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ORIGINAL ARTICLE

Tongue development in stillborns autopsied at different gestational ages☆,☆☆

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KEYWORDS
Gestational age;
Stillborn;
Autopsy

Abstract
Objectives: This study aimed to analyze, through the morphometric method, the perimeter and length of the tongue, the collagen fibers, and the perimeter of blood vessels at different gestational ages and fetal weights.
Material and methods: Tongues ($n = 55$) of stillborns autopsied at 23–40 weeks of gestational age were macroscopically analyzed, and their length and perimeter were measured. Fifty-five tongue fragments were collected through a longitudinal section in the region that accompanies the median lingual sulcus and histologically processed. Slides were stained with picrosirius and immunolabeled with CD31 antibody. Quantification was performed on collagen fibers under polarized light, and on the perimeter of vessels with the CD31.
Results: A positive and significant correlation of gestational age with tongue perimeter and length was found. There was a positive and significant correlation between collagen fibers and gestational age, as well as between gestational age and the perimeter of blood vessels. Between collagen fibers and fetal weight, a positive and significant increase was observed. Regarding the correlation between the perimeter of blood vessels and the fetal weight, an increase was observed.

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☆☆ Study conducted at Universidade Federal do Triângulo Mineiro (UFTM), Instituto de Ciências Biológicas e Naturais, Disciplina de Patologia Geral, Uberaba, MG, Brazil.

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Conclusion: As gestational age advances, there is an increase in tongue perimeter and length, in the percentage of collagen fibers, and in vascular perimeter, demonstrating that tongue formation is directly related to tongue growth and development.
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PALAVRAS-CHAVE
Idade gestacional;
Natimortos;
Autópsia

Desenvolvimento da língua em natimortos autopsiados em diferentes idades gestacionais

Resumo
Objetivos: Analisar, por meio do método morfométrico, o perímetro e o comprimento da língua, as fibras de colágeno, o perímetro dos vasos sanguíneos, em idades gestacionais e de acordo com o peso fetal.
Materiais e métodos: Línguas (n = 55) de natimortos autopsiados com 23-40 semanas de idade gestacional foram analisadas macroscopicamente, medidas em comprimento e perímetro; 55 fragmentos das línguas foram coletados por meio de uma secção longitudinal na região que acompanha o sulco lingual médio e processados histologicamente. As lâminas foram coloridas com picrosirius e imunomarcadas com o anticorpo CD31. A quantificação foi feita em fibras de colágeno examinadas com microscópio de luz polarizada e o perímetro dos vasos com o CD31.
Resultados: Foi encontrada uma correlação positiva e significativa da idade gestacional com o perímetro e o comprimento da língua. Houve uma correlação positiva e significativa entre as fibras de colágeno e a idade gestacional; bem como entre a idade gestacional e o perímetro dos vasos sanguíneos; e houve um aumento positivo e significativo entre as fibras de colágeno e o peso fetal. No que diz respeito à correlação entre o perímetro dos vasos sanguíneos e o peso fetal, houve um aumento.
Conclusão: Conforme a idade gestacional avança, há um aumento no perímetro e no comprimento da língua, um aumento no percentual de fibras de colágeno e um aumento no perímetro vascular, demonstra que a formação da língua está diretamente relacionada ao crescimento e ao desenvolvimento da língua.
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Introduction

Pediatric autopsy is an important study of the structural and functional differences of the organs according to the time of fetal development. In the autopsy, the estimation of the gestational age (GA) is important to identify whether fetal development was occurring normally, to determine the death time in relation to the birth, to ascertain the diagnosis of the diseases that are specific to that developmental stage, and to detect those children classified as risk in the neonatal period.[1]

During examination, the evaluation of the tongue provides a variety of information, as it is a special organ of reception, chewing, swallowing, speaking, and tasting. Evidences from mammalian studies suggests that it is composed of muscle cells that have different arrangements in origin and insertion, and different histochemical properties in comparison with other skeletal muscles.[2,3]

The development of the tongue is described as a relatively rapid process, which begins between the fourth and fifth week of intrauterine life. This process has a remarkable effect on the oral cavity[4]; therefore, it is extremely important for the development of the stomatognathic system that the tongue develops correctly.[5] There appears to be a synchrony in the formation of the orofacial complex since, from the 14th week onwards, the muscles of the oropharyngeal region are sufficiently advanced to move the tongue, coinciding with peaks in the growth of the head circumference that occur between the 15th and 17th week.[6,7]

Collagen is expressed in the tongue in the early stages of development, and is detectable in the mesenchyme derived from cranial neural crest cells (CNCC), adjacent to the tongue epithelium[8] and in tendons of the extrinsic muscles, which connect the tongue to the mandible.[9] The connective tissue and the vascular system of the tongue are derived from the CNCC, while most of its muscles originate from myoblasts that migrated from the occipital somites.[10]

Endothelial cells play a key role in the control of coagulation, thrombosis, vascular tone, permeability, inflammation, tissue repair, and angiogenesis.[11] They constitute a heterogeneous population of cells in the human body. Functions and molecular characteristics of endothelial cells vary along the vascular tree and in the same organ between different vessels, as for example, phenotypic variations can occur in the expression of the CD31 molecule in these cells.[12–14]

Ultrasound examinations have indicated a highly significant correlation between fetal tongue circumference and gestational age (14 to 26 weeks).[15] This data may be useful in the prenatal diagnosis of suspected congenital syndromes that include, among its manifestations, tongue growth disorders and GA estimation.[12]

The autopsy material is very rich for research, since through macro- and microscopic analyses it is possible to make feasible research studies with clinical diagnoses and detection of structural abnormalities. The autopsy is considered an important diagnostic method for the physician, since it allows documenting and comparing clinical and pathological cases.[1] In pediatric autopsies, the hallux-calcaneus length (HCL) is a reliable parameter to establish GA in fetuses and stillbirths. The GA obtained by HCL is taken by measuring the length of the foot, from the heel to the tip of the hallux.[16]

The aim of this study was to analyze, through the morphometric method, the perimeter and length of the tongue, the collagen fibers, the perimeter of the blood vessels, at different GAs and in relation to the fetal weight. It can contribute as a new method to estimate GA through the tongue development.

Material and methods

This was a retrospective study, approved by the Ethics Committee of the Federal University of Triângulo Mineiro, under protocol No. 1158.

Of the 152 pediatric autopsy reports analyzed, those of 55 stillborns autopsied by the General Pathology Discipline at the Clinical Hospital of the Federal University of Triângulo Mineiro (HC/UFTM), Uberaba, State of Minas Gerais, from 1994 to 2015 were selected. The anatomopathological examination was performed by two pathologists, and the information obtained from the autopsy reports was GA, determined using the HCL method (hallux-calcaneus length), and fetal weight.

Inclusion criteria were GA between 23 and 40 weeks, those with data of gender and fetal weight, and those in which the tongue was in good preservation condition. The exclusion criteria were stillborn infants with malformations and lack of data in reports such as GA, fetal weight and gender. Moreover, any cases with intrauterine growth restriction or another alteration were excluded.

Measurement of tongue length and perimeter

The 55 tongues analyzed were arranged on the macroscopy laboratory bench and identified individually, with the respective autopsy number, along with a ruler for later calibration in the ImageJ® Software (National Institutes of Health, USA). All photographs were taken from the same distance (30 cm) using a Canon Rebel® camera (Canon, Tokyo, Japan). Morphometric analyses were performed measuring the length from the glossoepiglottic fold to the apex of the tongue; to obtain the perimeter of the tongue, the contour was measured throughout the macroscopic extension.

Sample collection and histopathological processing

Fifty-five tongue fragments from autopsied stillborns recovered in the archive of biological material of the discipline of General Pathology (UFTM) were used. Fragments were removed through a longitudinal section in the region that accompanies the median lingual sulcus, with a thickness of approximately 0.5 cm. Serial cuts of 4 μm in thickness were then performed. Slides were stained with Picrosirius (PS; saturated aqueous solution of picric acid added with 0.1 g% Sirius red F3B) (Bayer, Leverkusen, Germany) with counterstaining by hematoxylin, and a slide was used for immunohistochemistry.

Morphometric analysis of collagen fibers

The PS-stained slide was analyzed for quantification of collagen fibers. The number of fields for evaluation and quantification of collagen fibers of the longitudinal section of the tongue, at different GAs, was defined as four quadrants and ten fields per quadrant of the histological section were analyzed. The area of collagen fibers under polarized light presented a birefringent appearance, ranging from orange to red (Fig. 1). Collagen fibers were marked by the observer to obtain the percentage of collagen per field analyzed. Thus, the field image was digitized using a camera coupled to a microscope with a Leica Qwin Plus® image analyzer (Leica Microsystems Inc, IL, USA). Morphometry was performed with Leica Qwin Plus® software image analyzer system (Leica Microsystems Inc, IL, USA), with a 10× objective lens (final magnification of 320×).

Immunohistochemical analysis

Immunohistochemistry was performed to identify anti-CD31 positivity. The number of fields for evaluation and quantification of the CD31 marker in the longitudinal section of the tongue, at different GAs, was defined as four quadrants and tend fields per quadrant of the histological section were analyzed. Measurements were taken using a video camera coupled to a light microscope, and these to a computer with the image analyzer system Axiovision SE64 Rel. 4.9.1® software. The perimeter of blood vessels was measured using ImageJ® Software (National Institutes of Health, USA), with an objective lens 100× (final magnification 3250×; Fig. 2).

Statistical analysis

For the statistical analysis, a spreadsheet of the program Microsoft Excel® was elaborated. The information was analyzed using the electronic program GraphPad Prism® version 5.0 (GraphPad Software, Inc, CA, USA). To verify the type of distribution of the variables the statistical test of Shapiro–Wilk was applied. For correlation, the Spearman correlation coefficient (rS) was used for non-normal distribution. The differences were considered statistically significant when p was less than 5% ($p < 0.05$).

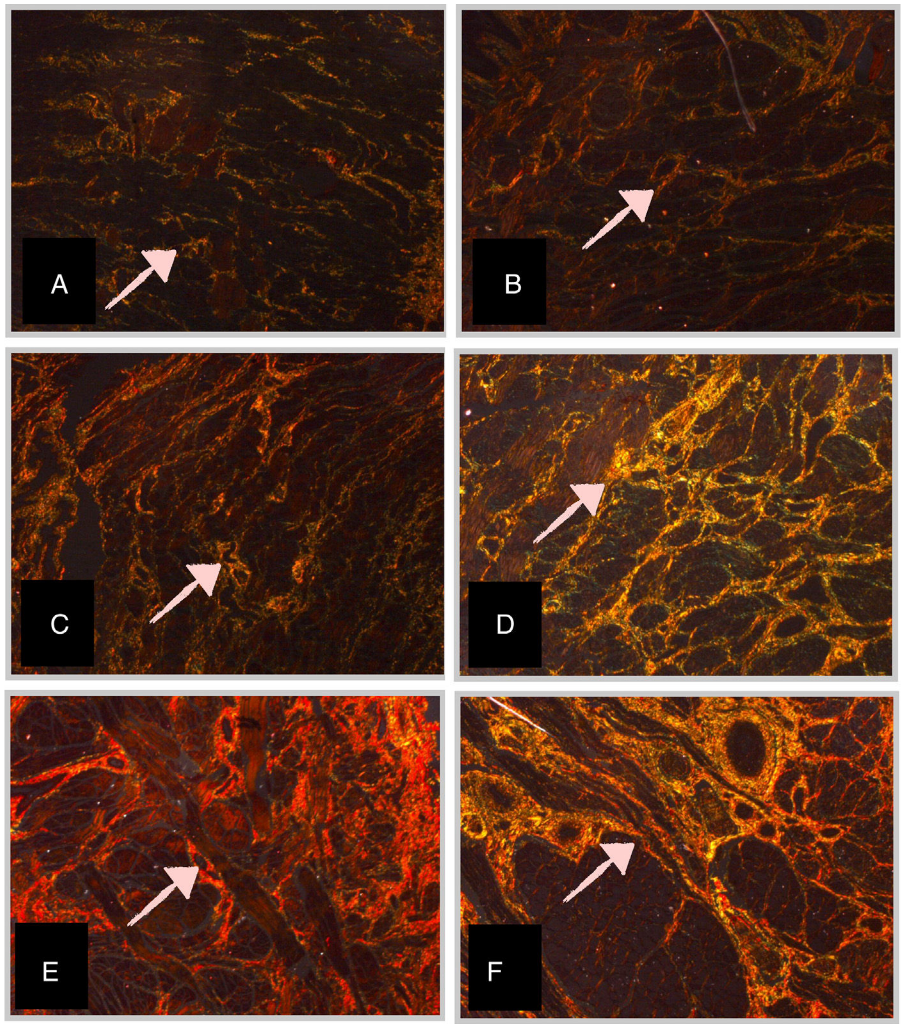

Figure 1 Micrographs of stillborn tongue fragments examined under polarized light, showing the birefringent collagen fibers (arrows) (Picrosirius – 10×–320× final magnification) at different gestational ages (GA).
(A) GA: 23 weeks; (B) GA: 28 weeks; (C) GA: 34 weeks; (D) GA: 37 weeks; (E) GA: 39 weeks, and (F) GA: 40 weeks.

Results

Of the 152 reports of pediatric autopsies analyzed, 55 were selected for evaluation, with a median GA of 33 weeks, ranging from 23 to 40 weeks. Regarding gender, 34 (60.71%) were male and 22 (39.28%) were female. The analyzed data were presented in Table 1.

The tongue perimeter presented a positive and significant correlation with GA (rS = 0.528; $p < 0.001$; Fig. 3).

The correlation of GA and tongue length was also positive and significant (rS = 0.527; $p < 0.001$; Fig. 3).

A positive and significant increase was observed in the correlation of the GA with collagen fibers (rS = −0.071; $p = 0.001$; Fig. 3).

Considering the relationship between collagen fibers and fetal weight, a positive and significant correlation was observed (rS = 0.143; $p < 0.001$; Fig. 3).

The correlation between the GA and the perimeter of the blood vessels was positive and significant (rS = 0.093; $p < 0.001$; Fig. 3).

A correlation was observed between the perimeter of blood vessels and the fetal weight: there was an increase in the perimeter of the vessels, in tendency significant (rS = 0.028; $p = 0.076$; Fig. 3).

Discussion

The present study corroborates the literature, as a positive and significant increase in perimeter and length of the tongue was reported at different GAs.[4,17] Fetal development is extremely important for the evaluation of the newborn, thus GA is an indispensable parameter for evaluation and survival after birth.[18] Foot length is an important element for the structural assessment of the fetus at different GAs, because it is a body measurement that is closely related to GA, weight, and length.[19]

A positive and significant correlation was observed between collagen fibers in the tongue of stillborns and GA (23–40 weeks) and weight. This finding indicate that CNCC

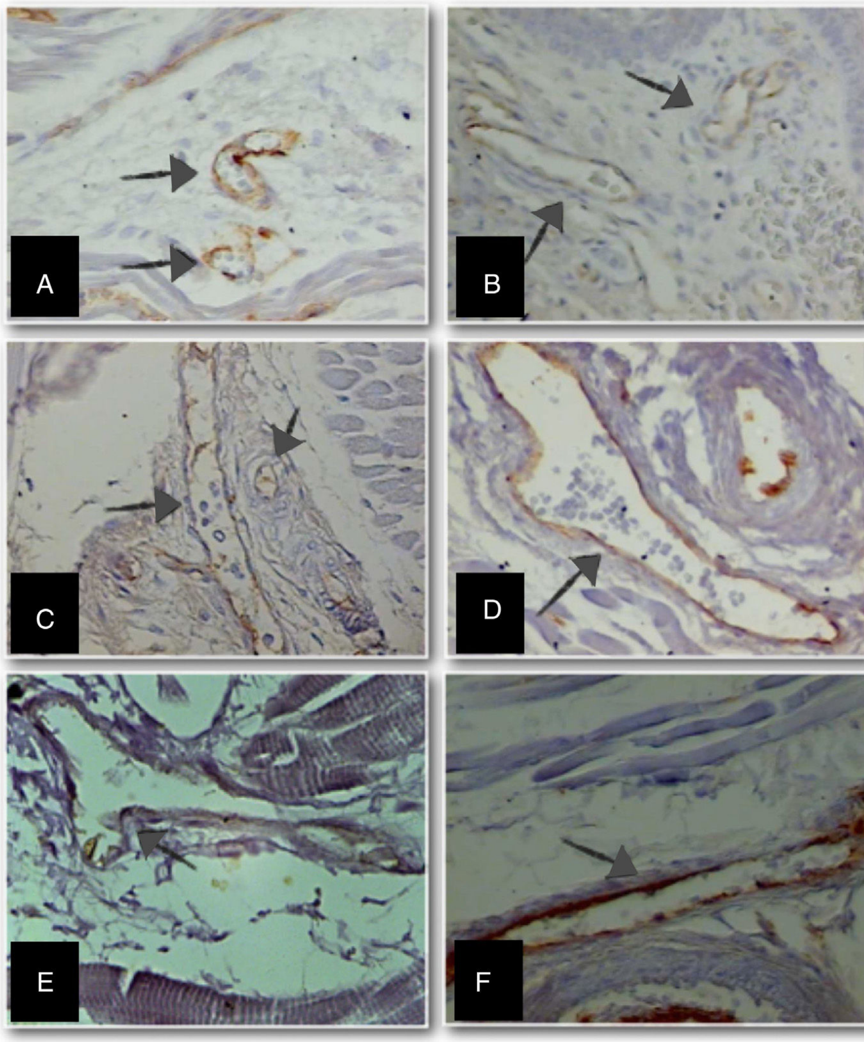

Figure 2 Micrographs of stillborn tongue fragments examined under light microscopy, showing the increase in anti-CD31 immunolabeled blood vessels (arrows) (objective 100×–3250× final magnification) at different gestational ages (GA).
(A) GA: 23 weeks; (B) GA: 28 weeks; (C) GA: 34 weeks; (D) GA: 37 weeks; (E) GA: 39 weeks, and (F) GA: 40 weeks.

initiates and directly potentiates tongue development and gives rise to fibroblasts that promote the development of connective tissue.[9,20]

In addition, blood vessels perimeter was positively correlated with different GAs. The authors chose to use the perimeter for vessel morphometry using the CD31 marker, which has not yet been described in the literature. As observed in a study on stillborns with 20 to 40 weeks GA, anti-CD31 is a marker of vessels in relation to the development of GA.[14,21]

The present study corroborates the literature, in which 23 tongues of autopsied stillborns were analyzed, demonstrating that after the seventh week, the vessels (whose walls are beginning to develop) increase progressively. In the posterior region of the tongue, the blood vessels are small and form a very dense capillary network. The anterior vascularization of the tongue is greater, the vessels have smaller calibers, providing the conditions for a rapid supply of energy and nutrients to the myocytes. This capillary network of the tongue has been described in the literature as an important element against diseases.[22–24]

In turn, the increase of the perimeter of blood vessels correlated with the fetal weight was positive and tendency significant. There is a natural tendency for fetal growth during the different GAs, but some factors may cause changes in fetal weight, since this variable is different in each GA and depends on external factors, such as maternal nutrition. Intrauterine complications resulting in newborns with low birth weight (<2500 g) are recognized as risk factors that contribute to the development of vascular diseases in adulthood.[25] Fetal weight and GA should be taken into account due to the influence of other characteristics (genetic and socioeconomic factors). The increase in fetal weight may be related to a severe fetal complication, which generates a fetal systemic response characterized by edema, inflammation, and alteration of chemical mediators.[26]

The precise evaluation for growth in the neonatal period is important to observe if the fetus was subjected to abnormal intrauterine conditions that resulted in delayed growth acceleration.[27] However, antenatal ultrasound detection and estimated fetal weight are far from straightforward,

Table 1 Constitutional and morphometric data of the 55 stillborns autopsied by the discipline of General Pathology at the Clinical Hospital of the Federal University of Triângulo Mineiro (HC/UFTM), Uberaba, State of Minas Gerais, Brazil, from 1994 to 2015.

Cases	Tongue length (cm)	Tongue perimeter (cm)	Collagen (%) $X \pm SD$	Vessels perimeter (μm) $X \pm SD$	Gestational age (weeks)	Fetal weight (kg)
N4210	3.314	8.227	2.084 ± 0.305	16.86 ± 1.343	23	0.54
N4156	3.287	9.065	13.231 ± 1.036	23.37 ± 2.147	23	0.7
N4301	3.295	8.161	2.881 ± 0.526	70.09 ± 7.397	23	0.545
N4051	3.901	10.182	2.572 ± 0.564	121.3 ± 15.25	24	1.3
N4162	3.542	8.861	4.987 ± 0.658	80.48 ± 10.23	24	0.64
N4238	3.891	10.172	20.588 ± 1.511	19.20 ± 1.555	24	1.08
N4279	3.622	9.572	2.319 ± 0.563	70.74 ± 6.36	24	0.620
N4149	4.135	11.021	1.493 ± 0.315	71.18 ± 15.62	25	0.57
N4313	4.378	11.46	2.527 ± 0.372	80.27 ± 9.780	26	1.04
N4265	2.951	7.354	7.383 ± 0.634	86.48 ± 12.30	26	0.985
N4269	3.994	10.333	11.299 ± 0.812	84.02 ± 12.44	26	0.8
N4090	3.344	9.422	2.833 ± 0.266	24.64 ± 2.078	27	0.85
N4199	3.237	8.339	3.737 ± 0.569	66.40 ± 4.911	27	0.9
N4159	4. 327	11.856	6.139 ± 0.791	15.62 ± 1.075	27	2.25
N3935	4.423	11.43	8.013 ± 1.430	18.38 ± 2.382	28	1.09
N4086	3.746	10.379	12.202 ± 0.747	25.90 ± 7.057	28	1.25
N4134	3.755	9.872	1.745 ± 0.218	89.27 ± 8.840	28	1.02
N4108	3.751	10.033	2.419 ± 0.638	54.88 ± 4.987	28	1.2
N4174	3.482	9.813	1.327 ± 0.199	70.18 ± 7.454	28	1.08
N4275	3.324	8.864	4.142 ± 0.401	105.1 ± 7.451	28	1
N4239	4.423	11.213	1.837 ± 0.305	21.89 ± 1.765	29	1.24
N3976	4.902	11.618	6.757 ± 1.049	20.22 ± 1.582	29	2.77
N4125	4.493	11.031	9.542 ± 0.941	96.22 ± 8.743	30	1.7
N4295	3.785	9.065	0.7795 ± 0.294	117.5 ± 10.20	30	0.780
N4325	4.134	11.322	4.25 ± 0.4896	73.24 ± 5.928	31	1.65
N4284	4.209	11.585	1.699 ± 0.1744	56.87 ± 4.441	31	1.85
N4145	4.026	11.131	0.993 ± 0.1784	124.0 ± 11.56	31	1.42
N3901	4.071	11.122	0.888 ± 0.139	28.68 ± 2.396	33	1.9
N4011	3.357	8.832	10.116 ± 0.551	47.02 ± 3.687	33	1.63
N4115	4.293	11.784	9.551 ± 1.126	75.32 ± 4.602	33	1.92
N4136	4.122	10.528	22.634 ± 1.795	22.12 ± 2.462	33	1.02
N4276	4.491	11.467	2.556 ± 0.448	65.22 ± 7.612	33	1.46
N4230	3.748	10.117	3.551 ± 0.322	59.95 ± 6.142	34	3.5
N3913	4.247	11.092	1.444 ± 0.233	30.04 ± 3.152	34	1.96
N3986	4.449	10.919	9.536 ± 1.255	50.56 ± 5.330	35	1.4
N4113	3.189	9.173	7.149 ± 1.022	75.35 ± 7.662	35	1.3
N4119	4.445	11.221	10.357 ± 1.056	22.57 ± 2.067	35	2.34
N4257	3.682	10.251	3.048 ± 0.472	51.36 ± 4.841	35	2.2
N4260	40.2	10.34	1.8 ± 0.305	47.83 ± 4.937	35	2.15
N4083A	4.848	11.893	2.255 ± 0.154	22.41 ± 1.893	35	2.25
N4232	4.138	11.905	16.117 ± 1.874	18.75 ± 1.413	36	2.66
N4147	4.07	10.951	2.863 ± 0.475	76.22 ± 6.190	36	3.88
N4111	3.914	11.641	20.782 ± 1.296	105.5 ± 8.113	37	4.23
N4150	3.986	10.208	11.147 ± 1.029	22.62 ± 3.218	37	2.16
N4297	4.313	11.555	5.233 ± 0.524	109.1 ± 9.913	37	1.4
N3989	4.879	13.47	12.774 ± 1.047	110.4 ± 9.032	38	3.55
N4019	5.865	14.985	10.028 ± 0.908	76.11 ± 10.24	38	3.44
N4204	4.851	11.971	6.958 ± 0.589	74.43 ± 8.284	39	2.8
N3894	3.623	11.214	3.701 ± 0.452	112.5 ± 9.923	40	3.56
N4100	3.353	8.512	1.889 ± 0.240	26.86 ± 2.116	40	0.9

Table 1 (*Continued*)

Cases	Tongue length (cm)	Tongue perimeter (cm)	Collagen (%) $X \pm SD$	Vessels perimeter (μm) $X \pm SD$	Gestational age (weeks)	Fetal weight (kg)
N4107	3.592	11.174	12.609 ± 1.94	124.1 ± 9.532	40	0.9
N4126	5.13	13.431	17.911 ± 3.24	33.13 ± 2.671	40	1.7
N4140	4.5	11.866	8 ± 0.917	24.90 ± 2.074	40	1.35
N4158	4.535	12.504	10.003 ± 0.755	18.18 ± 1.370	40	2.93
N4176	3.395	9.463	4.078 ± 0.327	64.04 ± 6.370	40	1.28

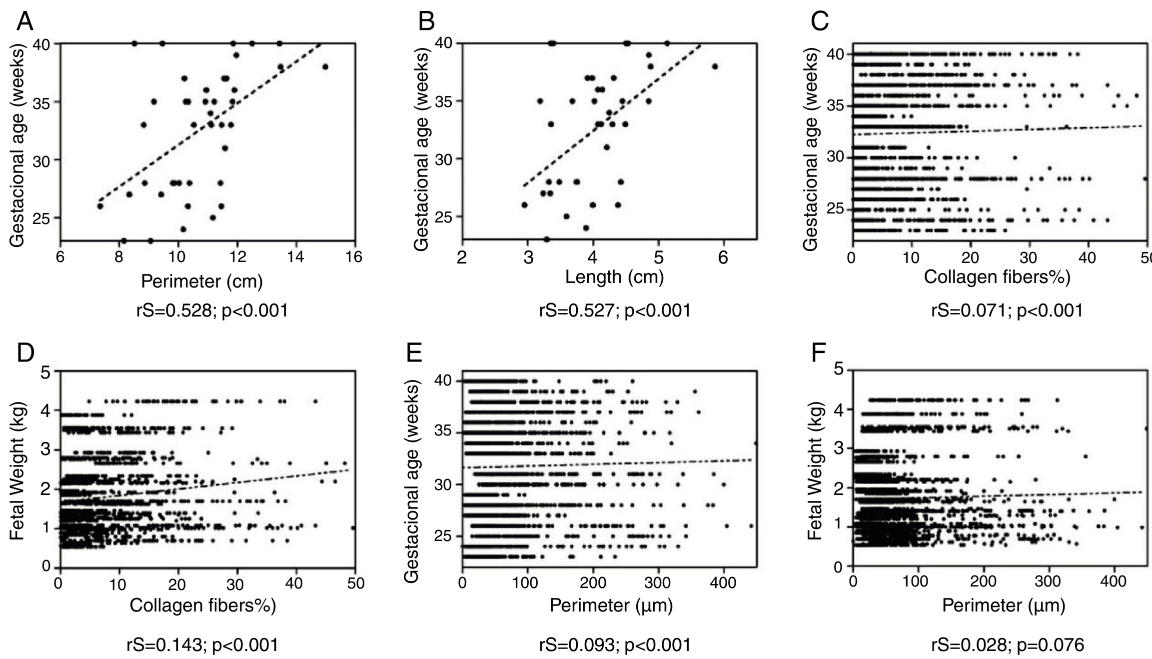

Figure 3 Graphs showing the association of GA with tongue perimeter (A), tongue length (B), collagen fibers (C), and vessel perimeter (E). Fetal weight was associated with the collagen fibers (D) and the perimeter of the vessels (F).

because these well-defined parameters are estimated using complex calculations that may give varying results for the same fetus. To further complicate matters, then obtained results can then be plotted on a number of different antenatal reference charts generated from local, national, or international cohorts, some of which are customized for maternal factors, such as parity, height, weight, and ethnicity. These variations contribute to large differences in antenatal detection abnormalities.[28,29]

Therefore, with the advance of GA, there is an increase in the perimeter and length of the tongue, an increase in the percentage of collagen fibers and an increase in the vascular perimeter, demonstrating that tongue formation is directly linked to fetal growth and development. Therefore, tongue embryogenesis would be a valid parameter to estimate GA in the pediatric autopsy, in conjunction with traditional methods.

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Conflicts of interest

The authors declare no conflicts of interest.

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References

1. Cohen MC, Drut R. La autopsia en pediatría. Diagnóstico de situación en un hospital de pediatría de referencia. Arch Argent Pediatr. 2003;101:166–70.

2. Sato I, Suzuki M, Sato M, Sato T, Inokuchi S. A histochemical study of lingual muscle fibers in rat. Okajimas Folia Anat Jpn. 1990;66:405–15.
3. Dalrymple KR, Prigozy TI, Shuler CF. Embryonic, fetal, and neonatal tongue myoblasts exhibit molecular heterogeneity *in vitro*. Differentiation. 2000;66:218–26.
4. Siebert JR. A morphometric study of normal and abnormal fetal to childhood tongue size. Arch Oral Biol. 1985;30:433–40.
5. Hong SJ, Cha BG, Kim YS, Lee SK, Chi JG. Tongue growth during prenatal development in Korean fetuses and embryos. J Pathol Transl Med. 2015;49:497–510.
6. Siebert JR. Prenatal growth of the median face. Am J Med Genet. 1986;25:369–79.
7. Inoue T, Nakayama K, Ihara Y, Tachikawa S, Nakamura S, Mochizuki A, et al. Coordinated control of the tongue during suckling-like activity and respiration. J Oral Sci. 2017;59:183–8.
8. Hosokawa R, Oka K, Yamaza T, Iwata J, Urata M, Xu X, et al. TGF-beta mediated FGF10 signaling in cranial neural crest cells controls development of myogenic progenitor cells through tissue-tissue interactions during tongue morphogenesis. Dev Biol. 2010;341:186–95.
9. Parada C, Chay Y. Mandible and tongue development. Curr Top Dev Biol. 2015;115:31–58.
10. Parada C, Han D, Chay Y. Molecular and cellular regulatory mechanisms of tongue myogenesis. J Dent Res. 2012;91:528–35.
11. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115:1285–95.
12. Naruse K, Fujieda M, Miyazaki E, Hayashi Y, Toi M, Fukui T, et al. An immunohistochemical study of developing glomeruli in human fetal kidneys. Kidney Int. 2000;57:1836–46.
13. Junqueira LC, Carneiro J. Histologia básica. In: Gama P, editor. O trato digestivo. 11th ed. Rio de Janeiro: Guanabara Koogan; 2008. p. 284–5.
14. Liu L, Shi GP. CD31: beyond a marker for endothelial cells. Cardiovasc Res. 2012;94:3–5.
15. Achiron R, Ben Arie A, Gabbay U, Mashiach S, Rotstein Z, Lipitz S. Development of the fetal tongue between 14 and 26 weeks of gestation: *in utero* ultrasonographic measurements. Ultrasound Obstet Gynecol. 1997;9:39–41.
16. Zago AF, Paravidine LM, Siqueira LM, Balbão LM, Reis MA, Castro EC. Estudo comparativo entre o comprimento hálux-calcâneo e outros métodos de avaliação de idade gestacional em recém-nascidos. Pediatr Mod. 2000;36:388–91.
17. Bronshtein M, Zimmer EZ, Tzidony D, Hajos J, Jaeger M, Blazer S. Transvaginal sonographic measurement of fetal lingual width in early pregnancy. Prenat Diagn. 1998;18:577–80.
18. Hutchinson EF, Kieser JA, Kramer B. Morphometric growth relationships of the immature human mandible and tongue. Eur J Oral Sci. 2014;122:181–9.
19. Salge AK, Rocha EL, Gaíva MA, Castral TC, Guimarães JV, Xavier RM. Medida do comprimento hálux-calcâneo de recém-nascidos em gestações de alto e baixo risco. Rev Esc Enferm USP. 2017;51:e03200.
20. Iwata J, Suzuki A, Pelikan RC, Ho TV, Chai Y. Noncanonical transforming growth factor β (TGFβ) signaling in cranial neural crest cells causes tongue muscle developmental defects. J Biol Chem. 2013;288:29760–70.
21. Fonseca Ferraz ML, Dos Santos AM, Cavellani CL, Rossi RC, Corrêa RR, Dos Reis MA, et al. Histochemical and immunohistochemical study of the glomerular development in human fetuses. Pediatr Nephrol. 2008;23:257–62.
22. Macleod RI, Soames JV. A morphometric study of age changes in the human lingual artery. Arch Oral Biol. 1988;33:455–7.
23. Granberg I, Lindell B, Eriksson PO, Pedrosa-Domellöf F, Stål P. Capillary supply in relation to myosin heavy chain fibre composition of human intrinsic tongue muscles. Cells Tissues Organs. 2010;192:303–13.
24. Mangold AR, Torgerson RR, Rogers RS. Diseases of the tongue. Clin Dermatol. 2016;34:458–69.
25. Kandasamy Y, Smith R, Wright IM, Hartley L. Relationship between birth weight and retinal microvasculature in newborn infants. J Perinatol. 2012;32:443–7.
26. Corrêa RR, Rocha LP, Petrini CG, Texieira VP, Castro EC. Influência da causa de morte no peso corporal e dos órgãos internos em autópsias perinatais. Rev Bras Ginecol Obstrt. 2014;36:23–8.
27. Thawani R, Dewan P, Faridi MM, Arora SK, Kumar R. Estimation of gestational age, using neonatal anthropometry: a cross-sectional study in India. J Health Popul Nutr. 2013;31:523–30.
28. Gardosi J, Mongelli M, Wilcox M, Chang A. An adjustable fetal weight standard. Ultrasound Obstet Gynecol. 1995;6:168–74.
29. Poljak B, Agarwal U, Jackson R, Alfirevic Z, Sharp A. Diagnostic accuracy of individual antenatal tools for prediction of small-for-gestational age at birth. Ultrasound Obstet Gynecol. 2017;49:493–9.