

Palatal shelf integrated into tissue segment derived from maxillary process: A comparative study of the growth of its parts during the human embryonic period

Josep Nebot-Cegarra^{a,*}, Anna Maria Cueto-González^{b,c,2}

^a Department of Morphological Sciences, Human Anatomy and Embryology Unit, Faculty of Medicine, Autonomous University of Barcelona. Bellaterra (Cerdanyola del Vallès), Barcelona, Catalonia, Spain

^b Department of Clinical and Molecular Genetics, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Catalonia, Spain

^c Medicine Genetics Group, Vall Hebron Research Institute, European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN ITHCA), Vall d'Hebron Barcelona Hospital Campus, Autonomous University of Barcelona, Barcelona, Catalonia, Spain

ARTICLE INFO

Keywords:

Palate
Palatal shelf
Palatogenesis
Development
Embryology
Human embryo
Stereology

ABSTRACT

Objective: To analyse the volumetric dynamics of the maxillary palatal segment (MPS), comprising the palatal shelf and adjacent tissues derived from the maxillary process.

Material and methods: Twenty-eight MPSs were delineated in coronal or quasi-coronal histological sections of the heads of 14 human embryos at Carnegie stages (CS) 18–23. These were divided into palatal and juxtapalatal zones and anteroposterior thirds. The MPSs were classified into seven developmental degrees, based on the palatal shelf phases (preprimordial, vertical, and horizontal) and the embryo's CS. The volume of each third was measured, and the percentage of the MPS volume it represented was calculated. The mean percentage of volume in the thirds of the MPSs at the same developmental degree served as the comparison parameter.

Results: During the vertical phase, the anterior palatal third exhibited the greatest volume percentage. Its primary growth occurred at the beginning of shelf formation, after which it stabilised. After the palatal elevation (horizontal phase), the percentages of the palatal thirds initially equalised, but later the middle and posterior thirds surpassed the value of the anterior third, with their internal parts increasing significantly, particularly in the posterior third; moreover, the concordance between the opposing sign volumetric changes in neighbouring thirds suggests mesenchymal transfer from the juxtapalatal thirds.

Conclusion: The percentage distribution of MPS volume during human embryonic palatogenesis emphasises: (1) stability in the anterior third; (2) expansion in the middle and posterior thirds, particularly in the internal parts, closely linked to the elevation and horizontal progress of the palatal shelf.

1. Introduction

Proper palate formation, or palatogenesis, is essential for basic biological functions such as eating, drinking, breathing, and vocalisation (Nohara et al., 2022). In humans, palatogenesis begins in the fifth week after fertilisation (O'Rahilly and Müller, 1992) and concludes by week 12 (Bush and Jiang, 2012), when all palatal primordia have fused. This process divides the oronasal cavity into the oral and nasal cavities and

separates the mouth from the nasopharynx.

According to the order of appearance, two parts are considered. The primary palate comprises the front and central parts of the palate and develops from the median palatal process, which forms from the fused medial nasal processes. The secondary palate is made by the right and left palatal shelves or lateral palatal processes, which originate from the maxillary processes.

The hard palate develops from both palatal parts, while, behind it,

* Correspondence to: Department of Morphological Sciences, Human Anatomy and Embryology Unit, Faculty of Medicine, Autonomous University of Barcelona. Av. Can Domènech, Edifici M, 08193 Cerdanyola del Vallès, Catalonia, Barcelona, Spain.

E-mail addresses: Josep.Nebot@uab.cat (J. Nebot-Cegarra), annam.cueto@vallhebron.cat (A.M. Cueto-González).

¹ Department of Morphological Sciences, Human Anatomy and Embryology Unit, Faculty of Medicine, Autonomous University of Barcelona. Av. Can Domènech, Edifici M, 08193 Cerdanyola del Vallès, Barcelona, Catalonia, Spain

² Department of Clinical and Molecular Genetics, Vall d'Hebron Barcelona Hospital Campus, Pg. de la Vall d'Hebron, 119, 08035 Barcelona, Catalonia, Spain

<https://doi.org/10.1016/j.aanat.2026.152858>

Received 28 January 2026; Received in revised form 8 May 2026; Accepted 28 May 2026

Available online 2 June 2026

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the soft palate originates solely from the secondary palate. Therefore, most of the palate is formed from the secondary palate.

The normal development of the secondary palate depends on the coordinated growth and movement of each palatal shelf with its contralateral counterpart, as well as with the surrounding craniofacial structures (Bush and Jiang, 2012; Lan et al., 2015; Li et al., 2017).

By the end of the fifth week, a vertical human palatal shelf develops on each side of the tongue, formed by the proliferation of mesenchyme from the medial part of the maxillary process. This mesenchymal core is covered by an ectodermal epithelium composed of a basal layer of cuboidal cells and a superficial layer of flat cells, known as the periderm (Hammond et al., 2019).

During the transition from the seventh to the eighth week of development, the palatal shelves rise rapidly until they reach a horizontal position above the tongue (O'Rahilly and Müller, 1992). Then, they grow inwards until their medial edges converge and come into contact in the eighth week (Bush and Jiang, 2012; O'Rahilly and Müller, 1992). Their fusion is completed in the 12th week (Bush and Jiang, 2012). Fibronectin and $\alpha 5$ -integrin play a crucial role in the adhesion of the palatal shelves (Martínez-Sanz et al., 2008). For this union to occur, the peridermal cells at the point of contact must migrate towards the oral and nasal surfaces of the shelves before undergoing apoptosis (Murray et al., 2007). Until then, the periderm prevents the shelves from fusing with the tongue or other nearby structures (Richardson et al., 2014). An epithelial seam then forms centrally and eventually disintegrates. In mouse embryos, the loss of this seam has been linked to apoptosis (Cuervo and Covarrubias, 2004; Vaziri Sani et al., 2005), cell migration (Aoyama et al., 2019; Carette and Ferguson, 1992), epithelial-mesenchymal transition (Martínez-Alvarez et al., 2000), and exosome-mediated interactions (Huang et al., 2024).

Data from human foetuses suggest that fusion in the soft palate occurs faster than in the hard palate (Danescu et al., 2015). Additionally, male foetuses seem to go through this process slightly earlier than females (O'Rahilly and Müller, 1992).

In the region that will become the hard palate, the palatal shelves fuse and join the primary palate at the front. Above, where the shelves join and merge, they also join the lower end of the posterior part of the nasal septum, which develops downwards from the middle part of the frontonasal prominence (Ferguson, 1978; O'Rahilly and Müller, 1992; Steding and Jian, 2010).

During the second trimester, in the soft palate, the posterior junction of the palatal shelves forms the uvula through mesenchymal growth that moves the epithelium between the two posterior tips of the palate backwards (Burdi and Faist, 1967).

Any alteration to palatogenesis can cause palatal clefts, which are characterised by abnormal connections between the oral cavity, nasal cavities, and nasopharynx (Meng et al., 2009; Vermeij-Keers et al., 1983; Yu and Ornitz, 2011).

In Europe, the prevalence of cleft lip and palate, cleft lip, and cleft palate was recorded as 0.7/1000, 0.3/1000, and 0.5/1000, respectively (Pérez-Hettinga et al., 2025). According to Yu and Ornitz (2011), about 90% of isolated posterior cleft palates result from defects in the elevation of the palatal shelf. The causes of these clefts are multifactorial, involving both genetic and environmental factors (Im et al., 2025; Maldonado et al., 2018; Weingärtner et al., 2007). The diagnosis, follow-up and treatment of these congenital anomalies require a multidisciplinary approach. Advances in prenatal ultrasound resolution have enabled earlier diagnosis of cleft lip and hard palate (Gillham et al., 2009; Shi et al., 2023).

Significant advances have been made in the current century in the understanding of the role of the mechanical forces and tissue physical properties (Brock et al., 2016; Du et al., 2021; Wang et al., 2020), and the molecular basis of normal and abnormal palatogenesis in murids and other vertebrates (Bush and Jiang, 2012; Cox, 2004; Dixon et al., 2011; Gao et al., 2009; Gritli-Linde, 2007; Im et al., 2025; Jin et al., 2010; Kohli and Kohli, 2012; Kouskoura et al., 2013; Lan et al., 2004, 2015,

2019; Lane and Kaartinen, 2014; Li et al., 2021; Meng et al., 2009; Okano et al., 2006; Seelan et al., 2012; Thyagarajan et al., 2003; Twigg and Wilkie, 2015; Wilkie, Morriss-Kay, 2001; Young et al., 2007).

Although some uncertainties remain regarding the functions of distinct molecular factors during palate development, evidence indicates heterogeneity among the molecules involved in various regional palatal interaction pathways. This aligns with the heterogeneous development of different parts of the palate (Bush and Jiang, 2012; Gao et al., 2009; He et al., 2008; Hilliard et al., 2005; Jin et al., 2010; Lan et al., 2004, 2015; Lane and Kaartinen, 2014; Li and Ding, 2007; Okano et al., 2006; Welsh et al., 2025).

The development of the mammalian palate involves various molecular mechanisms that are regulated by the reciprocal interactions between its epithelial and mesenchymal layers during differentiation along the anterior-posterior and medial-lateral axes (Bush and Jiang, 2012; Hammond and Dixon, 2022; He et al., 2008; Im et al., 2025; Won et al., 2023). In mouse embryos, *Shox2* and *Msx1* are expressed in the anterior secondary palate and *Meox2*, *Barx1* and *Tbx22*, in the posterior secondary palate under the control of specific genetic networks. The *Shh* gene, which is present in the oral epithelium of the palatal shelf, regulates the medial-lateral differentiation of the mesenchymal core. It becomes activated in the lateral region, while its activity remains limited in the medial region (Hammond and Dixon, 2022; He et al., 2008; Im et al., 2025).

Despite decades of research, the process of palatal shelf elevation remains highly controversial, as evidenced by the lack of consensus on the significance of the mechanisms involved (Yu and Ornitz, 2011). Various factors have been proposed to contribute to this process, including the rotation of the shelf (Brinkley and Vickerman, 1978; Chou et al., 2004; Coleman, 1965; Diewert, 1985; Ferguson, 1978; Greene and Pratt, 1976; Walker and Fraser, 1956; Wragg et al., 1972; Yu and Ornitz, 2011); the increased oedema caused by hydrophilia resulting from the progressive accumulation of glycosaminoglycans in the palatal shelf extracellular matrix (Brinkley and Vickerman, 1978; Chou et al., 2004; Ferguson, 1978; Lan et al., 2019; Pratt et al., 1973; Snyder-Warwick et al., 2010; Walker and Fraser, 1956; Yonemitsu et al., 2020), creating turgor that generates an intrinsic force that elevates the palatal shelves, thus clearing the way to overcome tongue interposition (Ferguson, 1978); the proliferation of the shelf mesenchyme (Yoshioka et al., 2021), especially at its medial edge (Ferguson, 1978; Gao et al., 2009; Jin et al., 2010; Lan et al., 2004; Schupbach et al., 1983; Walker and Fraser, 1956; Wragg et al., 1972); the palatal mesenchyme remodelling (Chou et al., 2004; Coleman, 1965; Jin et al., 2010; Walker and Fraser, 1956; Yasuda and Fujimoto, 1986; Yu and Ornitz, 2011); the combination of the anterior third of the palatal shelf rotating with mesenchymal remodelling of the middle and posterior thirds (Chiquet et al., 2016); the active and passive tongue movements (Diewert, 1985; Ferguson, 1978; Greene and Pratt, 1976; Humphrey, 1968; Jin et al., 2010; Nohara et al., 2022; Wragg et al., 1972; Yu and Ornitz, 2011), which cause the palatal shelf to “flow” over the tongue (Hammond and Dixon, 2022); the combination of intrinsic and extrinsic forces, including interactions between the palate and tongue (Yu and Yonemitsu, 2019); and the specific control of genes such as *Snail1*, *Snail2* (Murray et al., 2007), *Wnt5A* (He et al., 2008), and *Osr2* (Gritli-Linde, 2007; Lan et al., 2004).

Research articles focusing specifically on human palate development (Burdi and Faist, 1967; Diewert, 1983; Diewert and Lozanoff, 1993a, b; Diewert and Shiota, 1990; Diewert et al., 1993; Nohara et al., 2022; Radlanski, 2003; Radlanski et al., 2004; Vermeij-Keers et al., 1983; Yoon et al., 2000) have not definitively established the role of the mechanisms involved in the elevation of the palatal shelf. However, synchronised analyses of the growth of the secondary palate and neighbouring structures suggest that the rapid elevation of the palatal shelves and the anterior movement of the tongue, which accompanies the extension of Meckel's cartilage, could relieve tension that accumulates in this area during development (Nohara et al., 2022).

The wide range of results and interpretations concerning the embryology of the palate, especially the palatal shelf, has been attributed to the improper extrapolation of palatogenesis data from other species to humans (Chou et al., 2004; Ferguson, 1978; Yu and Ornitz, 2011), the risky extension of data from one part of the palate to the entire palate (Ferguson, 1978; Yu and Ornitz, 2011), and the use of different criteria for dividing the palate into analytical zones.

This article addresses these criticisms and introduces methodological innovations for analysing and comparing regional volumetric changes in the palatal shelf and the adjacent tissue segment during stages of human embryonic palatogenesis. It also considers the extent to which the results can be related to those of molecular and histomorphological studies conducted in animal models.

2. Material and methods

2.1. Embryos

Serial sections of a graded series of normal human embryos were examined, covering stages from the initial formation of the palatal shelves on both sides of the tongue to their confluence at the midline above it. Embryos were sourced from three collections: the Domènech-Mateu Collection at the Autonomous University of Barcelona in Bellaterra, Spain; the Carnegie Collection at the National Museum of Health and Medicine in Washington, DC, USA; and the Boyd Collection at the University of Cambridge in Cambridge, England.

Table 1 summarises the data on embryonic development and the details provided by these collections regarding the embryonic sections. All samples were sectioned in a series of transverse planes through the trunk, which were coronal or quasi-coronal planes in the head. Embryos exhibiting pronounced right-left asymmetry or those with excessively oblique cutting planes were excluded from the study. All selected sections of each embryo were stained consistently, except for H983, which was stained with three stains applied in an alternating pattern.

Table 1

Overview of the human embryos analysed in this study.

Embryo	Collection	Carnegie stage	Crown-rump length (mm)	Postovulation age (days)	Fixation / Embedding	Plane	Thickness (µm)	Stain	Sequential selection
E047	DM	18	15	43	F / P	TT/CH	10	HE	1 in 4
E051	DM	18	16	44	F / P	TT/CH	10	HE	1 in 4
E052	DM	18	16	44	F / P	TT/CH	10	HE	1 in 4
4430	C	18	14	44	CA / P	TT/CH	15	AC	1 in 6
E064	DM	19	18	45	F / P	TT/CH	10	HE	1 in 4
9325	C	19	17	45–47	F / P	TT/CH	15	A	1 in 10
E069	DM	20	19	51	F / P	TT/CH	10	HE	1 in 5
E079	DM	20	21	51	F / P	TT/CH	10	HE	1 in 4
462	C	20	20	49	F / P	TT/CH	40	AC	1 in 2
E090	DM	21	23	50	F / P	TT/CH	10	HE	1 in 5
4090	C	21	22.2	49–52	F / P	TT/CH	40	AC	1 in 2
H983	B	22	28	52–55	F / P, CE	TT/CH	10	HE, T, S	1 in 10
E104	DM	23	28	57	F / P	TT/CH	10	HE	1 in 6
9226	C	23	31	56	F / P, CE	TT/CH	12	A	1 in 10

Abbreviations: A, azan; AC, alum cochineal; B, Boyd; C, Carnegie; CA, corrosive acetic acid; CE, celloidin; CH, coronal, or close to it, in the head; DM, Domènech-Mateu; F, formol; HE, hematoxylin-eosin; P, paraffin; S, silver; T, trichrome; TT, transverse in the trunk.

2.2. Image acquisition

Serial sections of human embryos from the Domènech-Mateu Collection were scanned with a Zeiss Axioscan Z1 (Carl Zeiss Microscopy, Jena, Germany) and stored at the Digital Embryology Consortium (https://human-embryology.org/wiki/Main_Page). Digital images of serial sections from the Carnegie and Boyd collections were obtained through the Virtual Human Embryo Project (<https://virtualhumanembryo.lsuhsu.edu/>).

2.3. Image selection

Images containing a palatal shelf (right or left) or its presumed location (see 2.6, third paragraph) were preselected. A sequence was selected for analysis on each side of the images, beginning with a randomly selected section. For the Virtual Human Embryo Project images, the original section sequence was maintained. For the Domènech-Mateu collection, a separate sequence was created for each embryo (Table 1).

The selected images were saved in TIFF format and imported into Adobe Photoshop (version 12.0, 32-bit; San Jose, CA, USA). In each image, the mid-sagittal plane of the embryo was aligned with the Y-axis of the computer screen. To facilitate rapid identification of structural elements, stacks of photographs of the selected sections of each embryo were compiled in Fiji/ImageJ (version 1.54p; W. S. Rasband et al., National Institutes of Health, Bethesda, MD, USA).

2.4. Maxillary palatal fragment

Using Adobe Photoshop, a tissue fragment from the maxillary process was outlined on each selected image.

Three lines framed each fragment. The maxillary line followed the contours of the epithelial coverings of the oral and nasal cavities, as well as the nasopharyngeal lumen. It extended from the upper end, which was situated at the lowest point of the lateral wall of the nasal cavity, to

the lower end, at the point where it joined the tissue complex derived from the mandibular process. The other two lines were straight: the upper line, perpendicular to the mid-sagittal plane, passed through the upper end of the maxillary line. The lateral line, parallel to the mid-sagittal plane, extended through the lower end of the maxillary line (Fig. 1).

In some images, the maxillary line was interrupted by the fusion of the palatal shelf with the primary palate or the nasal septum. In others, the cutting procedure resulted in the omission of part of the maxillary line. In these cases, the missing part was reconstructed by superimposing and aligning the maxillary line from the nearest section in which it was visible. All these procedures were facilitated by the image stack.

2.5. Maxillary palatal segment (MPS)

The MPS was constituted by an anteroposteriorly ordered set of right or left maxillary palatal fragments from an embryo.

The maxillary palatal fragment was divided into three parts by tracing the intermediate and medial lines (Fig. 1). These straight lines were parallel to the sagittal midline and passed through the ends of the inner third of the upper line. The former passed through the outer end, separating the areas belonging to the palatal zone medially and the juxtapalatal zone laterally. The latter crossed the inner end, delimiting the areas of the internal and external parts of the palatal zone. The

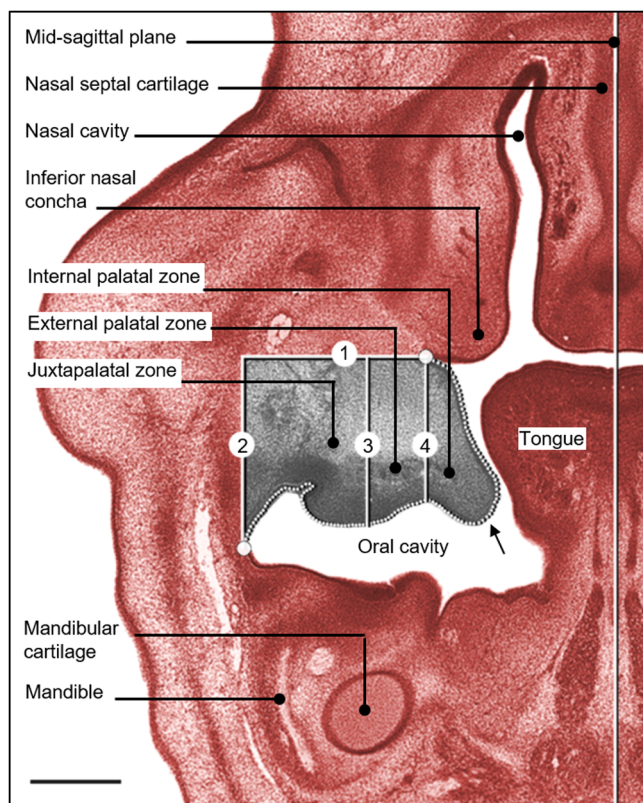


Fig. 1. Fragment (grey area) of the right maxillary palatal segment from a coronal section of the head of embryo number 4090 (Carnegie stage 21). Three lines frame the tissue fragment. The maxillary (dotted) line corresponds to the epithelial contour of the maxillary process. The upper (1) and lateral (2) lines are straight lines drawn from the end points (white circles) of the maxillary line: the former is perpendicular to the mid-sagittal plane (Msp), while the latter is parallel to it. The intermediate (3) and medial (4) lines are parallel to the Msp and divide the maxillary palatal fragment into sectors for measuring volumes. The former separates the areas belonging to the juxtapalatal and palatal zones of the maxillary palatal segment; the latter divides the area of the palatal zone into external and internal parts. The arrow points to the palatal shelf. Scale bar: 400 μm .

internal area was not always present.

The MPS zones and the parts of the palatal zone were formed by grouping their delimited areas in the tissue fragments. The palatal zone received this name because the palatal shelf or its expected site of formation was identified in all sections.

The selected images were grouped into thirds along the anterior-posterior axis. This was done separately for images containing right maxillary palatal fragments and for those with left maxillary palatal fragments. If the total number of selected images was divisible by three, each third contained the same number of maxillary palatal fragments. Otherwise, one or two thirds were randomly chosen to receive an extra image. The thirds in each zone were named palatal and juxtapalatal thirds, respectively.

2.6. Classification of the MPSs

The MPSs were classified according to a developmental scale from 0 to 6. The degree of MPS development (Fig. 2) was assessed by examining the phase of palatal shelf development and the Carnegie stage (CS) of the embryo (O'Rahilly and Müller, 1987, 2010). In all embryos, the classification of the two MPSs was the same.

Three phases have been identified in the development of the palatal shelf, from CS 18–23 (Fig. 2a–n).

The pre-primordial phase included two MPSs from one CS18 embryo, with no clear evidence of a palatal shelf. However, the area where it would form could be identified by its similarity to the region where it arose in other CS18 embryos. Due to the absence of the secondary palate primordium, these MPSs were considered to be at developmental degree 0 (Fig. 2a).

The vertical phase was characterised by the presence of the palatal shelf at the side of the tongue, with its distal end located below the proximal end. Twenty MPSs, from 10 embryos, had the palatal shelf at that phase of development: six, from three CS18 embryos, were classified as degree 1, with incipient palatal shelves (Fig. 2b–d); four, from two CS19 embryos, as degree 2 (Fig. 2e, f); six, from three CS20 embryos, as degree 3 (Fig. 2g–i); and four, from two CS21 embryos, as degree 4 (Fig. 2j, k).

In six MPSs, from three embryos, the palatal shelf was in a horizontal position above the tongue, with its distal ends pointing inward (horizontal phase). Four MPSs were classified as degree 5: two, from the embryo CS22 (Fig. 2l), and two, from an embryo CS23 (Fig. 2m). In both embryos, the palatal shelves were separated. Two MPSs from another CS23 embryo (Fig. 2n) were classified as degree 6 because their palatal shelves were in contact at the midline.

2.7. Measurement sectors in the MPS: volume calculation

Using Adobe Photoshop, three areas were extracted from each TIFF image of the maxillary palatal fragments: the juxtapalatal zone and the internal and external parts of the palatal zone of each MPS. These sectors were used for the volumetric measurements (Fig. 1). In Fiji/ImageJ, a uniform contrast enhancement threshold was applied to these images. The area (μm^2) was then measured using a calibrated scale. The results were exported to Microsoft Excel (version 2019; Redmond, WA, USA), where the areas of the generated images were summed to determine the values for each third of the measurement sectors. These values were then used to calculate the total area of each zone and of the MPS as a whole.

The following formula, based on Cavalieri's principle (Gundersen and Jensen, 1987; Mayhew, 1992), was used to calculate volumes (μm^3):

$$V_t = \sum A_t \times T \times SN$$

where V_t is the volume (μm^3) in a third of a measurement sector; $\sum A_t$ is the sum of the areas (μm^2) in the third of the sector; T is the thickness of the embryo sections; and SN is the sequence number in the section selection (Table 1).

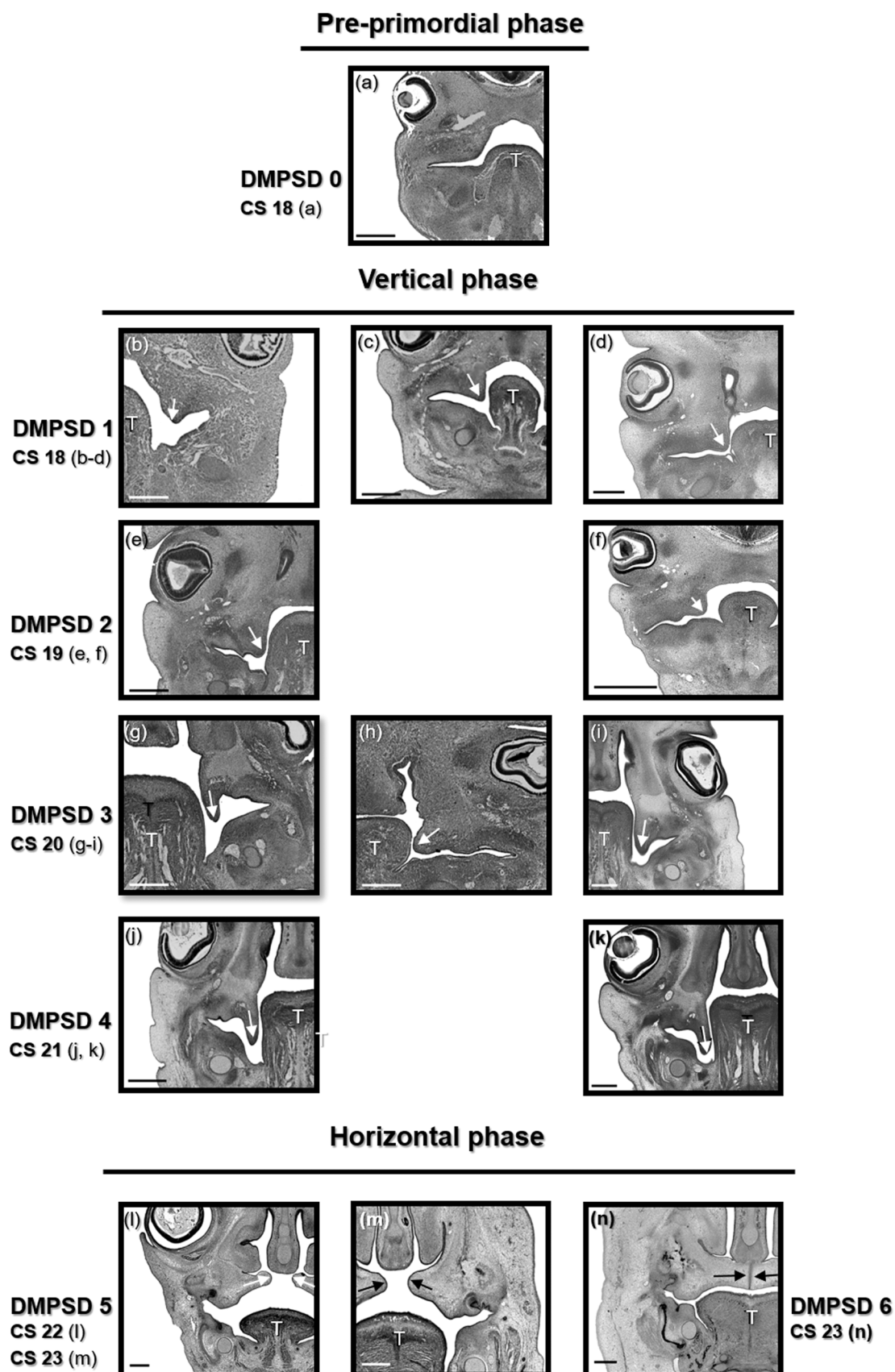


Fig. 2. Classification of maxillary palatal segments (MPSs) according to their developmental degree, based on the developmental phase of the palatal shelf (arrow) and the Carnegie stage (CS) of the embryo. Since these two parameters were identical in the MPSs of each embryo, only one image per embryo is displayed. The images correspond to sections in or near the coronal plane of the heads of the following embryos: E047 (panel a), E051 (b), E052 (c), 4430 (d), E064 (e), 9395 (f), E069 (g), E079 (h), 462 (i), E090 (j), 4090 (k), H983 (l), E104 (m), and 9226 (n). DMPSD, degree of MPS development; T, tongue. Scale bars: 500 μ m.

2.8. Percentage distribution of MPS volume

The percentage distribution of MPS volume across the thirds of the measurement sectors was determined. The total volume of the palatal zone was determined by summing the volumes of its constituent parts. The mean percentage of volume per third was then calculated for each sector from MPSs at the same degree of development. This percentage was then used as a comparative parameter to analyse volumetric changes (volumetric dynamics) in each sector.

2.9. Statistical analysis

Data were analysed using SPSS statistical software (IBM SPSS Statistics for Windows, Version 31.0.2.0; IBM Corp., Armonk, NY, USA; available at <https://www.ibm.com/products/spss-statistics>). For each third of the measurement sectors, descriptive statistics were calculated, including the mean with 95% confidence interval (CI), minimum and maximum values, and standard deviation.

Analysis of variance (ANOVA) was performed to assess differences in volumetric dynamics of the thirds among the measurement sectors. When the ANOVA showed statistically significant results, post hoc Bonferroni tests were applied to identify which thirds differed significantly.

In a secondary analysis, the same statistical procedures were applied to calculate volumetric differences in each measurement sector across the different embryonic intervals. Finally, Student's *t*-tests were used to analyse differences in the mean percentage volume of the thirds of each sector between two consecutive developmental degrees of the MPS.

Statistical significance was set at $p < 0.05$.

2.10. Changes in the mean percentage of volume during embryonic intervals

To analyse changes in the mean percentage of volume in the thirds of the measurement sectors, the difference in each embryonic interval between two consecutive developmental degrees of the MPS was measured. Percentage differences were expressed in percentage points (pp).

Each embryonic interval in the sequence of MPS development corresponded to a specific phase of the palatal shelf or a transition between two of them. Consequently, differences in the mean percentage of volume measured in each interval provided information on volumetric dynamics at each step of secondary palate formation.

Percentage changes during primordium formation, between the degrees 0 and 1 of MPS development, were evaluated in interval 1; the variations throughout the vertical phase, from the degrees 1–4, occurred in intervals 2–4; the changes during the elevation of the palatal shelf, between degrees 4 and 5, took place in interval 5; and the variations during the horizontal phase, between degrees 5 and 6, were observed in interval 6.

2.11. Concordance between the opposite sign changes in the mean percentage of volume of the palatal and adjacent thirds

For this Section, the two measurement sectors in the palatal zone have been unified.

A possible cause-and-effect relationship was considered when, during the same embryonic interval, changes of opposite sign occurred in the mean percentage of volume in a third of the palatal zone and juxtapalatal or palatal adjacent thirds, suggesting a transfer of mesenchyme.

Since it has not been possible to exclude the influence of other mechanisms on these changes, only the extent to which the loss in one third coincides with the gain in an adjacent third has been assessed. However, this does not clarify whether mesenchymal transfer occurred or, if so, its magnitude.

To analyse the most excellent concordance between percentage gains and losses in volume between the palatal and adjacent thirds during each embryonic interval, one of the following criteria was applied in each case: (1) If the percentage decrease in a third coincided with the percentage increase in a neighbouring third, the maximum percentage concordance would equal that value. (2) If the percentage decrease in a third exceeded the increase in an adjacent third, the greatest percentage concordance was based on the increase; otherwise, it corresponded to the decrease. (3) If the percentage decrease in a third coincided with growth in more than one adjacent third, the maximum percentage concordance was proportional to the respective increases. Conversely, if growth in a third coincided with decreases in several neighbouring thirds, the opposite applied.

3. Results

3.1. Distribution of the palatal shelf in the MPS zones

All maxillary palatal fragments contained tissue from the palatal shelf in the palatal zone. Only 11.45% of the fragments contained minimal portions from the palatal shelf's base in the juxtapalatal zone.

3.2. Statistical results

The statistical data are available in [Table 2](#).

Statistically significant global differences were observed in the juxtapalatal zone between the anterior and middle thirds (mean volume percentage difference = 4.67; 95% CI, 2.40–6.93; $p < 0.001$), as well as between the middle and posterior thirds (mean difference = 5.32 pp; 95% CI, 3.05–7.59; $p < 0.001$). In the palatal zone, statistically significant differences were also found between the anterior and posterior thirds (mean difference = 2.90 pp; 95% CI, 1.23–4.57; $p < 0.001$).

Analysis of the differences in mean volume percentages of the MPS thirds measured at each embryonic interval revealed the following statistically significant differences:

- The juxtapalatal zone, posterior third, showed in embryonic interval 1 a difference of -2.04 pp ($p = 0.003$) and a difference of -4.00 pp ($p = 0.024$) in interval 5.
- The palatal zone, middle third, showed in embryonic interval 5 a difference of 2.27 pp ($p = 0.009$) and 3.00 pp ($p = 0.006$) in interval 6. In the posterior third, the difference was 4.25 pp ($p = 0.028$) in interval 5.
- The external palatal zone, anterior third, showed in embryonic interval 5 a difference of -1.57 pp ($p = 0.047$). In the middle third, the difference was -1.02 pp ($p = 0.021$) in interval 5, and in the posterior third, -3.50 pp ($p = 0.008$) in the same interval.
- In the internal palatal zone, in the middle third, there was in interval 5 a difference of 3.29 pp ($p < 0.001$) and a difference of 3.61 pp ($p = 0.001$) in interval 6. In the posterior third, differences in intervals 5 and 6 were 7.74 pp ($p < 0.001$) and 3.87 pp ($p = 0.006$), respectively.

3.3. Volumetric dynamics in the thirds of the MPS zones: anterior-posterior axis

[Table 2](#) and [Fig. 3a, b](#), show the mean percentage of volume across the thirds of the MPS zones, by degree of MPS development. [Fig. 4](#) shows the differences between these average values at each embryonic interval. The results presented in the three following paragraphs of this Section have been calculated from the data in [Table 2](#) and [Fig. 4](#).

During the embryonic stages examined (from CS18 to CS23), the palatal zone of the MPS showed a general trend toward local growth, with an overall increase of 2.59 pp in the mean percentage of volume in the anterior third, 6.61 pp in the middle third, and 4.68 pp in the posterior third. In contrast, the juxtapalatal zone exhibited an insignificant

Table 2Descriptive statistics of the mean percentage of volume of the thirds of the maxillary palatal segments (MPSSs) at different degrees of MPS development (DMPSDs^a).

		95% Confidence Interval							
		DMPSD	N	Mean (%)	SD	Limit lower	Limit upper	Minimum value	Maximum value
Juxtapalatal zone	Anterior third	0	2	22.39	0.64	16.67	28.11	21.94	22.84
		1	6	23.30	4.62	18.45	28.14	14.36	27.06
		2	4	24.10	5.59	15.21	33.00	18.57	31.18
		3	6	26.65	3.53	22.95	30.36	21.38	30.00
		4	4	26.78	1.85	23.83	29.73	24.58	28.94
		5	4	24.28	2.29	20.63	27.91	21.01	26.32
	Middle third	6	2	22.44	0.53	17.67	27.20	22.06	22.81
		0	2	20.51	1.37	8.19	32.84	19.54	21.48
		1	6	22.38	2.54	19.72	25.04	19.47	26.58
		2	4	23.20	4.31	16.34	30.06	18.99	28.52
		3	6	18.91	3.83	14.90	22.93	12.81	23.28
		4	4	18.36	1.36	16.20	20.51	16.40	19.35
	Posterior third	5	4	18.11	1.92	15.06	21.16	15.81	20.29
		6	2	15.94	0.06	15.43	16.45	15.90	15.98
		0	2	18.22	1.51	4.68	31.75	17.15	19.28
		1	6	15.16	0.74	14.39	15.93	13.90	16.05
		2	4	16.40	4.16	9.78	23.02	12.86	22.41
		3	6	15.89	2.56	13.20	18.58	11.85	19.04
Palatal zone	Anterior third	4	4	15.04	1.78	12.21	17.87	13.30	17.52
		5	4	11.03	2.69	6.76	15.31	8.38	14.11
		6	2	8.87	0.09	8.04	9.69	8.80	8.93
		0	2	13.42	0.28	10.94	15.89	13.22	13.61
		1	6	15.40	2.21	13.08	17.72	12.90	18.30
		2	4	14.13	4.02	7.73	20.52	9.92	19.54
	Middle third	3	6	15.33	1.64	13.61	17.05	13.46	18.30
		4	4	15.11	1.60	12.58	17.65	13.25	17.12
		5	4	15.36	4.13	8.79	21.94	10.45	18.97
		6	2	16.01	0.78	8.95	23.06	15.45	16.56
		0	2	12.08	0.06	11.51	12.66	12.04	12.13
		1	6	12.52	1.62	10.81	14.22	11.23	15.63
	Posterior third	2	4	12.06	2.63	7.87	16.25	8.96	14.59
		3	6	12.80	1.03	11.73	13.89	11.64	14.46
		4	4	13.43	1.05	11.76	15.09	12.72	14.97
		5	4	15.70	0.93	14.22	17.17	14.40	16.49
		6	2	18.70	0.06	18.19	19.21	18.66	18.74
		0	2	13.38	0.71	7.03	19.73	12.88	13.88
External palatal zone	Anterior third	1	6	11.25	1.90	9.26	13.24	8.21	13.44
		2	4	10.11	1.54	7.66	12.55	8.46	12.02
		3	6	10.41	1.51	8.82	11.99	8.51	12.50
		4	4	11.28	1.59	8.75	13.81	8.94	12.28
		5	4	15.53	2.95	10.83	20.23	12.59	18.48
		6	2	18.06	0.06	17.55	18.57	18.02	18.10
	Middle third	0	2	10.38	0.06	9.80	10.95	10.33	10.42
		1	6	9.69	1.84	7.76	11.62	6.33	11.35
		2	4	10.23	3.09	5.30	15.15	7.73	14.66
		3	6	11.38	1.57	9.73	13.03	8.66	13.03
		4	4	11.63	1.15	9.80	13.45	10.01	12.53
		5	4	10.05	1.10	8.30	11.80	8.41	10.68
	Posterior third	6	2	10.35	0.05	9.90	10.79	10.31	10.38
		0	2	8.21	0.08	7.45	8.97	8.15	8.27
		1	6	9.66	1.05	8.56	10.76	8.47	11.30
		2	4	9.62	1.81	6.73	12.50	8.11	11.94
		3	6	10.38	1.07	9.26	11.50	9.07	11.75
		4	4	9.48	0.57	8.58	10.38	8.84	10.19
Internal palatal zone	Anterior third	5	4	8.46	0.55	7.58	9.34	7.83	9.17
		6	2	7.86	0.03	7.61	8.11	7.84	7.88
		0	2	9.08	0.88	1.20	16.96	8.46	9.70
		1	6	8.45	0.79	7.62	9.29	7.56	9.49
		2	4	8.90	1.13	7.09	10.70	7.51	10.15
		3	6	9.76	1.61	8.06	11.45	7.68	11.58
	Middle third	4	4	10.59	1.45	8.28	12.91	8.76	12.02
		5	4	7.09	1.56	4.61	9.57	5.26	8.78
		6	2	5.76	0.23	3.66	7.85	5.59	5.92
		0	2	3.04	0.34	-0.01	6.09	2.80	3.28
		1	6	5.71	3.14	2.42	9.00	2.95	11.49
		2	4	3.90	2.19	0.41	7.38	1.35	6.37
Internal palatal zone	Anterior third	3	6	3.95	1.33	2.56	5.34	2.18	5.59
		4	4	3.49	0.88	2.08	4.90	2.77	4.78
		5	4	5.31	3.37	-0.06	10.67	2.03	8.29
		6	2	5.66	0.83	-1.84	13.16	5.07	6.25
		0	2	3.87	0.16	2.47	5.27	3.76	3.98
		1	6	2.86	1.01	1.80	3.92	1.94	4.33

(continued on next page)

Table 2 (continued)

		95% Confidence Interval						
Posterior third	2	4	2.45	1.26	0.45	4.45	0.71	3.71
	3	6	2.42	1.53	0.81	4.03	0.83	5.27
	4	4	3.95	0.60	2.99	4.91	3.43	4.78
	5	4	7.24	0.81	5.94	8.53	6.50	7.99
	6	2	10.84	0.08	10.08	11.60	10.78	10.90
	0	2	4.30	0.17	2.78	5.82	4.18	4.42
	1	6	2.80	1.94	0.77	4.83	0.65	4.95
	2	4	1.21	1.56	-1.27	3.70	0.00	3.48
	3	6	0.65	0.98	-0.38	1.68	0.00	2.51
	4	4	0.69	0.61	-0.29	1.67	0.18	1.50
	5	4	8.44	1.48	6.08	10.79	6.99	9.73
	6	2	12.31	0.18	10.72	13.89	12.18	12.43

Abbreviations: N, number of the MPSS; SD, standard deviation.
Notes: ^a in each embryo, the two MPSS exhibited the same degree of development.

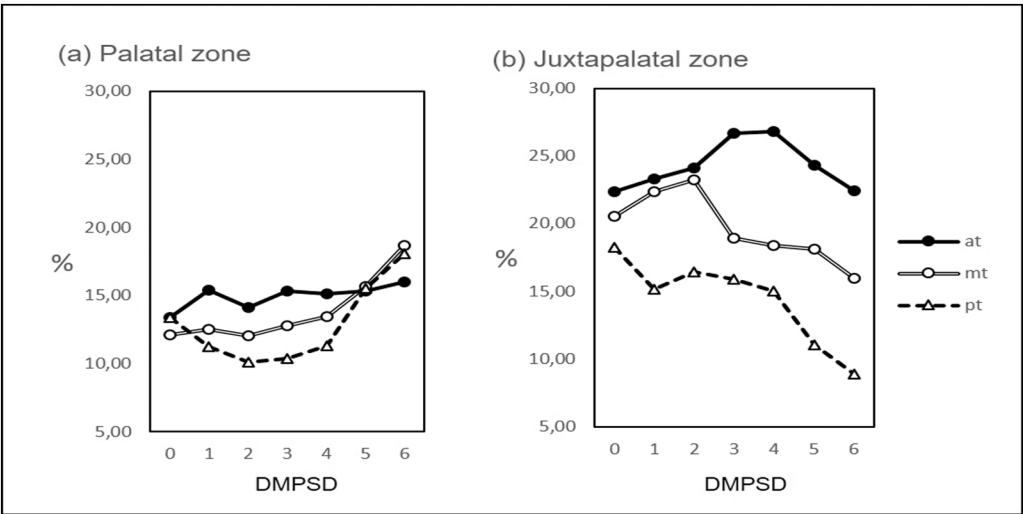


Fig. 3. Curves displaying the mean percentage distribution of volume in the maxillary palatal segment (MPS) at each degree of MPS development (DMPSD), divided into anterior (at), middle (mt), and posterior (pt) thirds of each zone. See values in Table 2.

overall increase in the anterior third (0.05 pp) and decreases in the middle (−4.57 pp) and posterior (−9.35 pp) thirds.

As shown in Fig. 3a, b, the anterior third displayed the most consistent mean volume percentage profile across both MPS zones. The profiles of the middle and posterior thirds showed some resemblance in both zones, especially in the palatal zone, except for interval 1.

Noteworthy among the variations in the mean percentage of volume in the palatal zone (Fig. 4) was the fact that positive changes in the anterior third were mainly concentrated (48.60%) in the embryonic interval 1 (beginning of the palatal shelf growth); in the middle third, the most considerable positive changes occurred in intervals 5 (32.08%) and 6 (42.50%), when the palatal shelf rose and grew horizontally, respectively; and in the posterior third, these increases were mainly seen in interval 5 (53.41%) and, to a lesser extent, in interval 6 (31.84%). During intervals 2–4 (the vertical phase), there was more variation in the direction of changes, but none exceeded 1.27 pp.

3.4. Volumetric dynamics in the thirds of the MPS palatal zone: medial-lateral axis

Table 2 presents the mean percentage of volume in the external and internal parts of the palatal zone of the MPS, and Fig. 5a-c shows a comparison between them. The results presented in the following two paragraphs are derived from the data in Table 2.

In this embryonic series, the mean percentage of volume in the external part of the palatal zone consistently surpassed that of the internal part throughout all developmental phases of the palatal shelf in

the anterior third, with differences of ≥ 3.98 pp (Fig. 5a), as well as in phases before the supralingual ascent of the palatal shelf (developmental degrees of the MPS 0–4) in the middle and posterior palatal thirds, with differences of ≥ 5.53 pp and ≥ 4.78 pp, respectively (Fig. 5b, c).

During the horizontal formation phase of the palatal shelf, before the shelves converged at the midline (developmental degree 5), the values of the two parts of the palatal zone were nearly equal, with a difference of 1.22 pp favouring the external part and 1.34 pp favouring the internal part in the middle and posterior palatal thirds, respectively (Fig. 5b, c). Once the shelves made contact at the midline (developmental degree 6), the internal part of the palatal zone dominated over the external part in the middle and posterior palatal thirds, with differences of 2.98 and 6.55 pp, respectively (Fig. 5b, c).

These radical changes in the percentage predominance of the palatal parts of the MPS were related to the volumetric dynamics that occurred during embryonic intervals 5 and 6. During interval 5, which corresponds to the elevation of the palatal shelf, the internal part of the palatal zone increased the mean percentage of volume, particularly in the posterior third (7.74 pp), and, to a lesser extent, in the middle and anterior thirds (3.29 pp and 1.82 pp, respectively). During the horizontal phase of palatal shelf formation, in interval 6, the percentage value of the internal part continued to increase in the middle (3.61 pp) and posterior (3.87 pp) palatal thirds. Meanwhile, in the anterior third, it remained stable (0.35 pp).

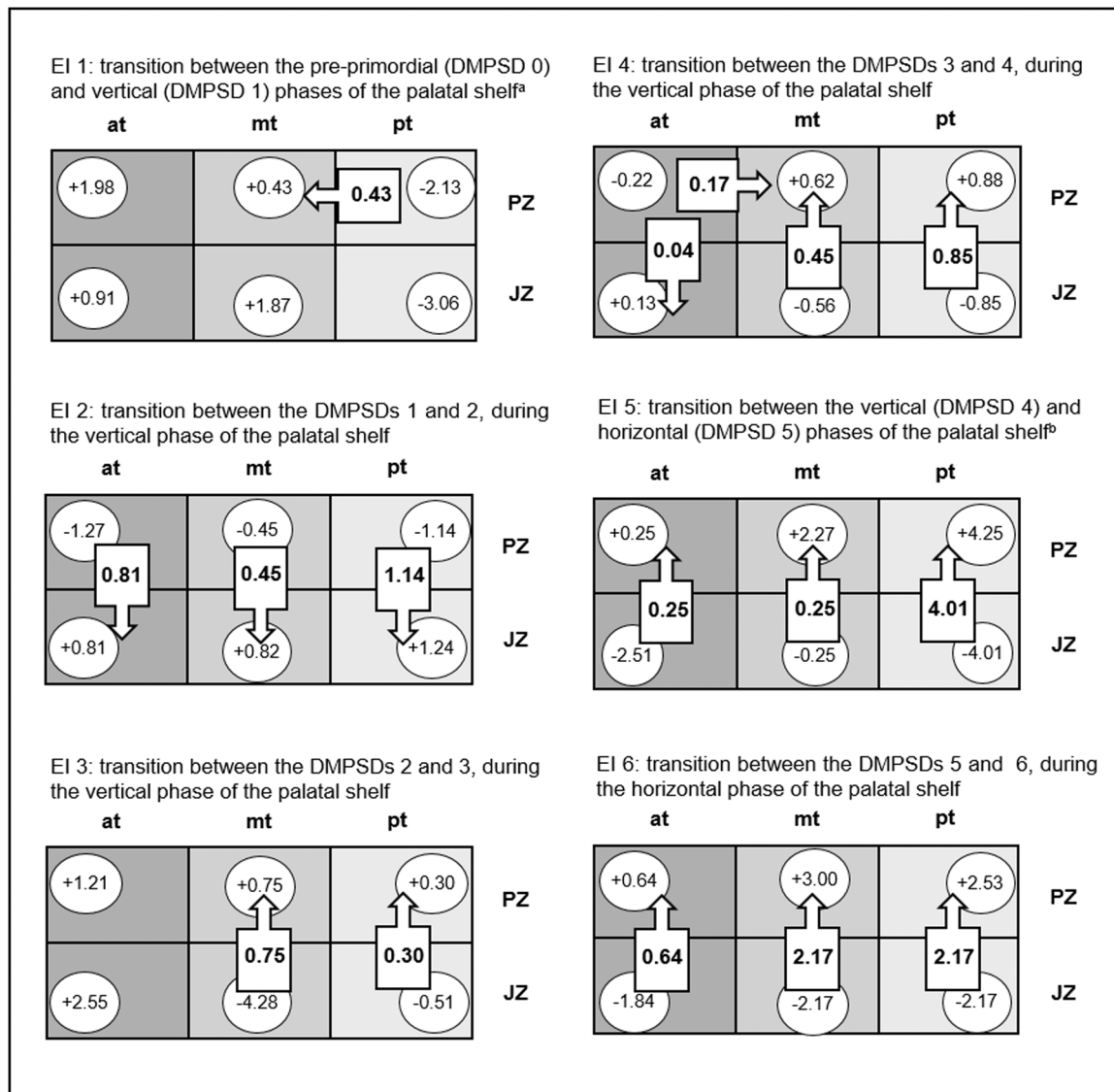


Fig. 4. Changes in the mean percentage distribution of volume in the maxillary palatal segment (MPS), measured at each embryonic interval (EI). Unit of measurement: percentage points. Each box with arrow shows the most significant level of concordance between the gains and losses in a palatal third and an adjacent one. The arrows indicate the direction of the hypothetical mesenchymal transfer suggested by these data. at, anterior third; DMPD, degree of MPS development; JZ, juxtapalatal zone; mt, middle third; pt, posterior third; PZ, palatal zone. Note: ^a formation of the palatal shelf primordium; ^b elevation of the palatal shelf.

3.5. Volume percentage changes with opposite sign in the palatal and adjacent thirds

Based on the differences in the average percentages of volume in the palatal and adjacent thirds measured in each embryonic interval, Fig. 4 shows the best concordance between percentage gains and losses.

Analysis of the data in Table 2 and Fig. 4 reveals that 68.95% of percentage changes in the palatal thirds during the embryonic period aligned with one of the criteria outlined in 2.11 regarding the concordance between gains and losses in the percentage of neighbouring thirds. 68.37% of cases occurred during the elevation of the palatal shelf above the tongue (embryonic interval 5) and its subsequent horizontal growth (embryonic interval 6).

4. Discussion

4.1. Methodological aspects

The study introduces innovations in methodology for assessing

volumetric dynamics during embryonic development of the palatal shelf and adjacent tissues within the MPS.

Firstly, this Section discusses aspects related to the series of human embryos used; secondly, it examines the methodological innovations employed to obtain and analyse volumetric data.

4.1.1. About the embryo series

The MPSs examined in this study were categorised into seven developmental degrees based on the palatal shelf phase and the embryo's CS (Fig. 2; Table 2). The phase observed in the two shelves of each embryo was the same.

The Carnegie staging system for classifying human embryos is based on morphological characteristics rather than chronological age or length, which can vary significantly among human embryos (Flierman et al., 2023; O'Rahilly and Müller, 2010). It is not possible to order embryos in a perfect series, as each may be advanced in some respects and delayed in others. As individual differences become less pronounced, it can sometimes be impossible to accurately match a new embryo to any of the illustrated standards. For this reason, some

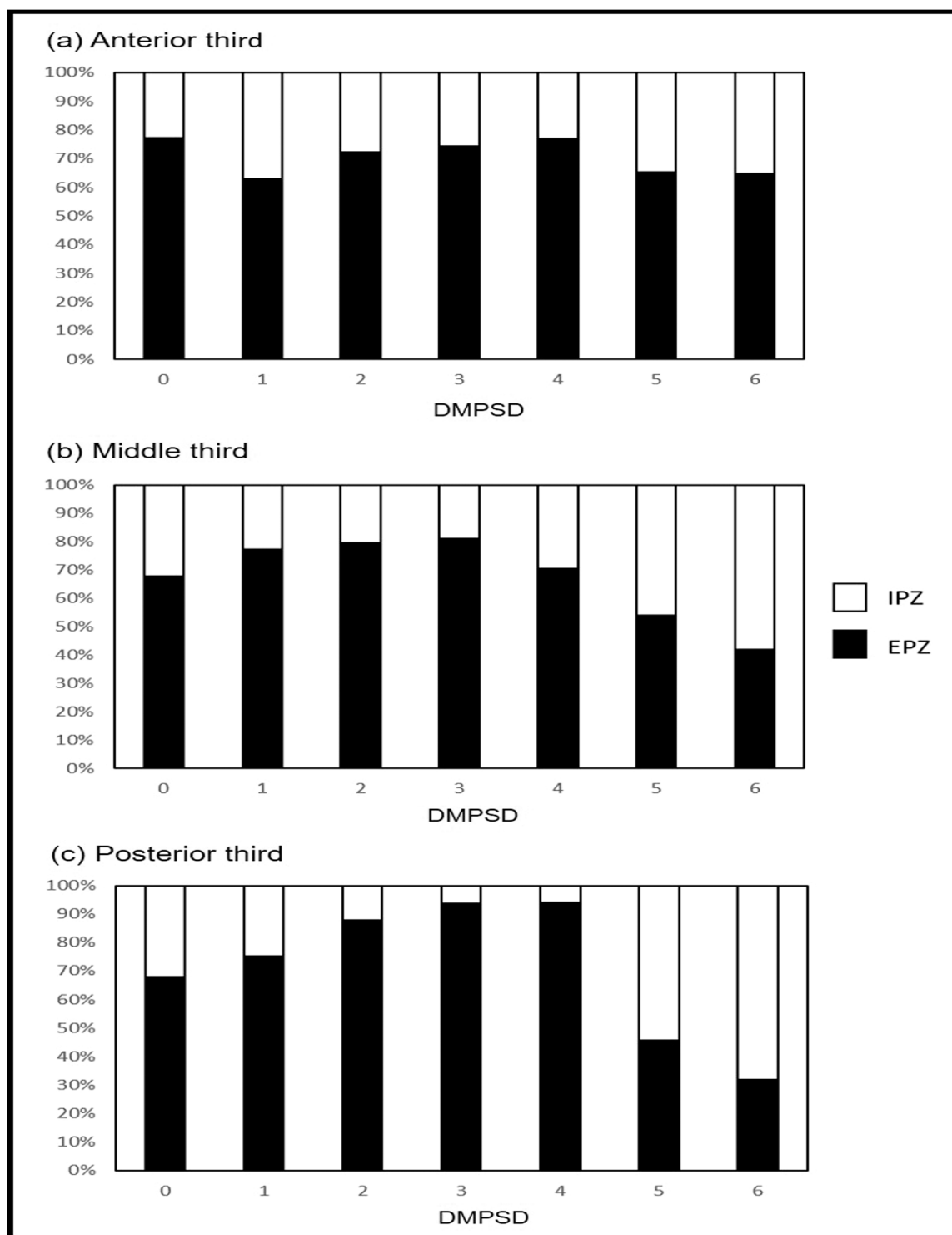


Fig. 5. Comparison of the mean percentage of volume in the parts of the palatal zone of the maxillary palatal segment (MPS), using 100% stacked columns. See values in Table 2. DMPSD, degree of MPS development; EPZ, external palatal zone; IPZ, internal palatal zone.

allowance is made for variation in the developmental staging process (O'Rahilly and Müller, 1987). These nuances clarify the early formation of the palatal shelf and its horizontal positioning above the tongue in CS18 and CS22 of the current series, respectively.

These events occur one stage later and one stage earlier than those described by O'Rahilly and Müller (1992). Additionally, it was found that not all CS18 and CS23 embryos showed the same level of MPS development. Consistent with this, Nohara et al. (2022) observed no palatal elevation in any CS22 embryos and in only three of eight CS23

embryos. They observed elevation in the remaining five embryos, but in one case, the palatal shelves remained separated. This was also observed in the E104 embryo (CS23) of the present series, and in 50% of the CS23 embryos of Diewert (1981), as cited by O'Rahilly and Müller (1987).

4.1.2. About the volumetric dynamics in the MPS

This study has provided new data on the regional volumetric dynamics of the palatal shelf and adjacent tissues during the human embryonic period. This was achieved by applying methodological

innovations that took into account the observation of specific histomorphological and molecular patterns in the regional development of the secondary palate in mice and rats (Bush and Jiang, 2012; Chiquet et al. 2016; Coleman, 1965; Ferguson, 1978; Gao et al., 2009; Gritli-Linde, 2007; Hammond and Dixon, 2022; He et al., 2008; Im et al., 2025; Jin et al., 2008, 2010; Lan et al., 2004; Nagasaka et al., 2022; Yu and Ornitz, 2011).

In most images, the edges of the lines used to frame the fragments included in each MPS were easily identifiable. When this was difficult, alternative methods were employed (see 2.4). This has reduced observer bias and improved reproducibility.

The broad division of the MPS into two zones and anteroposterior thirds, and the separation of the palatal zone into two mediolateral parts (Fig. 1), avoids the methodological error identified by Ferguson (1978) and Yu and Ornitz (2011) of extrapolating data obtained from a specific region to the entire palate. This palatal subdivision is among the most extensive published on human embryonic palatogenesis (Burdí and Faist, 1967; Burdí et al., 1988; Diewert and Lozanoff, 1993a, b; Diewert and Shiota, 1990; Diewert et al., 1993; Nohara et al., 2022; Radlanski, 2003; Radlanski et al., 2004; Vermeij-Keers et al., 1983).

The volumetric values of the present series were determined using stereological techniques (Gundersen and Jensen, 1987; Mayhew, 1992) applied to images of histological sections following the same method used in previous studies with human embryos and fetuses (Macarulla-Sanz et al., 1996; Nebot-Cegarra et al., 1999).

Since post-mortem changes and histological procedures can influence embryo size or sections dimensions, and because embryos at the same developmental stage can vary considerably in size (Flierman et al., 2023; O'Rahilly and Müller, 2010), it was decided not to compare MPS part volumes directly between embryos. The percentage distribution of MPS volume across the thirds of its zones has been used as an alternative measure. The reason is that, in a hypothetical scenario in which two embryos had developed identically but differed in size, their MPSs would reveal differences in volume but not in the percentage distribution across the thirds of the measurement sectors (the juxtapalatal and palatal zones and the two parts of the palatal zone).

The mean of these percentages was calculated from MPSs at the same developmental degree (Table 2) and used as a comparative parameter during the embryonic development of the human secondary palate.

Consequently, the changes measured in this way removed the influence of secondary factors affecting the size of the embryonic body and histological sections, while providing a more precise view of the specific tissue dynamics within each measurement sector of the MPS zones.

The data obtained in this study could serve as a reference for future *in vivo* studies. Technical improvements in ultrasound technology enable more precise imaging of embryos and fetuses during pregnancy, as well as measurement of their volumes. This is a more reliable indicator of embryonic and foetal growth than crown-rump length (Rousian et al., 2010) and a more accurate parameter for detecting growth restriction in subjects with congenital anomalies during prenatal development (Baken et al., 2017).

The new approach represented by this study has avoided the need to resort to methods that have produced inconsistent results and interpretations (Ferguson, 1978; Yu and Ornitz, 2011), has revealed the extent to which growth patterns of the palatal and juxtapalatal zones of the human MPS are specific, and has helped to minimise the risk of misinterpreting human palatogenesis by extrapolating, without sufficient nuance, the findings obtained in other species using techniques that are difficult to apply in human embryos (Chou et al., 2004; Ferguson, 1978; Yu and Ornitz, 2011). Therefore, it has helped identify connections between volumetric data during human palatogenesis and the outcomes of molecular and histomorphological studies in murine embryos. In the future, applying these methodological innovations to research with embryos of these species should enhance our understanding of their homologies.

4.2. Volumetric dynamics of the MPS palatal thirds: anterior-posterior axis

During the analysed period, the percentage distribution of MPS volume in the palatal zone showed a more consistent change profile in the anterior third than in the remaining two thirds. Similar profiles were observed in the middle and posterior thirds during the vertical and horizontal phases of palatal shelf formation (Fig. 3a).

The most notable growth in the anterior third of the palatal zone occurred at the beginning of palatal shelf formation (Figs. 3a, 4; Table 2). During the vertical phase of the palatal shelf, the anterior third remained the primary volumetric part of the palatal zone (Fig. 3a). After the sudden elevation of the palatal shelf (horizontal phase), the middle and posterior thirds first equalled and then surpassed the anterior third in volumetric terms with statistically significant increases in both (see 3.2; Fig. 3; Table 2). A significant difference was observed between the anterior and posterior thirds throughout the period under review.

These data reveal the importance of each third of the human palatal zone in achieving optimal tissue mass at each developmental phase of the palatal shelf. They also demonstrate the fundamental role of growth in the middle and posterior thirds in preventing delays or slow progress in the elevation and horizontal growth of the palatal shelf. This is crucial in preventing cleft palate. (Bukova et al., 2021; Geetha-Loganathan et al., 2022). However, these findings differ from those of Nohara et al. (2022), who noted an “unnatural” slowing and arrest of palatal shelf elevation and fusion in normal human embryos at CS22 and early CS23, which they termed the “approach period” in palatogenesis.

Caution is warranted when considering potential similarities between species, as anatomical differences in the palate arise from their specific developmental processes. However, the difficulties involved in molecular and experimental research on human palate development have prompted the exploration of a possible link between the findings of this study on the anteroposterior growth of the human palatal shelf and research primarily conducted on mouse embryos, which are the model most utilised (94%) in studies on palatogenesis (Levi et al., 2011).

In a recent paper using mouse embryos (Welsh et al., 2025), it was found that the anterior part of the secondary palate contributes most to overall palatal lengthening. This growth occurs through the formation of palatal tissue on each side from the growth zone of the palatal rugae, which is located just in front of the first ruga to appear. This generative zone is initially located at the very front, but its position shifts backwards as tissue accumulates in front of it. In mice, palatal rugae form during palatogenesis (Welsh et al., 2025); in humans, these structures appear in the third month of gestation (Chong et al., 2020). Therefore, in the embryos analysed in this study, rugae had not yet developed.

Rugae cover the entire oral surface of the hard palate in mice and are often twice as numerous as in humans, who have them only on the anterior part. The varying extent of the rugae growth zone on each side of the hard palate may be linked to differences in maxillary prognathism between the two species (Welsh et al., 2025).

These comparative data suggest that, as in mice, the part of each palatal shelf associated with the palatal rugae (the anterior third) forms a specific developmental region in humans. The remaining part (the middle and posterior thirds, which have similar growth profiles) constitutes another region. As in mice, there may be specific genetic control of development within each of these regions (see paragraph 14, 1).

Regarding chronology, the late growth of the middle and posterior parts of the human secondary palate (Fig. 3a) contrasts with the early horizontal movement towards the midline observed in the posterior part of the mouse (Walker and Fraser, 1956; Yu and Ornitz, 2011) and rat (Ferguson, 1978; Schupbach et al., 1983) palatal shelf.

4.3. Volume dynamics of the MPS palatal thirds: medial-lateral axis

In this embryonic series, the average percentage of volume in the external part of the MPS palatal zone was clearly greater than that in the

internal part in the anterior third, at all the developmental degrees of the MPS (Fig. 5a). The same predominance was also observed in the middle and posterior thirds before the elevation of the palatal shelf (Fig. 5b, c).

However, a radical change in this parameter occurred (Fig. 5b, c) when the shelf shifted from a vertical to a horizontal position (embryonic interval 5) and rested on the tongue. This sudden change primarily occurred in the posterior third of the palatal zone, with a marked and statistically significant increase in the percentage of the internal part. This growth was about twice that in the middle palatal third, also significant, and four times that in the anterior palatal third (see 3.4). During the horizontal phase, when the shelves were still separated, the percentage values of the two parts of the palatal zone were almost identical in the middle and posterior thirds. However, once the right and left palatal shelves made contact at the midline, the internal part became dominant, as it had grown significantly during embryonic interval 6 in its middle and posterior thirds (Fig. 5b, c).

These data demonstrate a close link between the elevation and horizontal growth of the human palatal shelf and the growth of the internal part of the palatal zone in the middle and posterior thirds.

Furthermore, the results of this study using human embryos could be linked to the findings of previous experiments involving mouse embryos, that investigated which part of the vertical palatal shelf develops into the horizontal palatal axis. In homozygous *Osr2*^{-/-} mouse embryos, a selective reduction in cell proliferation was observed in the medial region of the palatal shelf. This was associated with the formation of a palatal cleft, caused by a lack of elevation in the middle and posterior thirds of the palatal shelf (Bush and Jiang, 2012; Gao et al., 2009; Gritti-Linde, 2007; Lan et al., 2004). In *Zfhx-1a* and *Jag2* mutant mice, it has been demonstrated that the medial edge of the horizontal palatal shelf develops from the inner side of the vertical shelf, near the tongue, rather than from the distal end. Furthermore, the horizontal axis of the palatal shelf is formed by the growth of mesenchyme adjacent to the inner side of the vertical shelf, rather than by the rotation of the pre-existing vertical axis (Jin et al., 2008, 2010). Chiquet et al. (2016) mapped the expression domains of specific extracellular matrix components in the palatal shelf of mouse embryos, confirming the role of mesenchymal growth in the shelf elevation, except in the anterior third, where rotation was proposed as an alternative explanation for the ascent of the shelf from its vertical position.

These similarities suggest that, as in the mouse embryo, differential genetic regulation of secondary palate development occurs along the medial-lateral axis in the human embryo (see paragraph 14, 1).

4.4. About the concordance between opposite sign changes in the mean percentage of volume of the palatal and adjacent thirds

There is evidence that mesenchymal remodelling contributes to palate formation and elevation in murids (Chiquet et al., 2016; Chou et al., 2004; Coleman, 1965; Ferguson, 1978; He et al., 2008; Jin et al., 2010; Walker and Fraser, 1956; Yasuda and Fujimoto, 1986; Yu and Ornitz, 2011). Furthermore, primary mesenchymal cells from the mouse embryonic palate in culture have been shown to migrate (Fantauzzo and Soriano, 2017; Gao et al., 2019; Goering et al., 2021a, b; He and Soriano, 2013).

As in the present embryonic series, 68.95% of changes in the average percentage of volume in the palatal thirds aligned with some of the criteria outlined in 2.11 about concordance between percentage increases and decreases in neighbouring thirds (see 3.5) suggests that mesenchymal transfer might occur between the palatal thirds and their adjacent thirds during the human secondary palate development, among other mechanisms. If this tissue remodelling were to occur, it would take place within the broad mesenchymal core of the MPS. However, MPS volume measurements also included the maxillary line, a thin epithelial layer. The prominent presence of mesenchyme confirmed the accuracy of the calculations used to assess the correlation between the percentage increase or decrease in the volume of the mesenchymal nucleus of

adjacent thirds in each embryonic interval. This served as the basis for suggesting potential mesenchymal transfer from the “donor” to the “recipient” third.

The level of concordance indicates the likelihood of transfer but does not confirm that this mechanism is responsible for the percentage variations. Conversely, it can be inferred that the percentage of volume lost by the “donor” third exceeding the simultaneous gain in the neighbouring thirds cannot be attributed to mesenchymal transfer.

During the elevation of the palatal shelf, between the vertical and horizontal phases (embryonic interval 5), the posterior palatal third showed the most excellent concordance with the neighbouring thirds regarding opposite sign changes in volume percentage (Fig. 4). In contrast, during the horizontal phase (interval 6), this agreement was evenly distributed between the middle and posterior palatal thirds (Fig. 4). If the equivalence between these correspondences and mesenchymal transferability were confirmed, it would align with the observed mesenchymal remodelling in the middle and posterior thirds of the secondary palate during mouse (Chiquet et al., 2016; Jin et al., 2010) and rat (Coleman, 1965) development.

In this series of embryos, the elevation and horizontal expansion of the palatal shelf were accompanied by notable growth in the internal part of the palatal zone in the middle and posterior thirds (Fig. 5b, c; see Sections 3.4, 4.3). Simultaneously, there were decreases in the volume percentage of the juxtaapalatal zone consistent with the growth of these two palatal thirds (Fig. 4). If these correlations were due to mesenchymal transfers between the thirds, it would be reasonable to suggest that the percentage increase in the internal part of the palatal zone and the transfers to these palatal thirds were related (see Sections 3.4, 3.5).

This observation is consistent with studies on mouse embryos, which suggest that the mesenchymal condensation observed next to the medial border epithelium of the palatal shelf results from mesenchymal remodelling rather than differential subepithelial cell growth (Jin et al., 2010; Yu and Ornitz, 2011).

It seems reasonable to suggest that the role of hypothetical mesenchymal transfer in the ascent and subsequent horizontal growth of the palatal shelf is smaller than that shown in Fig. 4. The figure shows the strongest correlation between opposite-sign percentage changes in volume in adjacent thirds. Furthermore, these correlations could be explained by other mechanisms, such as local differential proliferation and remodelling of cellular and extracellular components (Chiquet et al., 2016; Nagasaka et al., 2022).

4.5. Limitations

The primary limitation has been the reduced number of human embryos studied. Although digitising collections makes more embryos and fetuses accessible, the limited availability of embryos at various developmental stages remains a significant constraint on human embryology research (Kong et al., 2023; Que et al., 2024). However, Radlanski et al. (2004) noted that this reality enables studies to be carried out with small numbers of human embryos, provided that the quality of the analysed histological sections is sufficient. The fourteen embryos in this series met this requirement and had no congenital physical defects. They also satisfied the morphological criteria (O’Rahilly and Müller, 1987, 2010) for each CS involved in the embryonic development of the palatal shelf (O’Rahilly and Müller, 1992). Although the sample size (all MPSs) in this study is twice the number of embryos (Table 2), it remains small, which limits the statistical power of the data.

Another limitation concerns the variability in the plane of histological sections of the embryos. The images of the embryonic sections used in this study were grouped into anterior-posterior thirds because the microtome cutting plane cross-sectioned the head along a coronal or quasi-coronal plane, although it was aligned to produce transverse sections of the trunk (Table 1). However, careful embryo selection has minimised slight variations in the angles of the cutting planes on their

heads.

The proposed link between the volumetric dynamics of the MPSs in the embryos of this study and some data from molecular analysis of palatogenesis in mouse and rat embryos, although argued, is limited in verifying by the use of digitised images from collections of human embryos, where molecular identification techniques were not used.

The final limitation concerns the analysis of mesenchymal transferability in the MPS palatal zone. As this study used histologically processed samples from human embryo collections (Table 1), the specific methods that have demonstrated, in mouse and rat embryos, the importance of tissue reorganisation during palatogenesis, particularly concerning palatal elevation, could not be employed. These methods include immunofluorescent analysis of actin fibres in the palatal mesenchyme and nuclear orientation (Chiquet et al., 2016), induction of cleft palate in embryos (Coleman, 1965; Jin et al., 2010), tracking carbon particles (Chou et al., 2004) or DiI-labelled grafted cells within the mesenchymal core of the palatal shelves cultured *in vitro* (He et al., 2008), and scratch labelling on the palate of whole embryos cultivated *in vitro* (Yasuda and Fujimoto, 1986). Therefore, although the calculations in this study indicate the temporal relationship between opposite sign changes in the average percentage of volume detected in adjacent thirds, they cannot determine the extent to which mesenchymal transfer and other mechanisms contribute to these results (see Sections 2.11, 3.5, and 4.4).

5. Conclusions

In the present study, conducted on histological sections of 14 human embryos (CS18-CS23), regionalisation in volumetric dynamics in the anteroposterior thirds of the 28 MPSs is highlighted. During the vertical phase of the palatal shelf development, the anterior third exhibits notable dominance in the volumetric percentage distribution in the MPS palatal zone. Expansion in this third is relevant only at the beginning of palatal shelf development; thereafter, its volumetric percentage stabilises. From the point at which the palatal shelf rises abruptly above the tongue (horizontal phase), the middle and posterior palatal thirds, with almost identical growth profiles, first equal and then exceed the anterior third in volumetric terms. The notable growth in the internal part of the middle and posterior palatal thirds during the elevation and horizontal formation of the palatal shelf indicates a close link with these processes. This relationship could be explained, with nuances, by a mechanism of mesenchymal transfer from the adjacent juxtapalatal thirds to the middle and posterior palatal thirds. This is suggested by the notable level of concordance observed between positive changes in the average percentage of volume of the middle and posterior palatal thirds and negative changes in the neighbouring juxtapalatal thirds. The findings also reveal substantial correlations with the development of the secondary palate observed in mouse and rat embryos.

CRedit authorship contribution statement

Anna Maria Cueto-González: Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Methodology, Investigation, Data curation. **Josep Nebot-Cegarra:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

Ethical statement

According to the report SCER201 issued by the Research Ethics Committee of the Autonomous University of Barcelona (CERec), this research does not require ethical approval from the CERec.

Submission declaration

The authors declare that the work described has not been published previously, is not under consideration for publication elsewhere, and has been approved for publication by all the authors.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors

Declaration of Competing Interest

The authors declare no conflicts of interests associated with this study.

Acknowledgement

The authors would like to thank the Autonomous University of Barcelona for funding the open-access publication of this article, and the Human Anatomy and Embryology Unit (Autonomous University of Barcelona) and the Virtual Human Embryo Project for supplying the histological images of the embryos used in this study. We would also like to thank Dr. Sílvia Ricart, paediatrician and postgraduate in Biostatistics (Paediatrics Department, Sant Joan de Déu Barcelona Hospital, University of Barcelona) for her valuable comments on the manuscript and her invaluable assistance with the statistical analysis of the data.

Data availability statement

Data is available upon request from the corresponding author.

References

- Aoyama, G., Kurosaka, H., Oka, A., Nakatsugawa, K., Yamamoto, S., Sarper, S.E., Usami, Y., Toyosawa, S., Inubushi, T., Isogai, Y., Yamashiro, T., 2019. Observation of dynamic cellular migration of the medial edge epithelium of the palatal shelf *in vitro*. *Front. Physiol.* 10, 698. <https://doi.org/10.3389/fphys.2019.00698>.
- Baken, L., Benoit, B., Koning, A.H.J., van der Spek, P.J., Steegers, E.A.P., Exalto, N., 2017. First-trimester crown-rump length and embryonic volume of fetuses with structural congenital abnormalities measured in virtual reality: an observational study. *Biomed. Res. Int.* 2017, 1953076. <https://doi.org/10.1155/2017/1953076>.
- Brinkley, L.L., Vickerman, M.M., 1978. The mechanical role of the cranial base in palatal shelf movement: an experimental re-examination. *J. Embryol. Exp. Morphol.* 48, 93–100.
- Brock, L.J., Economou, A.D., Cobourne, M.T., Green, J.B., 2016. Mapping cellular processes in the mesenchyme during palatal development in the absence of Tbx1 reveals complex proliferation changes and perturbed cell packing and polarity. *J. Anat.* 228, 464–473. <https://doi.org/10.1111/joa.12425>.
- Bukova, I., Szczerkowska, K.I., Prochazkova, M., Beck, I.M., Prochazka, J., Sedlacek, R., 2021. Loss of Wif function affects methylation pattern in palate development and leads to cleft palate. *Front. Cell. Dev. Biol.* 9, 620692. <https://doi.org/10.3389/fcell.2021.620692>.
- Burdi, A.R., Faist, K., 1967. Morphogenesis of the palate in normal human embryos with special emphasis on the mechanisms involved. *Am. J. Anat.* 120, 149–160.
- Burdi, A.R., Lawton, T.J., Grosslight, J., 1988. Prenatal pattern emergence in early human facial development. *Cleft Palate J.* 25, 8–15.
- Bush, J.O., Jiang, R., 2012. Palatogenesis: morphogenetic and molecular mechanisms of secondary palate development. *Development* 139, 231–243. <https://doi.org/10.1242/dev.067082>.
- Carette, M.J., Ferguson, M.W., 1992. The fate of medial edge epithelial cells during palatal fusion *in vitro*: an analysis by DiI labelling and confocal microscopy. *Development* 114, 379–388. <https://doi.org/10.1242/dev.114.2.379>.
- Chiquet, M., Blumer, S., Angelini, M., Mitsiadis, T.A., Katsaros, C., 2016. Mesenchymal remodeling during palatal shelf elevation revealed by extracellular matrix and F-Actin expression patterns. *Front. Physiol.* 7, 392. <https://doi.org/10.3389/fphys.2016.00392>.
- Chong, J.A., Mohamed, A.M.F.S., Pau, A., 2020. Morphological patterns of the palatal rugae: a review. *J. Oral. Biosci.* 62, 249–259. <https://doi.org/10.1016/j.job.2020.06.003>.
- Chou, M.J., Kosazuma, T., Takigawa, T., Yamada, S., Takahara, S., Shiota, K., 2004. Palatal shelf movement during palatogenesis: a fate map of the fetal mouse palate cultured *in vitro*. *Anat. Embryol.* 208, 19–25. <https://doi.org/10.1007/s00429-004-0379-0>.
- Coleman, R.D., 1965. Development of the rat palate. *Anat. Rec.* 151, 107–117. <https://doi.org/10.1002/ar.1091510202>.

- Cox, T.C., 2004. Taking it to the max: the genetic and developmental mechanisms coordinating midfacial morphogenesis and dysmorphology. *Clin. Genet.* 65, 163–176. <https://doi.org/10.1111/j.0009-9163.2004.00225.x>.
- Cuervo, R., Covarrubias, L., 2004. Death is the major fate of medial edge epithelial cells and the cause of basal lamina degradation during palatogenesis. *Development* 131, 15–24. <https://doi.org/10.1242/dev.00907>.
- Danescu, A., Mattson, M., Dool, C., Diewert, V.M., Richman, J.M., 2015. Analysis of human soft palate morphogenesis supports regional regulation of palatal fusion. *J. Anat.* 227, 474–486. <https://doi.org/10.1111/joa.12365>.
- Diewert, V.M., 1981. Active contributions of differential craniofacial growth to secondary palate development in man. *Anat. Rec.* 199, 69A–70A.
- Diewert, V.M., 1983. A morphometric analysis of craniofacial growth and changes in spatial relations during secondary palatal development in human embryos and fetuses. *Am. J. Anat.* 167, 495–522. <https://doi.org/10.1002/aja.1001670407>.
- Diewert, V.M., 1985. Development of human craniofacial morphology during the late embryonic and early fetal periods. *Am. J. Orthod.* 88, 64–76. [https://doi.org/10.1016/0002-9416\(85\)90107-1](https://doi.org/10.1016/0002-9416(85)90107-1).
- Diewert, V.M., Lozanoff, S., Choy, V., 1993. Computer reconstructions of human embryonic craniofacial morphology showing changes in relations between the face and brain during primary palate formation. *J. Craniofac. Genet. Dev. Biol.* 13, 193–201.
- Diewert, V.M., Lozanoff, S., 1993b. Growth and morphogenesis of the human embryonic midface during primary palate formation analyzed in frontal sections. *J. Craniofac. Genet. Dev. Biol.* 13, 162–183.
- Diewert, V.M., Lozanoff, S., 1993a. A morphometric analysis of human embryonic craniofacial growth in the median plane during primary palate formation. *J. Craniofac. Genet. Dev. Biol.* 13, 147–161.
- Diewert, V.M., Shiota, K., 1990. Morphological observations in normal primary palate and cleft lip embryos in the Kyoto collection. *Teratology* 41, 663–677. <https://doi.org/10.1002/tera.1420410603>.
- Dixon, M.J., Marazita, M.L., Beaty, T.H., Murray, J.C., 2011. Cleft lip and palate: understanding genetic and environmental influences. *Nat. Rev. Genet.* 12, 167–178. <https://doi.org/10.1038/nrg2933>.
- Du, W., Bhojwani, A., Hu, J.K., 2021. FAcETs of mechanical regulation in the morphogenesis of craniofacial structures. *Int. J. Oral. Sci.* 13, 4. <https://doi.org/10.1038/s41368-020-00110-4>.
- Fantauzzo, K.A., Soriano, P., 2017. Generation of an immortalized mouse embryonic palatal mesenchyme cell line. *PloS. One* 12, e0179078. <https://doi.org/10.1371/journal.pone.0179078>.
- Ferguson, M.W., 1978. Palatal shelf elevation in the Wistar rat fetus. *J. Anat.* 125, 555–577.
- Flierman, S., Tijsterman, M., Rousian, M., de Bakker, B.S., 2023. Discrepancies in embryonic staging: towards a gold standard. *Life* 13, 1084. <https://doi.org/10.3390/life13051084>.
- Gao, Y., Lan, Y., Ovitt, C.E., Jiang, R., 2009. Functional equivalence of the zinc finger transcription factors *Osrl* and *Osrl2* in mouse development. *Dev. Biol.* 328, 200–209. <https://doi.org/10.1016/j.ydbio.2009.01.008>.
- Gao, L., Xu, J., Li, X., Wang, T., Wu, W., Cao, J., 2019. 2,3,7,8-Tetrachlorodibenzo-p-dioxin and *TGFβ3*-mediated mouse embryonic palatal mesenchymal cells. *Dose Response* 17, 1559325818786822. <https://doi.org/10.1177/1559325818786822>.
- Geetha-Loganathan, P., Abramyan, J., Buchtová, M., 2022. Editorial: cellular mechanisms during normal and abnormal craniofacial development. *Front. Cell. Dev. Biol.* 10, 872038. <https://doi.org/10.3389/fcell.2022.872038>.
- Gillham, J.C., Anand, S., Bullen, P.J., 2009. Antenatal detection of cleft lip with or without cleft palate: incidence of associated chromosomal and structural anomalies. *Ultrasound Obstet. Gynecol.* 34, 410–415. <https://doi.org/10.1002/ultr.6447>.
- Goering, J.P., Isai, D.G., Czirok, A., Saadi, I., 2021a. Isolation and time-lapse imaging of primary mouse embryonic palatal mesenchyme cells to analyze collective movement attributes. *J. Vis. Exp.* (168), e62151. <https://doi.org/10.3791/62151>.
- Goering, J.P., Isai, D.G., Hall, E.G., Wilson, N.R., Kosa, E., Wenger, L.W., Umar, Z., Yousaf, A., Czirok, A., Saadi, I., 2021b. *SPECC1L*-deficient primary mouse embryonic palatal mesenchyme cells show speed and directionality defects. *Sci. Rep.* 11, 1452. <https://doi.org/10.1038/s41598-021-81123-9>.
- Greene, R.M., Pratt, R.M., 1976. Developmental aspects of secondary palate formation. *J. Embryol. Exp. Morphol.* 36, 225–245.
- Gritli-Linde, A., 2007. Molecular control of secondary palate development. *Dev. Biol.* 301, 309–326. <https://doi.org/10.1016/j.ydbio.2006.07.042>.
- Gundersen, H.J., Jensen, E.B., 1987. The efficiency of systematic sampling in stereology and its prediction. *J. Microsc.* 147, 229–263. <https://doi.org/10.1111/j.1365-2818.1987.tb02837.x>.
- Hammond, N.L., Dixon, J., Dixon, M.J., 2019. Periderm: life-cycle and function during orofacial and epidermal development. *Semin. Cell. Dev. Biol.* 91, 75–83. <https://doi.org/10.1016/j.semedb.2017.08.021>.
- Hammond, N.L., Dixon, M.J., 2022. Revisiting the embryogenesis of lip and palate development. *Oral. Dis.* 28, 1306–1326. <https://doi.org/10.1111/odi.14174>.
- He, F., Soriano, P., 2013. A critical role for *PDGFRα* signaling in medial nasal process development. *PLoS. Genet.* 9, e1003851. <https://doi.org/10.1371/journal.pgen.1003851>.
- He, F., Xiong, W., Yu, X., Espinoza-Lewis, R., Liu, C., Gu, S., Nishita, M., Suzuki, K., Yamada, G., Minami, Y., Chen, Y., 2008. *Wnt5a* regulates directional cell migration and cell proliferation via *Ror2*-mediated noncanonical pathway in mammalian palate development. *Development* 135, 3871–3879. <https://doi.org/10.1242/dev.025767>.
- Hilliard, S.A., Yu, L., Gu, S., Zhang, Z., Chen, Y.P., 2005. Regional regulation of palatal growth and patterning along the anterior-posterior axis in mice. *J. Anat.* 207, 655–667. <https://doi.org/10.1111/j.1469-7580.2005.00474.x>.
- Huang, Z., Zhi, Y., Cao, H., Bian, Z., He, M., 2024. Exosomes derived from human palatal mesenchymal cells mediate intercellular communication during palatal fusion by promoting oral epithelial cell migration. *Int. J. Nanomed.* 19, 3109–3121. <https://doi.org/10.2147/IJN.S451491>.
- Humphrey, T., 1968. The development of mouth opening and related reflexes involving the oral area of human fetuses. *Ala. J. Med. Sci.* 5, 126–157.
- Im, H., Song, Y., Kim, J.K., Park, D.K., Kim, D.S., Kim, H., Shin, J.O., 2025. Molecular regulation of palatogenesis and clefting: an integrative analysis of genetic, epigenetic networks, and environmental interactions. *Int. J. Mol. Sci.* 26, 1382. <https://doi.org/10.3390/ijms26031382>.
- Jin, J.Z., Li, Q., Higashi, Y., Darling, D.S., Ding, J., 2008. Analysis of *Zfhx1a* mutant mice reveals palatal shelf contact-independent medial edge epithelial differentiation during palate fusion. *Cell. Tissue Res.* 333, 29–38. <https://doi.org/10.1007/s00441-008-0612-x>.
- Jin, J.Z., Tan, M., Warner, D.R., Darling, D.S., Higashi, Y., Gridley, T., Ding, J., 2010. Mesenchymal cell remodelling during mouse secondary palate reorientation. *Dev. Dyn.* 239, 2110–2117. <https://doi.org/10.1002/dvdy.22339>.
- Kohli, S.S., Kohli, V.S., 2012. A comprehensive review of the genetic basis of cleft lip and palate. *J. Oral. Maxillofac. Pathol.* 16, 64–72. <https://doi.org/10.4103/0973-029X.92976>.
- Kong, S., Li, R., Tian, Y., Zhang, Y., Lu, Y., Ou, Q., Gao, P., Li, K., Zhang, Y., 2023. Single-cell omics: a new direction for functional genetic research in human diseases and animal models. *Front. Genet.* 13, 1100016. <https://doi.org/10.3389/fgene.2022.1100016>.
- Kouskoura, T., Kozlova, A., Alexiou, M., Blumer, S., Zouvelou, V., Katsaros, C., Chiquet, M., Mitsiadis, T.A., Graf, D., 2013. The etiology of cleft palate formation in *BMP7*-deficient mice. *PloS. One* 8, e59463. <https://doi.org/10.1371/journal.pone.0059463>.
- Lan, Y., Ovitt, C.E., Cho, E.S., Maltby, K.M., Wang, Q., Jiang, R., 2004. Odd-skipped related 2, (*Osrl2*) encodes a key intrinsic regulator of secondary palate growth and morphogenesis. *Development* 131, 3207–3216. <https://doi.org/10.1242/dev.01175>.
- Lan, Y., Qin, C., Jiang, R., 2019. Requirement of hyaluronan synthase-2 in craniofacial and palate development. *J. Dent. Res.* 98, 1367–1375. <https://doi.org/10.1177/0022034519872478>.
- Lan, Y., Xu, J., Jiang, R., 2015. Cellular and molecular mechanisms of palatogenesis. *Curr. Top. Dev. Biol.* 115, 59–84. <https://doi.org/10.1016/bs.ctdb.2015.07.002>.
- Lane, J., Kaartinen, V., 2014. Signaling networks in palate development. *Wiley Interdiscip. Rev. Syst. Biol. Med.* 6, 271–278. <https://doi.org/10.1002/wsbm.1265>.
- Levi, B., Brugman, S., Wong, V.W., Grova, M., Longaker, M.T., Wan, D.C., 2011. Palatogenesis: engineering, pathways and pathologies. *Organogenesis* 7, 242–254. <https://doi.org/10.4161/org.7.4.17926>.
- Li, Q., Ding, J., 2007. Gene expression analysis reveals that formation of the mouse anterior secondary palate involves recruitment of cells from the posterior side. *Int. J. Dev. Biol.* 51, 167–172. <https://doi.org/10.1387/ijdb.062212ql>.
- Li, C., Lan, Y., Jiang, R., 2017. Molecular and cellular mechanisms of palate development. *J. Dent. Res.* 96, 1184–1191. <https://doi.org/10.1177/0022034517703580>.
- Li, R., Sun, Y., Chen, Z., Zheng, M., Shan, Y., Ying, X., Weng, M., Chen, Z., 2021. *The Fibroblast Growth Factor 9 (Fgf9)* Participates in palatogenesis by promoting palatal growth and elevation. *Front. Physiol.* 12, 653040. <https://doi.org/10.3389/fphys.2021.653040>.
- Macarulla-Sanz, E., Nebot-Cegarra, J., Reina-de la Torre, F., 1996. Computer-assisted stereological analysis of gastric volume during the human embryonic period. *J. Anat.* 188, 395–401.
- Maldonado, E., López, Y., Herrera, M., Martínez-Sanz, E., Martínez-Álvarez, C., Pérez-Miguelsanz, J., 2018. Craniofacial structure alterations of foetuses from folic acid deficient pregnant mice. *Ann. Anat.* 218, 59–68. <https://doi.org/10.1016/j.aanat.2018.02.010>.
- Martínez-Álvarez, C., Tudela, C., Pérez-Miguelsanz, J., O’Kane, S., Puerta, J., Ferguson, M.W., 2000. Medial edge epithelial cell fate during palatal fusion. *Dev. Biol.* 220, 343–357. <https://doi.org/10.1006/dbio.2000.9644>.
- Martínez-Sanz, E., Del Río, A., Barrio, C., Murillo, J., Maldonado, E., Garcillán, B., Amorós, M., Fuerte, T., Fernández, A., Trinidad, E., Rabadán, M.A., López, Y., Martínez, M.L., Martínez-Álvarez, C., 2008. Alteration of medial-edge epithelium cell adhesion in two *Tgf-beta3* null mouse strains. *Differentiation* 76, 417–430. <https://doi.org/10.1111/j.1432-0436.2007.00226.x>.
- Mayhew, T.M., 1992. A review of recent advances in stereology for quantifying neural structure. *J. Neurocytol.* 21, 313–328. <https://doi.org/10.1007/BF01191700>.
- Meng, L., Bian, Z., Torensmas, R., Von den Hoff, J.W., 2009. Biological mechanisms in palatogenesis and cleft palate. *J. Dent. Res.* 88, 22–33. <https://doi.org/10.1177/0022034508327868>.
- Murray, S.A., Oram, K.F., Gridley, T., 2007. Multiple functions of *Snail* family genes during palate development in mice. *Development* 134, 1789–1797. <https://doi.org/10.1242/dev.02837>.
- Nagasaka, A., Sakiyama, K., Bando, Y., Yamamoto, M., Abe, S., Amano, O., 2022. Spatiotemporal gene expression regions along the anterior-posterior axis in mouse embryos before and after palatal elevation. *Int. J. Mol. Sci.* 23, 5160. <https://doi.org/10.3390/ijms23095160>.
- Nebot-Cegarra, J., Maraculla-Sanz, E., Reina-De La Torre, F., 1999. Factors involved in the 'rotation' of the human embryonic stomach around its longitudinal axis: computer-assisted morphometric analysis. *J. Anat.* 194, 61–69. <https://doi.org/10.1046/j.1469-7580.1999.19410061.x>.
- Nohara, A., Owaki, N., Matsubayashi, J., Katsube, M., Imai, H., Yoneyama, A., Yamada, S., Kanahashi, T., Takakuwa, T., 2022. Morphometric analysis of secondary palate development in human embryos. *J. Anat.* 241, 1287–1302. <https://doi.org/10.1111/joa.13745>.

- Okano, J., Suzuki, S., Shiota, K., 2006. Regional heterogeneity in the developing palate: morphological and molecular evidence for normal and abnormal palatogenesis. *Congenit. Anom.* 46, 49–54. <https://doi.org/10.1111/j.1741-4520.2006.00103.x>.
- O'Rahilly, R., Müller, F., 1987. *Developmental Stages in Human Embryos: Including A Revision of Streeter's Horizons and A Survey of the Carnegie Collection*. Carnegie Institution of Washington 637, Washington.
- O'Rahilly, R., Müller, F., 1992. *Human Embryology and Teratology*. John Wiley and Sons Inc, New York.
- O'Rahilly, R., Müller, F., 2010. Developmental stages in human embryos: revised and new measurements. *Cells Tissues Organs* 192, 73–84. <https://doi.org/10.1159/000289817>.
- Pérez-Hettinga, A.C., González-Carrera, M.C., Duque-García, E.A., Mora-Díaz, I.I., Rengifo-Reina, H.A., Díaz-Báez, D., 2025. Prevalence and incidence of cleft lip and/or palate in Europe: a scoping review and meta-analysis. *Cleft Palate Craniofac. J.* 18, 10556656241304210. <https://doi.org/10.1177/10556656241304210>.
- Pratt, R.M., Goggins, J.F., Wilk, A.L., King, C.T., 1973. Acid mucopolysaccharide synthesis in the secondary palate of the developing rat at the time of rotation and fusion. *Dev. Biol.* 32, 230–237. [https://doi.org/10.1016/0012-1606\(73\)90237-6](https://doi.org/10.1016/0012-1606(73)90237-6).
- Que, Y.C., Liu, Q.Q., Xu, Y.C., 2024. Progress and challenges in human developmental cell atlas. *Yi Chuan* 46, 760–778. <https://doi.org/10.16288/j.ycz.24-153>.
- Radlanski, R.J., 2003. Prenatal craniofacial morphogenesis: four-dimensional visualization of morphogenetic processes. *Orthod. Craniofac. Res.* 6 (1), 89–94. <https://doi.org/10.1034/j.1600-0544.2003.240.x>.
- Radlanski, R.J., Emmerich, S., Renz, H., 2004. Prenatal morphogenesis of the human incisive canal. *Anat. Embryol.* 208, 265–271. <https://doi.org/10.1007/s00429-004-0389-y>.
- Richardson, R.J., Hammond, N.L., Coulombe, P.A., Saloranta, C., Nousiainen, H.O., Salonen, R., Berry, A., Hanley, N., Headon, D., Karikoski, R., Dixon, M.J., 2014. Periderm prevents pathological epithelial adhesions during embryogenesis. *J. Clin. Invest.* 124, 3891–3900. <https://doi.org/10.1172/JCI71946>.
- Rousian, M., Koning, A.H., van Oppenraaij, R.H., Hop, W.C., Verwoerd-Dikkeboom, C. M., van der Spek, P.J., Exalto, N., Steegers, E.A., 2010. An innovative virtual reality technique for automated human embryonic volume measurements. *Hum. Reprod.* 25, 2210–2216. <https://doi.org/10.1093/humrep/deq175>.
- Schupbach, P.M., Chamberlain, J.G., Schroeder, H.E., 1983. Development of the secondary palate in the rat: a scanning electron microscopic study. *J. Craniofacial Genet. Dev. Biol.* 3, 159–177.
- Seelan, R.S., Mukhopadhyay, P., Pisano, M.M., Greene, R.M., 2012. Developmental epigenetics of the murine secondary palate. *ILAR J.* 53, 240–252. <https://doi.org/10.1093/ilar.53.3-4.240>.
- Shi, Z., Wen, H., Leng, J., Wang, J., Wang, Y., Luo, D., Chen, Z., Qin, Y., Liang, M., Tan, Y., Li, S., 2023. Cleft palate in fetuses: feasibility of early diagnosis by Crystal and Realistic Vue rendering 3D ultrasound technology in the first trimester. *Front. Pediatr.* 11, 1199965. <https://doi.org/10.3389/fped.2023.1199965>.
- Snyder-Warwick, A.K., Perlyn, C.A., Pan, J., Yu, K., Zhang, L., Ornitz, D.M., 2010. Analysis of a gain-of-function FGFR2 Crouzon mutation provides evidence of loss of function activity in the etiology of cleft palate. *Proc. Natl. Acad. Sci. USA* 107, 2515–2520. <https://doi.org/10.1073/pnas.0913985107>.
- Steding, G., Jian, Y., 2010. The origin and early development of the nasal septum in human embryos. *Ann. Anat.* 192, 82–85. <https://doi.org/10.1016/j.aanat.2010.01.002>.
- Thyagarajan, T., Totev, S., Danton, M.J., Kulkarni, A.B., 2003. Genetically altered mouse models: the good, the bad, and the ugly. *Crit. Rev. Oral. Biol. Med.* 14, 154–174. <https://doi.org/10.1177/154411130301400302>.
- Twigg, S.R., Wilkie, A.O., 2015. New insights into craniofacial malformations. *Hum. Mol. Genet.* 24, R50–R59. <https://doi.org/10.1093/hmg/ddv228>.
- Vaziri Sani, F., Hallberg, K., Harfe, B.D., McMahon, A.P., Linde, A., Gritti-Linde, A., 2005. Fate-mapping of the epithelial seam during palatal fusion rules out epithelial-mesenchymal transformation. *Dev. Biol.* 285, 490–495. <https://doi.org/10.1016/j.ydbio.2005.07.027>.
- Vermeij-Keers, C., Mazzola, R.F., Van der Meulen, J.C., Strickler, M., 1983. Cerebro-craniofacial and craniofacial malformations: an embryological analysis. *Cleft Palate J.* 20, 128–145.
- Walker, B.E., Fraser, F.C., 1956. Closure of secondary palate in the three strains of mice. *J. Embryol. Exp. Morphol.* 4, 176–189.
- Wang, X., Li, C., Zhu, Z., Yuan, L., Chan, W.Y., Sha, O., 2020. Extracellular matrix remodeling during palate development. *Organogenesis* 16, 43–60. <https://doi.org/10.1080/15476278.2020.1735239>.
- Weingärtner, J., Maile, S., Proff, P., Reicheneder, C., Fanghänel, J., Gedrange, T., 2007. Secondary palatal closure in rats in association with relative maternofetal levels of folic acid, vitamin B12, and homocysteine. *Ann. Anat.* 189, 229–233. <https://doi.org/10.1016/j.aanat.2006.10.006>.
- Welsh, I.C., Feiler, M.E., Lipman, D., Mormile, I., Hansen, K., Percival, C.J., 2025. Palatal segment contributions to midfacial anterior-posterior growth. *J. Anat.* 247, 52–67. <https://doi.org/10.1111/joa.14222>.
- Wilkie, A.O., Morriss-Kay, G.M., 2001. Genetics of craniofacial development and malformation. *Nat. Rev. Genet.* 2, 458–468. <https://doi.org/10.1038/35076601>.
- Won, H.J., Kim, J.W., Won, H.S., Shin, J.O., 2023. Gene regulatory networks and signaling pathways in palatogenesis and cleft palate: a comprehensive review. *Cells* 12, 1954. <https://doi.org/10.3390/cells12151954>.
- Wragg, L.E., Diewert, V.M., Klein, M., 1972. Spatial relations in the oral cavity and the mechanism of secondary palate closure in the rat. *Arch. Oral. Biol.* 17, 683–690. [https://doi.org/10.1016/0003-9969\(72\)90194-x](https://doi.org/10.1016/0003-9969(72)90194-x).
- Yasuda, Y., Fujimoto, T., 1986. The anterior half of mouse palatal shelf is elevated by a remodeling movement. *Acta Anat.* 125, 37–41. <https://doi.org/10.1159/000146134>.
- Yonemitsu, M.A., Lin, T.Y., Yu, K., 2020. Hyaluronic acid is required for palatal shelf movement and its interaction with the tongue during palatal shelf elevation. *Dev. Biol.* 457, 57–68. <https://doi.org/10.1016/j.ydbio.2019.09.004>.
- Yoon, H., Chung, I.S., Seol, E.Y., Park, B.Y., Park, H.W., 2000. Development of the lip and palate in staged human embryos and early fetuses. *Yonsei Med. J.* 41, 477–484. <https://doi.org/10.3349/ymj.2000.41.4.477>.
- Yoshioka, H., Ramakrishnan, S.S., Shim, J., Suzuki, A., Iwata, J., 2021. Excessive *all-trans* retinoic acid inhibits cell proliferation through upregulated microRNA-4680-3p in cultured human palate cells. *Front. Cell. Dev. Biol.* 9, 618876. <https://doi.org/10.3389/fcell.2021.618876>.
- Young, N.M., Wat, S., Diewert, V.M., Browder, L.W., Hallgrímsson, B., 2007. Comparative morphometrics embryonic facial morphogenesis: implications for cleft lip etiology. *Anat. Rec.* 290, 123–139. <https://doi.org/10.1002/ar.20415>.
- Yu, K., Ornitz, D.M., 2011. Histomorphological study of palatal shelf elevation during murine secondary palate formation. *Dev. Dynam.* 240, 1737–1744. <https://doi.org/10.1002/dvdy.22670>.
- Yu, K., Yonemitsu, M.A., 2019. *in vitro* analysis of palatal shelf elevation during secondary palate formation. *Anat. Rec.* 302, 1594–1604. <https://doi.org/10.1002/ar.24076>.