fulfilled the parameters to autopsy.

**Results:** Twenty four fetuses were submitted to histological evaluation and were determined the exact number, morphology, and mineralization of their tooth germs. All tooth germs were identifiable with ultrasonography as early as the 13<sup>th</sup> week of gestation. Of the fetuses autopsied, 41.7% had hypodontia (29.1% maxillary hypodontia and 20.9% mandibular hypodontia).

**Conclusions:** This results indicate that prenatal ultrasound is a reliable method for detecting of hypodontia an early gestational ages. Further studies with larger samples are needed to confirm these results.

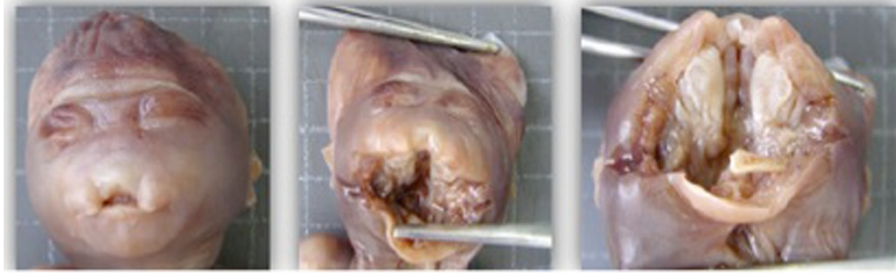
**Keywords:** Tooth germ, Tooth buds, Ultrasound, Prenatal, Diagnosis, Histology

\* Correspondence: marianaseabr@gmail.com

<sup>1</sup>Faculty of Dentistry, University of Porto (FMDUP), 4200-393 Porto, Portugal

<sup>7</sup>Portuguese Catholic University (UCP), Viseu, Portugal

Full list of author information is available at the end of the article



**Figure 1** Fetus at 21 weeks GA with removal of the upper jaw and mandible. Source: CGC Genetics/Embryofetal Pathology Laboratory, Portugal.

## Introduction

Hypodontia or tooth agenesis is the most frequent developmental malformation of the orofacial complex [1] and is commonly associated with other abnormalities in many syndromes [2,3]. Detection of hypodontia in prenatal ultrasound may therefore be a sign of congenital malformations, genetic syndromes and chromosomal abnormalities [4,5].

Human odontogenesis occurs over a long period, approximately from the 6th intrauterine week until late adolescence, when the roots of the third molars are formed [6]. In the mammalian embryo, teeth develop via a series of interactions between the odontogenic epithelium and the neural crest-derived ectomesenchyme of the early jaw [7-11].

Studies about intrauterine tooth development in humans are scarce and most work in this area to date has been conducted in rodents [12]. Although mouse models have been extremely valuable to gain a better understanding of human craniofacial development, rodent and human dental formulas diverge [13], making it difficult to extrapolate the results to our understanding of human dental development. Therefore, research with human fetuses is essential to expand our knowledge in

this area (Figure 1). Fetal or perinatal autopsy is useful to determine different developmental parameters, to detect congenital abnormalities, to identify the cause of death and the risk of possible recurrence, and to identify possible genetic syndromes [14].

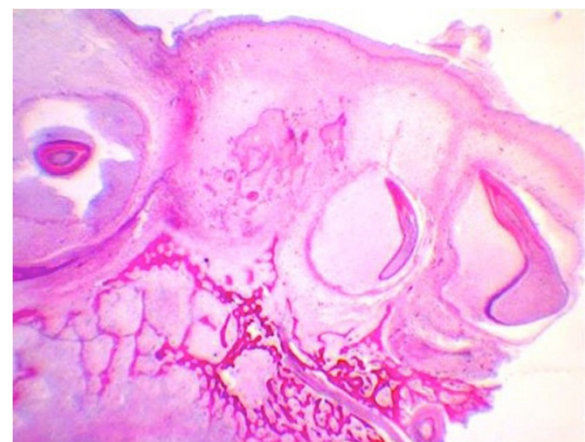
To date, only a few studies have evaluated the histological relationship of human tooth germs identified by two-dimensional (2D) ultrasonography. Most of these studies, conducted with a limited number of pregnant women and abortion products [4,5,15], failed to visualize tooth germs with ultrasonography during the early gestational stages and to identify all tooth germs in fetal autopsies. To analyze whether 2D ultrasonography was an adequate tool for evaluating tooth germs and identify genetic syndromes, our study investigated whether prenatal ultrasound images of fetal tooth germs obtained from a Portuguese population sample correlated with histological images obtained from fetal autopsies.

## Methods

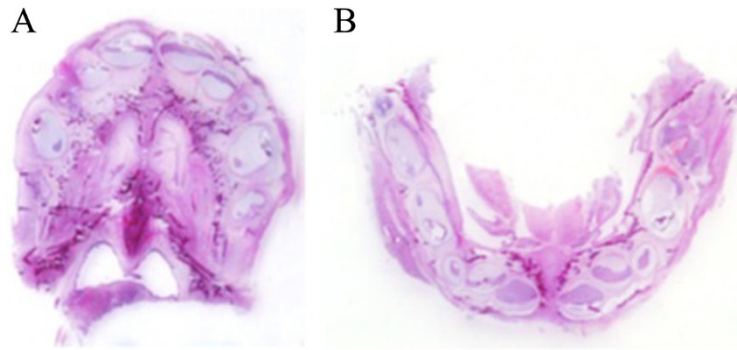
The study protocol followed the ethical principles outlined by the Helsinki Declaration and was approved by

**Table 1** Distribution of fetuses according to gestational ages (GA)

GA	N	%
13	1	4.2
14	4	16.6
15	4	16.7
16	2	8.3
17	1	4.2
18	1	4.2
21	5	20.9
22	3	12.5
26	1	4.2
28	1	4.2
30	1	4.2
Total	24	100.0



**Figure 2** Histological section of maxilla from a fetus at 30 GA weeks. Initial mineralization of dental germs from temporary dentition (HE 10x). Source: CGC Genetics/Embryofetal Pathology Laboratory, Portugal.

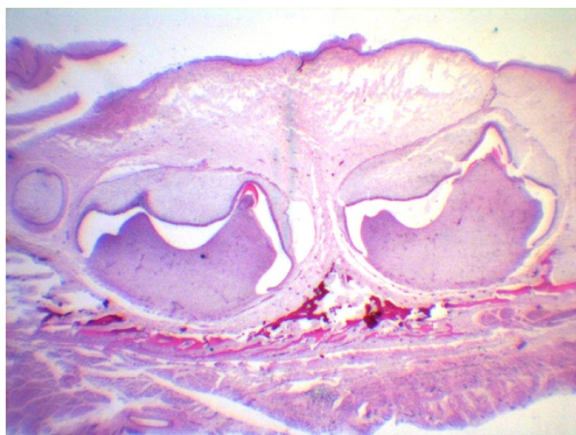


**Figure 3** Fetus at 21 GA weeks. All temporary tooth germs are present at maxilla (A) and mandibula (B); (HE macro). Source: CGC Genetics/Embryofetal Pathology Laboratory, Porto, Portugal.

the Ethics Committee of the Dental Medicine Faculty, University of Porto (FMDUP, Porto, Portugal), and of the Centro Hospitalar de Vila Nova de Gaia/Espinho (CHVNG/EPE, Porto, Portugal) as well as by the CGC Genetics Embryofetal Pathology Laboratory.

Fetal tooth germs were visualized with 2D prenatal ultrasound (GE E8 Voluson® equipment, serial number 0123, 2010, Austria) with C512D, Rab4-8D, 11LD, and C1-5 probes, with normal harmonic frequency. The images were viewed, captured, and archived, using the Astraia® program (version 1.23.0, Astraia Software GmbH and parents, Germany), and processed on the same equipment with dimensions of 640 \* 480 VGA pixels.

Two operators were reliable for conducting examinations and recording data. They were both specialists in fetal medicine and had equivalent levels of practice in obstetric ultrasound and prenatal diagnosis. Calibration was achieved on both operators to ensure a correct understanding of the ultrasound images.



**Figure 4** Histological section of jaw from fetus at 21 weeks GA – (HE 10x). Source: CGC Genetics/Embryofetal Pathology Laboratory, Portugal.

Fetal tooth germs in both maxilla and mandible were visualized with 2D prenatal ultrasound (GE E8 Voluson® equipment) in a group of 157 pregnant Portuguese participants being seen at the CHVNG/EPE. Informed consent was obtained from participants before the exam.

Eighty-five fetuses examined by prenatal ultrasound screening from May 2011 to August 2012 had an indication for autopsy following spontaneous fetal death or medical termination of pregnancy. Of these, 37 (43.5%) were randomly selected for tooth germ evaluation by routine histopathological analysis. Of these 13 were excluded because they did not meet the study's inclusion criteria, which were the following: 1) fetuses who were up to 30 weeks of gestation, and 2) fetuses whose histological pieces were not representative of all maxillary tooth germs. Therefore, the final sample included 24 autopsied fetuses who were between the 13<sup>th</sup> and 30<sup>th</sup> weeks of gestation (Table 1). Each fetus was examined according to a predesigned protocol from the Embryofetal Pathology Laboratory CGC Genetics, which included a photograph, a whole body radiograph, and external and internal examination and microscopic study. Prior to post-mortem examination, written consent was obtained from the father or from another relative.

The statistical analysis was performed using IBM® SPSS (Statistical Package for Social Sciences) version 22.0 and R version 2.15.1 (2012-06-22). Given the nature of the variables involved, it was decided to use statistical tools based on the analysis most appropriate to the scales of measurement. Thus, the analysis consisted of the prevalence study in which estimates were determined for all parameters evaluated, as well as interval

**Table 2** Prevalence of hypodontia in fetal autopsies

		N	Percent	95% Confidence interval
Valid	Absence	14	58.3	-
	Presence	10	41.7	[22.8%; 3.1%]
	<b>Total</b>	24	100.0	



estimates with 95% confidence, and analytical study of the data for qualitative variables, where the association between two variables was established using the chi-square test of independence (for 2x2 tables the exact test of Fisher was used). The decision rule consists of detecting statistically significant evidence for probability value (p-value of the test) less than 0.05.

**Results**

For the 157 fetuses who were initially examined by 2D ultrasound, the median gestational age (GA) at which all 10 maxillary and mandibular tooth germs were identified was the 13th week. In 25% of cases, GA was equal to or less than 12 weeks.

Only fetuses from interrupted pregnancies or cases of fetal death were autopsied. The remaining clinical cases followed a normal pregnancy. We analyzed 37 cases of fetal death/pregnancy medical interruption. Of these, 13 were excluded because they did not meet the inclusion criteria.

Of the 24 autopsied fetuses (Table 1), 14 (58.3%) were male and 10 (41.7%) were female. The chi-square test revealed no significant differences related to gender distribution ( $\chi^2 = 0.75$ ,  $df = 1$ ,  $p \text{ value} = 0.3865 > 0.05$ ). Thirteen (13) of the fetuses were medical abortions (MA) and 11

**Table 3 Hypodontia and fetus gender**

		Gender		Total
		Female	Male	
Hypodontia	No	Count	4	10
	Yes	Count	6	4
Total		Count	10	14

**Table 4 Maxillary hypodontia and clinical information**

		Clinical information		Total
		MA	SA	
Maxillary hypodontia	No	Count	9	8
	Yes	Count	4	3
Total		Count	13	11

were spontaneous abortions (SA). The MA cases were associated with relatively earlier gestational stages. A Fisher's exact test revealed that spontaneous abortions were more frequent in male fetuses ( $p = 0.004$ ).

It was determined the exact number, morphology, and mineralization of tooth germs in the 24 fetuses who underwent histological evaluations (Figures 2, 3, and 4). All tooth germs were histologically identified during the 13th week of gestation. Of the 24 fetuses autopsied, 41.7% had hypodontia (Table 2) (Figure 5).

Females and males were similarly affected by hypodontia ( $p = 0.132$ ) (Table 3). Maxillary and mandibular hypodontia were observed in 29.1% and 20.9% of fetuses, respectively (Tables 4 and 5).

A Fisher's exact test showed no significant association between maxillary ( $p = 1.000$ ) or mandibular ( $p = 0.630$ ) hypodontia and clinical information concerning the number of spontaneous or medical abortions (Tables 4 and 5).

The teeth most frequently missing in the maxilla were 65 and 55, while 81 was the most frequently missing tooth in the mandible.

**Discussion**

In this study, we showed that all tooth germs were histologically present at the 13th week of gestation, as revealed by prenatal ultrasonography (Figure 6). Therefore, we believe that visualization of tooth germs with 2D ultrasonography during the 13th week of gestation is a useful method of identifying genetic syndromes.

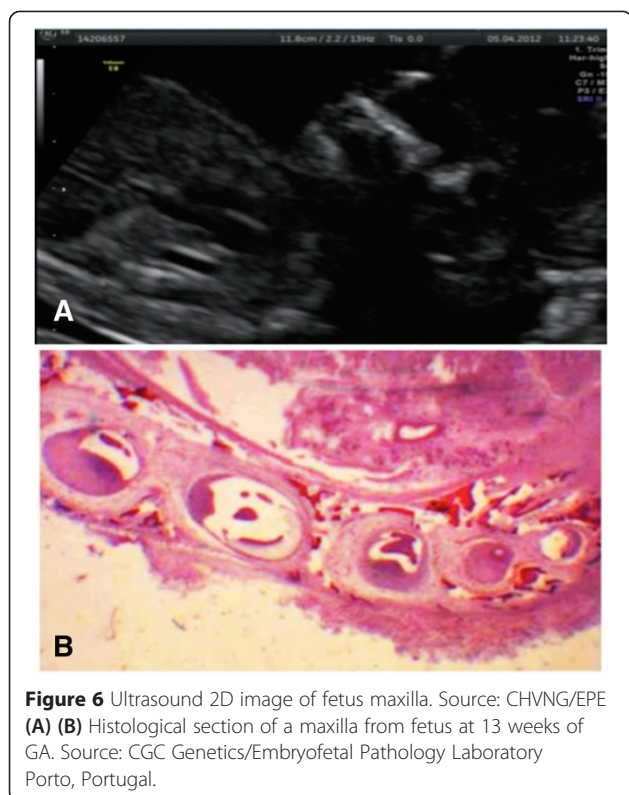
To our knowledge, this study was the first to investigate the occurrence of hypodontia in temporary dentition in autopsied fetuses. The number, morphology, and mineralization of the tooth germs were identified in all histological evaluations of our sample.

Although tooth agenesis is the most frequent developmental malformation of the orofacial complex, its prevalence varies considerably between generations and classes

**Table 5 Mandibular hypodontia and clinical information**

		Clinical information		Total
		MA	SA	
Mandibular hypodontia	No	Count	11	8
	Yes	Count	2	3
Total		Count	13	11





**Figure 6** Ultrasound 2D image of fetus maxilla. Source: CHVNG/EPE (A) (B) Histological section of a maxilla from fetus at 13 weeks of GA. Source: CGC Genetics/Embryofetal Pathology Laboratory Porto, Portugal.

of teeth. Agenesis of primary teeth is very rare, occurring at a frequency of less than 1% [16–18].

Some cases of tooth agenesis occur independently of developmental defects in other organs and are referred to as non-syndromic. However, missing teeth are also observed in association with other malformations, most noticeably with cleft lip, with or without cleft palate [18]. We detected hypodontia in 41.7% of autopsied fetuses, a much higher prevalence than that described for other non-syndromic populations, which varied from 0.1 to 2.63% [19–28]. This value may be related to the fact that tooth agenesis is usually associated with a variety of syndromes. As our sample consisted of fetuses who had suffered spontaneous death or medical termination of pregnancy, it is reasonable to assume that this group would present a higher number of syndromes associated with hypodontia.

Although it has been reported that females are generally more affected by hypodontia than males [29], we did not detect significant gender differences in the autopsied fetuses evaluated in the current study. Further studies with larger samples are needed to determine whether the prevalence of hypodontia is associated with gender in aborted fetuses with congenital malformations and genetic syndromes. Whether a specific absence of tooth germs relates to some kind of fetal abnormality is another important question that remains unanswered.

## Conclusions

Our results indicate the reliability of the ultrasound method for the prenatal detection of hypodontia at early gestational ages. The assessment of tooth germs in prenatal ultrasound may allow for the early identification of genetic syndromes. We were able to visualize, identify, and count fetal tooth germs using prenatal 2D ultrasound, around the 13th week of gestation.

The early prenatal identification of tooth germs enables the diagnosis of genetic syndromes with tooth number abnormalities and may represent a new approach for the early intervention of these syndromes in pediatric dentistry. The early diagnosis of these genetic syndromes would allow the pediatric dentist to prepare for the correct interception treatment, which could occur at birth (oral cleft, applying the nasoalveolar molding and the oral plaque), during the first months of life or even after the third year of life.

## Competing interests

The author(s) declare that they have no competing interests.

## Authors' contributions

MS carried out the studies and drafted the manuscript. AF, FV, PV participated in the design of the study and performed the statistical analysis. RN, AB conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

## Author details

<sup>1</sup>Faculty of Dentistry, University of Porto (FMDUP), 4200-393 Porto, Portugal. <sup>2</sup>Department of Oral Surgery of FMDUP, Porto, Portugal. <sup>3</sup>Pathology Laboratory of CGC Genetics/Centro de Genética Clínica, Porto, Portugal. <sup>4</sup>Prenatal Diagnosis Unit, Hospital of Vila Nova de Gaia/Espinho (CHVNG/E), Porto, Portugal. <sup>5</sup>Department of Production and Systems Engineering, and researcher in Algoritmi Centre University of Minho, Braga, Portugal. <sup>6</sup>Department of Medical and Orofacial Genetics of FMDUP, Porto, Portugal. <sup>7</sup>Portuguese Catholic University (UCP), Viseu, Portugal. <sup>8</sup>Life and Health Sciences Research Institute (ICVS), School of Health Sciences (ECS), Campus of Gualtar, University of Minho, Braga, Portugal. <sup>9</sup>ICVS/3B's - PT Government Associate Laboratory, Braga, Guimarães, Portugal.

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