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Article

Topography of the inferior alveolar nerve in human embryos and fetuses. An histomorphological study.

Sergey Lvovich Kabak, [1] Natallia Victorovna Zhuravleva[1] & Yuliya Michailovna Melnichenko.[1]

Affiliations: [1]Human Morphology Department, Belarusian State Medical University, Minsk, Belarus.

Corresponding author: Yuliya Michailovna Melnichenko. Human Morphology Department, Belarusian State Medical University, Dzerzhinskogo Avenue 83, Minsk, Belarus. Phone: (375)291637867. Email: mjm1980@yandex.ru

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Abstract: The aim of this study is to establish the position of the inferior alveolar nerve in relation to the Meckel's cartilage, the anlage of the mandibular body and primordia of the teeth, and also to trace the change in nerve trunk structure in the human prenatal ontogenesis. Serial sections (20µm) from thirty-two 6-12 weeks-old entire human embryos and serial sections (10µm) of six mandibles of 13-20 weeks-old human fetuses without developmental abnormalities were studied. Histological sections were impregnated with silver nitrate according to Bilshovsky-Buke and stained with hematoxylin and eosin. During embryonic development, the number of branches of the inferior alveolar nerve increases and its fascicular structure changes. In conclusion, the architecture of intraosseous canals in the body of the mandible, as well as the location of the foramina, is predetermined by the course and pattern of the vessel/nerve branching in the mandibular arch, even before the formation of bony trabeculae. Particularly, the formation of the incisive canal of the mandible can be explained by the presence of the incisive nerve as the extension of the inferior alveolar nerve. It has also been established that Meckel's cartilage does not participate in mandibular canal morphogenesis.

Keywords: Mandibular nerve; mandible; embryo research.

Conflict of interests: The authors declare no conflict of interest.

Ethics approval: The present study was approved by the Medical Ethics Committee of the Belarusian State Medical University, Minsk, Belarus (Protocol No.: 2017/2).

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INTRODUCTION.

The inferior alveolar nerve is one of the terminal branches of the mandibular nerve. After exiting the cranium through foramen ovale, it lies posterior and lateral to lingual nerve; then it enters the mandibular canal, in which it is accompanied by the artery and vein of the same name, as well as lymphatic vessels.[1] The branches of the inferior alveolar nerve are the mylohyoid nerve and the mental nerve that along with the dental branches form the inferior dental plexus. The mylohyoid nerve and the mental nerve are located outside the mandible, whereas the dental branches originate in the bone.

According to the literature there are several types of branching of the inferior alveolar nerve inside the bone.[2] In most of the cases it is one thick trunk, which splits into two terminal branches in the region of the mental foramen: the mental and incisive nerves. The branches innervating lower molars and the second premolar start from the inferior alveolar nerve before branching into its terminal subdivisions. Mandibular incisors receive innervation from the incisive nerve, or the incisive plexus can be formed instead. In many cases, the terminal branches of the inferior alveolar nerve can be surrounded by their own perineurium and lie separately from the beginning of the mandibular canal.[3] In such case, the incisive nerve, consisting of three or four bundles of nerve fibers, spreads its branches to dental alveoli.

Various aspects of the human prenatal morphogenesis of the mandible, such as histogenesis of bone tissue and the role of Meckel's cartilage, have been previously discussed by some authors.[4-6] Evidence about the position of the inferior alveolar nerve inside the mandible in the process of its embryonic development is scarce.

These data are necessary to explain the origin of an additional mental foramen, as well as the presence of individual alterations in anatomy of the mandibular canal containing the inferior alveolar nerve. This canal can bifurcate or have lateral branches that are well visualized *in vivo* when studied with the use of modern radiographic techniques.[7,8]

The aim of the study is to establish the position of the inferior alveolar nerve in relation to the Meckel's cartilage, the anlage of the mandibular body and primordia of the teeth, and to trace the change in nerve trunk structure in human prenatal ontogenesis.

MATERIALS AND METHODS.

Serial sections (20µm) from 32 entire human embryos and fetuses without developmental abnormalities (6-12 weeks of gestation, 13-70mm of crown-rump length [CRL]) impregnated with silver nitrate according to Bilshovsky-Buke and stained with hematoxylin and eosin were studied.

All samples were taken from the collection of the Belarusian State Medical University. In addition, mandibles from six fetuses without developmental abnormalities (13-20 weeks of gestation) were cut into 3-4 pieces which were used for making serial transverse, frontal and sagittal histological sections (10µm) stained with hematoxylin and eosin.

Age of human embryos and fetuses was determined by the crown-rump length or obstetric anamnesis (Table 1).

The present study was approved by the Medical Ethics Committee of the Belarusian State Medical University, Minsk, Belarus (Protocol No.: 2017/2).

Table 1. Embryos and fetuses used for this study.

Size (CRL, mm)	Embryonal age (days or weeks)	Number of embryos
13	44 days	1
17	44 days	6
22	51 days	4
23	54 days	7
24	54 days	5
27	56,5 days	3
33	10+1 weeks	3
34	10+2 weeks	3
-	13 weeks ◊◊	1
-	14 weeks◊◊	1
-	16 weeks ◊◊	1
-	20 weeks◊◊	3

◊◊ Gestational age.

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RESULTS.

In 13mm (CRL) human embryos, a Meckel's cartilage anlage is found in the form of a small condensation of mesenchymal cells within the mandibular processes (Figure 1A). The anlage is located near the inferior alveolar nerve, which is almost twice as large as its cross-sectional area and is a direct continuation of the mandibular nerve, originating from the trigeminal ganglion. The ganglion is a cluster of rounded neurons near the ventral surface of the neural tube. The branches exiting the ganglion lie in the same plane and can be clearly identified in the direction of their course.

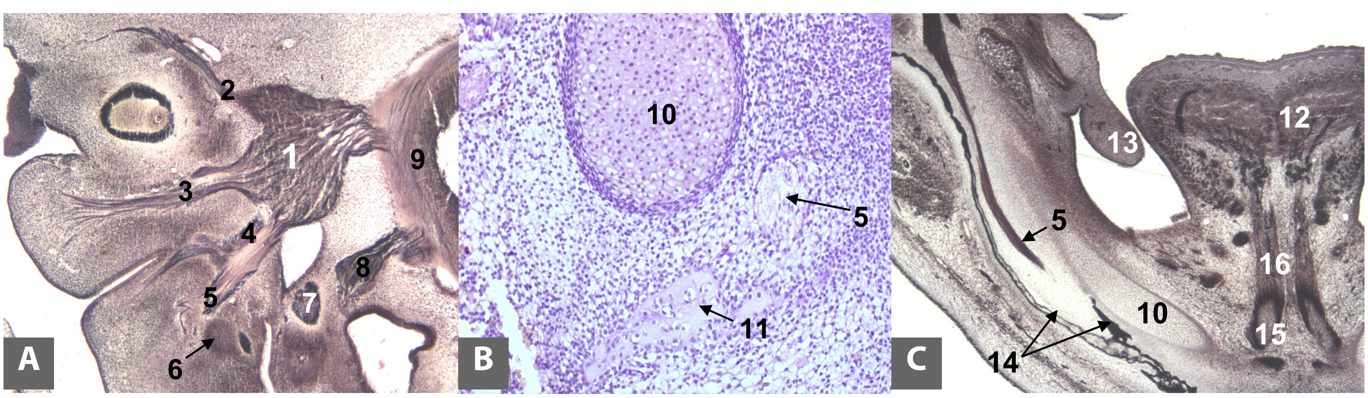
The mandibular nerve is the largest branch of the trigeminal nerve and consists of thin, convoluted, loosely packed nerve fibers forming one bundle. The distal end of the mandibular nerve is divided into the inferior alveolar and the lingual nerve. The inferior alveolar nerve is composed of loosely arranged nerve fibers and can be seen in the mandibular process to a considerable extent. Nerve divisions into secondary branches have not been identified.

In 17mm (CRL) human embryos, outside of Meckel's cartilage within mesenchyme of the mandibular processes, the primary center of ossification can be identified (Figure 1B). Initially, it has the shape of a plate, which is located laterally from the Meckel's cartilage and is separated from it by the inferior alveolar nerve. Subsequently, the process of intramembranous ossification spreads radially, and at the end of the embryonic and early fetal periods the anlage of mandibular body consists of lateral and medial bone plates. They partially merge, resulting in the formation of a Y-shaped structure, which covers the lower part of the inferior alveolar nerve below by forming a groove.

In 22-24mm (CRL) human embryos, the terminal branches of the inferior alveolar nerve are revealed. They are located on both sides of the lateral bone plate. Nerve fibers within the mental nerve are convoluted, loosely packed, but run parallel to each other. In one embryo with a CRL of 24mm the incisive nerve consists of loosely connected convoluted nerve fibers. In another embryo (23-mm CRL) it is a compact trunk that divides into lateral branches from several nerve fibers towards the dental lamina. In an embryo with a CRL of 27mm, the inferior alveolar nerve, lying between the two bone plates (outside the Meckel's cartilage), is characterized by a compact arrangement of nerve fibers that are forming one bundle (Figure 1C). Lateral branches of the nerve are not revealed.

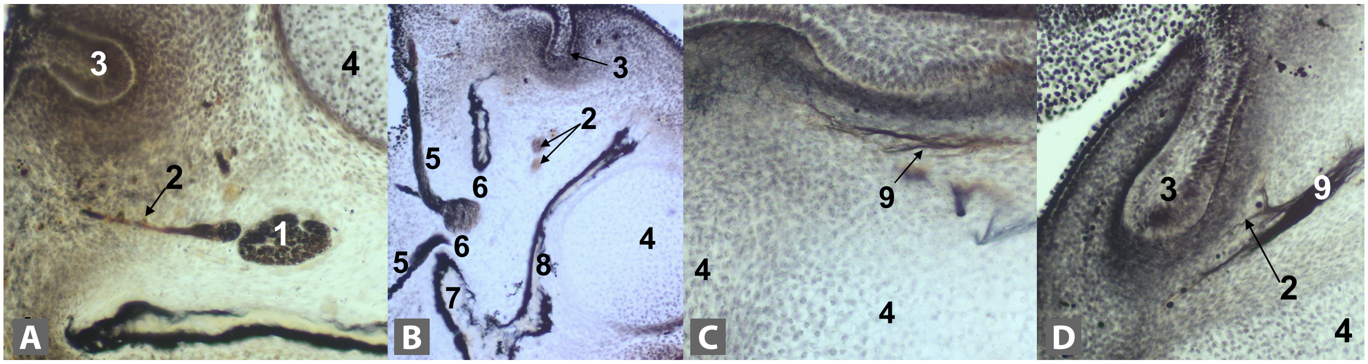
In the fetuses with a CRL of 33-34mm the lateral dental branches extending from the inferior alveolar nerve first run parallel to the main trunk and then follow towards the tooth germ (Figure 2A). The lateral plate retains a wide gap (the area of the future mental foramen). At this point, the major part of the nerve fibers of the inferior alveolar nerve are turned

Figure 1. Topography of trigeminal nerve and its branches in human embryos of 13mm CRL (A), 17mm CRL (B) and 27mm CRL (C).



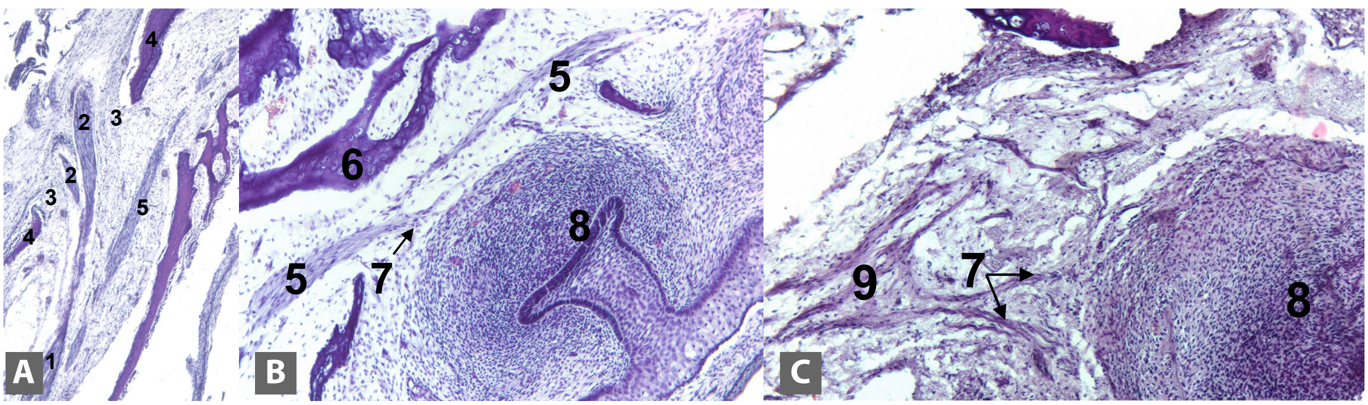
1. Trigeminal ganglion. 2. Ophthalmic nerve. 3. Maxillary nerve. 4. Mandibular nerve. 5. Inferior alveolar nerve. 6. Meckel's cartilage anlage. 7. Facial nerve. 8. Vestibulocochlear nerve. 9. Neural tube. 10. Meckel's cartilage. 11. Primary center of ossification. 12. Tongue. 13. Lateral palatine shelf. 14. Lateral and medial plates of mandibular body anlage. 15. Hyoid bone anlage. 16. Hyoglossus muscle.
Sagittal (A) and frontal (C) sections impregnated with silver nitrate according to Bilshovsky-Buke.
Sagittal section (B) stained with H&E.
Magnification, (A) x100; (B) x200; (C) x40.

Figure 2. Inferior alveolar nerve and its branches in human embryo of 33mm CRL at the level of the posterior tooth germ (A), the mental foramen (B), the mental symphysis (C) and at the level of the anterior tooth germ (D).



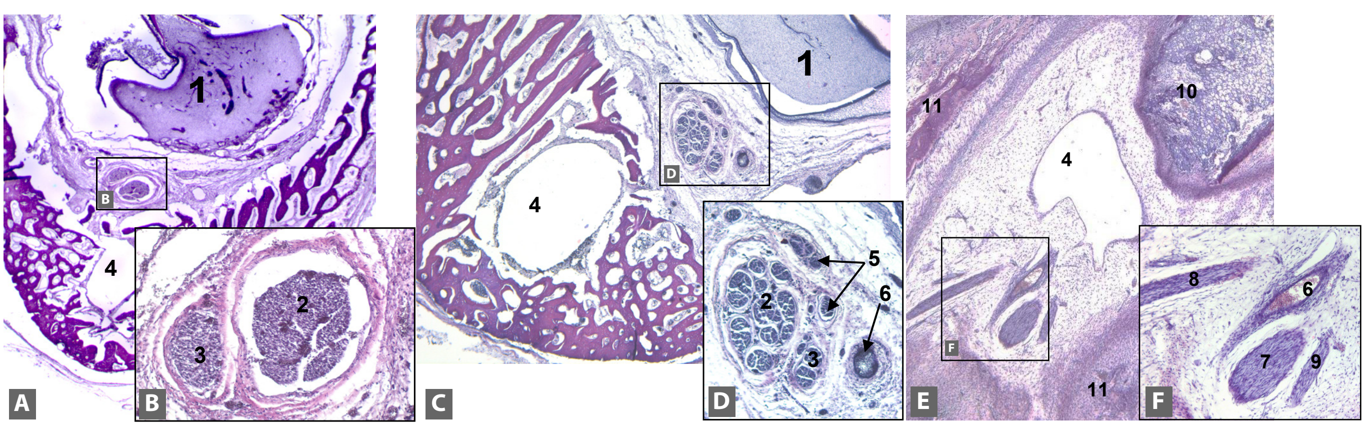
1. Inferior alveolar nerve. 2. Dental branch. 3. Tooth germ. 4. Meckel's cartilage. 5. Mental nerve branch. 6. Mental foramen. 7. Lateral plate of mandibular body. 8. Medial plate of mandibular body. 9. Incisive nerve.
Sagittal sections impregnated with silver nitrate according to Bilshovsky-Buke.
Magnification, (A-D) x200.

Figure 3. Topography of the inferior alveolar nerve and its branches in human fetuses of 16 weeks (A) and 13 weeks (B, C) of gestation.



1. Inferior alveolar nerve. 2. Mental nerve branches. 3. Mental foramen. 4. Lateral plate of mandibular body anlage. 5. Incisive nerve. 6. Bone trabecula. 7. Dental branch. 8. Tooth germ. 9. Dental plexus.
Sagittal sections of mandible, stained with H&E.
Magnification, (A) x40; (B,C) x100.

Figure 4. Neurovascular bundle of mandible in 20-week-old fetuses at the level of posterior tooth germs (A-D) and mandibular foramen (E,F).



1. Tooth germ. 2. Mental nerve. 3. Incisive nerve. 4. Serre's' vein in the separate osseous canal. 5. Dental branches of the inferior alveolar nerve. 6. Inferior alveolar artery.7. Inferior alveolar nerve. 8. Lingual nerve. 9. Mylohyoid nerve. 10. Mandibular condylar cartilage. 11. Membranous part of mandible.
Sagittal sections of mandible, stained with H&E.
Magnification, (A,C,E) x40; (B,D,F) x100.

to the side in the form of the mental nerve and divide into branches that are outside the lateral bone plate (Figure 2B).

In one of the embryos (34-mm CRL), the mental nerve division occurred before it passed through the described above gap. On the mesial side of the bone plate the incisive nerve can be observed. This nerve is an extension of the inferior alveolar nerve in its course that can be traced to the midline of the lower alveolar arch in the form of loosely arranged nerve fibers (Figure 2C). Nerve fibers that originate from the incisive nerve and follow to the anterior teeth are also revealed on this stage of development (Figure 2D).

In the fetuses of 13-16 weeks in utero, the inferior alveolar nerve becomes a compact nerve trunk, which splits at its mesial end into the mental and incisive nerve (Figure 3A). The nerve itself and its branches lie freely in the loose mesenchyme at a considerable distance from the bone trabeculae. The mental nerve emerges on the outer side of the lateral bone plate through a wide mental foramen as one trunk or several branches. The incisive nerve is presented by one trunk consisting of several nerve bundles with dental branches to tooth germs. Nerve bundles lie parallel or form a plexus (Figures 3B and 3C).

Dimensions of mandibular body increase in the fetal period parallel to a partial involution of Meckel's cartilage. The mandibular body consists of mesiodistally oriented trabeculae composing the woven bone. At the same time, the body of the mandible retains the appearance of a trough-like gutter containing a neurovascular bundle located at the bottom, and tooth germs at the top. The canal of the mandible is not present as such because bone tissue surrounds the inferior alveolar nerve, artery and vein on only three sides. The neurovascular bundle of a 20-week-old fetus molar is located closer to the alveolar ridge than to the base of the mandible and the distance from the inferior alveolar nerve to the buccal surface is approximately twice as long as from the lingual surface of the body of the mandible.

At this stage of development, the main connective tissue components of the inferior alveolar nerve are identified. On the surface, it is covered by epineurium that extends without a sharp boundary into connective tissue around the other elements of the neurovascular bundle. The nerve consists of two main components (primary bundles) (Figure 4A and 4B). Two bundles are in contact with each other by the perineurium. In turn, each of primary bundles splits into secondary bundles separated by layers of connective tissue. The larger primary bundle is located on the buccal side and has a multi-bundle structure.

At the site of localization, it is identified as the mental nerve. The dental branch of the inferior alveolar nerve localized on the lingual side of the nerve, presents less bundle structure.

We observed one outstanding case. A few dental branches were located at the level of the molar tooth germ adjacent to the primary bundle of the inferior alveolar nerve. They were determined within the composition of the neurovascular bundle located between the two plates of the mandibular body within the general epineurium. Dental branches were presented as separate bundles of nerve fibers surrounded by perineurium (Figure 4C and 4D).

In 20-week-old human fetuses, in the lower part of the groove between the lateral and medial plates forming the mandibular body, a transversely oriented bony trabecula was identified. Due to this trabecula, the intraosseous canal containing a wide thin-walled (venous) vessel is getting separated (Figure 4C).

In 20-week-old fetuses the mandibular foramen is not defined as a closed bone ring. The location of its future position is approached in the contact area of the proximal end of condyle and the membranous part of the lower jaw. The inferior alveolar nerve, artery and vein are positioned in that area at a great distance from each other (Figures 4E and 4F). Above these structures, separate from the neurovascular bundle, there is another large venous vessel. That represents a continuation of the vein (Serre's vein) located in a separate intraosseous canal at the bottom of the groove between the lateral and medial bone plates constituting the body of the mandible. The inferior alveolar nerve's branch, the mylohyoid nerve, is located in the future mandibular foramen region, slightly higher and laterally to the nerve. The lingual nerve is detected on its medial side.

DISCUSSION.

In human embryos in the early stages of prenatal development, thick nerve trunks are present in undifferentiated mesenchymal mandibular processes. We have found that the anlage of Meckel's cartilage, which is a primary supporting structure of the mandible and a crucial element in initiation of further development of the mandibular body, appears after the division of mandibular nerve into inferior alveolar nerve and lingual nerve. Although the Meckel's cartilage was previously supposed to serve as a template for bone deposition and to control endochondral and intramembranous bone formation during mammalian mandibular development, the phenotypes of mutant mice do not directly support this hypothesis.[9]

During embryonic development, the number of branches of the inferior alveolar nerve increases, and its fascicular structure changes. Beginning from the end of the embryonic period of development, the inferior alveolar nerve situated in a wide groove between two bone plates from the lateral side of the Meckel's cartilage looks like a thick trunk without lateral branches. The mental nerve as its direct extension appears. At the beginning of the fetal period separate nerve fibers heading into the tooth germs are revealed.

In 20-week-old fetuses, at the level of the first molar the inferior alveolar nerve is somewhat closer to the alveolar ridge than to the base of the mandible. In contrast, in adults, when mandibular teeth are present, the canal containing the inferior alveolar nerve is located closer to the base of the mandible than to the alveolar ridge.[10] In the fetal period of development, the distance from the inferior alveolar nerve to the buccal surface is approximately twice as long as from the lingual surface of the body of the mandible. Adults have a similar asymmetry in the location of the mandible canal containing the inferior alveolar nerve.[11,12]

In 20-week-old human fetuses, the inferior alveolar nerve usually appears as one compact trunk consisting of two unequal parts. Each of these adjacent parts is surrounded by connective tissue. Some cross sections have small bundles of nerve fibers (dental branches) lying at certain distance from the main nerve trunk. Near the mental foramen the inferior alveolar nerve separates into a larger part (the mental nerve) and a smaller part that goes to the anterior teeth. Due to this fact, the cross-sectional area of the nerve is becoming considerably reduced. Branching of the mental nerve can take place on the inner side with respect to the lateral bone plate. Probably, in such cases an accessory mental foramen can subsequently be formed around one of the branches of the mental nerve.

The type of the inferior alveolar nerve branching observed in fetuses in our study was described as the most frequent in cadaver studies in adults.[3,13,14] The mental nerve occupies the buccal two-thirds of the inferior alveolar nerve, with the rest filled with the incisive nerve of the mandible.[8] According to Hur *et al.,*[14] the lingual one-third of the inferior alveolar nerve contains several fractions (bundles of nerve fibers). The superior buccal portion innervates molars; the superior portion, the premolars; and the upper and lower lingual portions innervate canine and incisors. In our study, the lingual part of the inferior alveolar nerve of fetuses had several bundles of nerve fibers, but it was not possible to trace their paths accurately. It can only be assumed that the bundles lying somewhat apart are dental branches that extend from the trunk of the inferior alveolar nerve and then proceed to the tooth germs.

Formally, the incisive nerve is one of the dental branches of the inferior alveolar nerve. It is detected in human embryos even before the appearance of the dental lamina, *i.e.* earlier than other dental branches. This gives grounds to consider it as an independent anatomical structure intended for innervation of the anterior teeth. In adults the incisive nerve is in the same canal in the anterior part of the body of the mandible. The incisive canal and nerve are not identified when the nerve passes through the gaps between bone trabeculae while running to the anterior teeth. The nerve plexus providing an alternative to the incisive nerve innervation of the canine and incisors, represents individual anatomical variability and is formed at the early stages of embryonic development, even before the formation of bone trabeculae in that area.

Chavez *et al.,*[15] described several individual foramina on the lingual surface of the mandibular ramus in human fetuses, which communicated with canals leading to three groups of teeth: incisors, canines/premolars, and molars. It was assumed that those foramina and canals contain independent branches of the inferior alveolar nerve. The authors believed that in prenatal ontogenesis, due to the rapid remodeling of the bone tissue, canals and foramina join together, and nerves merge.

The assumption of the existence of three independent dental branches of the inferior alveolar nerve in human fetuses is widely used to explain the possible mechanism of the additional mandibular canal formation in adults. In our opinion, location of foramina and canals in the body of the mandible is genetically predetermined and related to blood vessel and nerve branching in the mandibular processes at the early stages of prenatal ontogenesis. In our study we did not find three independent branches passing to the teeth in embryos and fetuses in the first half of pregnancy.

Moreover, the general trend of the peripheral nervous system genesis was shifting to the successive appearance of new smaller branches, rather than their fusion with each other. The formation of bone tissue in the mandibular body occurs in the fetal period of development of human embryos around already existing vascular and nerve structures.

Fetuses in the first half of pregnancy have the inferior alveolar nerve located not in the canal, but in the groove, which is surrounded by bone on three sides. However, starting at the gestational age of 20 weeks, a closed intraosseous canal containing a large venous vessel was detected in the mandibular body. This is the Serres canal with a vein of the same name, which is present in 60-73.7% of human fetuses, newborns and children under 13 years of age. The vein starts in the mental foramen area and it is not a tributary of the inferior alveolar vein. According to Rodríguez-Vásquez *et al.,*[16] the vein of Serres drains blood from the base of mandible, while the inferior alveolar vein receives blood from the alveolar part of the developing bone.

CONCLUSION.

The architecture of intraosseous canals in the body of the mandible, as well as the location of the foramina, is predetermined by the course and pattern of the vessel/nerve branching in the mandibular arch, even before the formation of bony trabeculae. Particularly, the formation of the incisive canal of the mandible can be explained by the presence of the incisive nerve as the extension of the inferior alveolar nerve. It has also been established that Meckel's cartilage does not participate in mandibular canal morphogenesis.

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