

## ORIGINAL ARTICLE

# Two-Dimensional Identification of Fetal Tooth Germs

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**Objective:** To demonstrate the efficiency and applicability of two-dimensional ultrasonography in the identification of tooth germs and in the assessment of potential pathology.

**Design:** Observational, descriptive, cross-sectional study.

**Setting:** Prenatal Diagnosis Unit of Centro Hospitalar de Vila Nova de Gaia / Espinho–Empresa Pública in Portugal.

**Patients:** A total of 157 white pregnant women (median age, 32 years; range, 14 to 47 years) undergoing routine ultrasound exams.

**Main Outcome Measure(s):** Description of the fetal tooth germs, as visualized by two-dimensional ultrasonography, including results from prior fetal biometry and detailed screening for malformations.

**Results:** In the first trimester group, ultrasonography identified 10 tooth germs in the maxilla and 10 tooth germs in the mandible in all fetuses except for one who presented eight maxillary tooth germs. This case was associated with a chromosomal abnormality (trisomy 13) with a bilateral cleft palate. In the second and third trimesters group, ultrasonography identified a larger range of tooth germs: 81.2% of fetuses showed 10 tooth germs in the maxilla and 85.0% of fetuses had 10 tooth germs in the mandible. Hypodontia was more prevalent in the maxilla than in the mandible, which led us to use qualitative two-dimensional ultrasonography to analyze the possible association between hypodontia and other variables such as fetal pathology, markers, head, nuchal, face, and spine.

**Conclusions:** We recommend using this method as the first exam to evaluate fetal morphology and also to help establish accurate diagnosis of abnormalities in pregnancy.

KEY WORDS: *diagnosis, prenatal, tooth buds, tooth germ, ultrasound*

The field of craniofacial development has generated many divergent views and theories regarding the causes for normal and abnormal developmental profiles (Evans and Francis-West, 2005). The identification of facial abnormalities during pregnancy may be critical for the diagnosis of various genetic and polymalformative syndromes and chromosomal abnormalities (Clementi et al., 2000; Rotten and Levaillant, 2004a).

The visualization and characterization of the jaws should be part of the routine ultrasound examination of the fetus

(Andresen et al., 2012). The midsagittal plane allows one to study facial dysmorphology, analyze the facial profile, and measure several biometric parameters, such as facial angles and nasal bone length. The posterior coronal nose-mouth plane, on the other hand, is essential for evaluating lip continuity (or its interruption), nostril deformation, and alveolar crest alignment. Finally, serial axial images are used to analyze the eyes, lips, jaws, and tongue (Babcook et al., 1996; Cash et al., 2001; Chen et al., 2001; Ghi et al., 2002).

Characterization of both jaws and assessment of their size and position is crucial for investigating the presence of a number of genetic syndromes. Similarly, the continuity of the alveolar crests (especially in the upper jaw) may reveal the presence of cleft palate, particularly when there is a diagnosis of cleft lip. The jaw bones define the oral cavity and should therefore be evaluated as early as possible (Babcook and McGahan, 1997; Rotten and Levaillant, 2004b).

According to some researchers, the assessment of tooth germs with prenatal ultrasound may also contribute to the early identification of chromosomal syndromes (Ulm et al., 1995; Ulm et al., 1998; Ulm et al., 1999). Because there is little information regarding the usefulness of visualizing tooth germs, there is still controversy regarding the validity

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of using two-dimensional (2D) and three-dimensional (3D) ultrasound to identify tooth germs, which might facilitate the prenatal diagnosis of craniofacial anomalies (Ulm et al., 1995; Ulm et al., 1998; Ulm et al. 1999).

In this study, we aimed to demonstrate the efficiency and applicability of 2D ultrasonography in evaluating tooth germs during routine fetal prenatal screening. The 2D ultrasound has fewer side effects than magnetic scans, making it safe to use during gestation, which is when tooth germs can be identified.

## METHODS

Between May 2011 and August 2012, we randomly selected study participants from a group of pregnant women undergoing routine ultrasound exams at the Prenatal Diagnosis Unit of Centro Hospitalar de Vila Nova de Gaia / Espinho—Empresa Pública (CHVNG/EPE) in Portugal, according to the following inclusion criteria: white pregnant women with unifetal pregnancies of either sex, who were between the 11th and 36th weeks of gestation. The final study sample included 157 women.

The 2D prenatal ultrasounds were performed with GEE8 Voluson equipment (serial number 0123, General Electric Healthcare, West Milwaukee, WI) with C512D, Rab4-8D, 11LD, and C1-5 probes and with a normal harmonic frequency. Study participants were at rest during testing and received the transabdominal approach. The images were viewed, captured, and archived using the Astraia program (version 1.23.0, Astraia Software GmbH, Munich, Germany) and were processed on the same equipment with a resolution of  $640 \times 480$  VGA pixels.

The exams were performed as part of routine pregnancy visits and thus were not designed with the intention of addressing our specific research questions. This study was therefore observational, descriptive, and cross-sectional. Informed consent was obtained from each woman before the ultrasonographic exam. The study protocol followed the ethical principles outlined in the Declaration of Helsinki and was approved by the ethics committees of the School of Dental Medicine, University of Porto (Porto, Portugal) and of the CHVNG/EPE, Portugal.

Two operators were responsible for conducting examinations and recording data. They were both experts in fetal medicine and had comparable levels of experience in obstetric ultrasound and prenatal diagnosis. Calibration was performed on both operators to ensure a correct interpretation of the ultrasound images analyzed here.

The description of the fetal tooth germs, as visualized by 2D ultrasonography, included results from prior fetal biometry and detailed screening for malformations. The jaws were visualized with sagittal, coronal, and axial cuts, using the plane of the nuchal translucency and nasal bone as a starting point. With this plane, and through oblique deviations, we were able to study the entire maxilla and mandible. The acquired coronal section should include the



**FIGURE 1** Ultrasound image of tooth germs in the maxilla of a fetus (13th week of gestation). Source: CHVNG/EPE.

mandible, maxilla, and nasal triangle. We identified a groove separating the fetal tooth germ from the alveolar crest. The presence of this hypoechoic groove is believed to indicate the presence of dental germs (Fig. 1).

The data were analyzed using the independent Fisher exact test for contingency  $2 \times 2$  tables with SPSS, version 20.0 (IBM Corp., Armonk, NY).

## RESULTS

The original study group consisted of 161 pregnant women, including 77 in their first trimester and 84 in the second and third trimesters of pregnancy. In four women in the second and third trimesters group, the dental germs were not observed due to the fetus's position and poor echogenicity. These women were therefore excluded from the study, resulting in a final sample of 157 women (77 in the first trimester and 80 in the second and third trimesters). The rate of nonobservation error was 2.5% and was found mainly for later gestational periods.

The median age of the entire study group was 32 years, and the age range was 14 to 47 years. The average age of mothers in the first trimester group was 32.1 years (range, 18 to 43;  $SD = 6.2$ ), and in the second and third trimesters group, the average age was 31.4 years (range, 14 to 47;  $SD = 6.7$ ). These values were not statistically different (two independent samples  $t$  test:  $t = 40.721$ , degree of freedom [ $df$ ] = 152,  $P = .472 > .05$ ).

Whether at the level of the maxilla or mandible, the median gestational age for the detection of 10 tooth germs was 13 weeks. We found that in 25% of the women, the detection of dental germs was possible before the 12th week of gestation.

In the first trimester group, ultrasonography identified 10 tooth germs in the maxilla and 10 tooth germs in the mandible in all fetuses except one who presented eight maxillary tooth germs. This case was associated with a

**TABLE 1** Distribution of Tooth Germs by Location and Gestation Period

Evaluation Period	Location	Tooth Germs	n	%	95% CI
1st trimester	Maxilla	8	1	1.3	0.03 to 7.02
		10	76	98.7	92.97 to 99.97
		Total	77	100.0	
	Mandible	10	77	100.0	
		Total	77	100.0	
2nd and 3rd trimesters	Maxilla	8	1	1.2	0.03 to 6.77
		9	2	2.5	0.30 to 8.74
		10	65	81.2	70.96 to 89.11
		12	12	15.0	8.00 to 24.74
		Total	80	100.0	
	Mandible	10	68	85.0	
		12	12	15.0	
		Total	80	100.0	

chromosomal abnormality (trisomy 13) with a bilateral cleft palate.

In the second and third trimesters group, ultrasonography identified a larger range of tooth germs: 81.2% of fetuses showed 10 tooth germs in the maxilla and 85.0% of fetuses had 10 tooth germs in the mandible. In a few fetuses, eight and nine tooth germs were identified (1.2% and 2.5%, respectively). In another 15.0%, 12 tooth germs were observed in the maxilla and in the mandible, corresponding to the identification of the first permanent molar (Table 1).

Hypodontia was more prevalent in the maxilla (2.5%) than in the mandible. This observation led us to use qualitative 2D ultrasonography to analyze the possible association between hypodontia and other variables, such as fetal pathology, markers, head, nuchal, face, and spine.

We conducted the independent Fisher exact test for  $2 \times 2$  tables, which revealed that hypodontia in the maxilla was not significantly correlated with fetal pathology ( $P = .324$ ), head anomalies ( $P = .872$ ), fetal face anomalies ( $P = .956$ ), or fetal spinal anomalies ( $P = .956$ ) during the second and third trimesters of pregnancy.

## DISCUSSION

Few studies have evaluated fetal tooth germ parameters. In a study by Ulm et al. (1998), the percentage of tooth germs observed varied according to the type of ultrasonography used (2D or 3D). Whereas 3D ultrasonography yielded a success rate of 86% to 94% in identifying tooth germs at 19 weeks of gestation, 2D ultrasonography was only 56% to 62% successful.

Although we used 2D ultrasound, we achieved a nonobservation error rate of 2.5%, which was lower than that of other studies such as those of Ulm and colleagues. In some cases, we were able to detect up to 12 tooth germs: the 10 tooth germs corresponding to the temporary teeth as well as the very beginning of the two first permanent molar teeth, in the maxilla as well as the mandible.

Another discrepancy between our study and that of Ulm et al. (1998) is that those authors state that the number of

tooth germs remains constant throughout pregnancy and are visible as early as the 16th week of gestation, becoming progressively easier to identify as the pregnancy progresses. This difference between studies might be explained by a possibly sharper learning curve among the operators in our study—who received daily training for 3 months prior to the start of data collection—as well as differences in the equipment used, because our equipment has a wider range of options that probably optimized our images. The option HD Live, for instance, provides anatomical realism and helps increase depth perception. This helps achieve a deeper understanding of relational anatomy. Speckle Reduction Imaging suppresses speckle artifacts and maintains tissue architecture. The option CrossXBeamCRI enhances tissue and border differentiation. The HD-Flow reduces overwriting. The Advanced Volume Contrast Imaging With OmniView helps improve contrast resolution and visualization of the rendered anatomy with clarity in any image plane, even when viewing irregularly shaped structures. These options greatly contributed to the quality of the acquired images of our study and may explain the greater number and earlier detection of tooth germs in our study as compared with results reported elsewhere.

We would like to emphasize that the number of tooth germs we identified in our study was not constant over the gestational period, which is in line with Ten Cate (2008), who suggested that the development of all temporary teeth starts *in utero* and that the first permanent molar teeth start developing somewhere around the 20th week of gestation.

As mentioned herein, some fetuses in the second and third trimesters presented six tooth germs in each quarter; whereas, in the first trimester, all fetuses presented five tooth germs, except in the case of anomalies, such as one case of bilateral orofacial cleft.

Because we observed that the maxilla was more affected by hypodontia (2.5%) than the mandible, we used 2D ultrasonography to analyze the association between hypodontia and the other variables studied, such as fetal pathology markers, head, neck, face, and spine. We did not find any significant associations. To our knowledge, this is the first study reporting such results because this is an emerging area of research. We believe that significant correlations will emerge with larger study samples. In fact, the prenatal characterization of orofacial cleft and the evaluation of its severity have been previously suggested by some authors to be facilitated with visualization of the tooth germs using 2D ultrasonography (Rotten and Levaillant, 2004a; Sommerlad et al., 2010).

In this study, we were also able to diagnose orofacial cleft prenatally by using 2D ultrasonography. Usually, four-dimensional and magnetic ultrasound are used to further test the suspected diagnosis, which is mostly done by the end of the first trimester or during the second trimester. We believe that the visualization of fetal tooth germs using 2D ultrasound at 13 weeks of gestation will be a reality in the near future, and it may be used as an additional control or

even a marker in the diagnosis of genetic syndromes. There are still difficulties associated with the diagnosis of orofacial clefts. A step toward accurately identifying orofacial clefts in the general population may be a systematic assessment of the fetal jaws during the first trimester of pregnancy.

Therefore, we recommend that studying tooth germs should be included in the echographic examination performed during the first trimester assessment of changes in the upper face bones. The use of 2D ultrasonography to visualize fetal tooth germs represents a potentially useful complementary method that would assist in the diagnosis of severe syndromes associated with hypodontia or supernumerary teeth. However, more studies are needed, with larger samples, to confirm the usefulness of this method.

### CONCLUSION

Tooth germs have a similar echogenicity to that of bone. Their identification is possible through the observation of an ultrasound structure—a hypoechogenic groove—that in this exam separates the germs from the dental alveolar bone. Visualizing tooth germs may be hampered by the fetus' position and maternal echogenicity.

In this study we were able to visualize, identify, and count fetal tooth germs through the use of 2D ultrasound around the 13th week, with some cases in which visualization was possible earlier in pregnancy. In a very near future, this technique could be used as a standard tool in assessing genetic syndromes. More cases are needed to better assess the information regarding congenital malformations, genetic syndromes, and chromosomal abnormalities. This knowledge could be integrated into routine pregnancy evaluations as a means of improving the accuracy of prenatal diagnoses.

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