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Development of the dynamic structure (force lines) of the middle ear ossicles in human foetuses

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Summary. Objectives: To study the ontogenic development of the organisation of the human middle ear ossicles structure. Material and methods: 46 human temporal bones of ages varying from 32 days post-conception to newborns. Results: The development of the structural organisation of the malleus begins at 16 weeks via two cortical fascicles situated in the neck; at 21 weeks they extend towards the head, at 23 weeks to the lateral process and at 24 weeks to the handle. In the handle, the force lines are transmitted via three cardinal fascicles, two of them via the cortical fascicle and one via the centre, which starts after 29 weeks' development and is consolidated after 31 weeks.

In the incus the force lines start at 16 weeks via two cortical fascicles situated in the long process, which progressively extend in a rostro-caudal direction between 17 and 20 weeks. At 21 weeks they occupy the whole extension of the long process and at 22 weeks the fusion of both cortical fascicles begins. From 30 weeks onwards it is strengthened by the crossing of bone trabeculae from one cortical to another. Two fascicles come out of the incus body, surrounding the medullary cavity and going in the direction of the short process.

In the beginning, the stapes have two cortical fascicles in their crura. The remodelling process makes the internal cortical fascicle disappear and after 31 weeks all the force lines run through the external cortical fascicle. The tympanic membrane of the stapes footplate undergoes a remodelling process and after 28 weeks bony trabeculae are deposited.

In newborns (40 weeks), the ossicles' structure is cavitary and has not been completed. The fan-shaped trabecular fascicle, which starts in the articular facets of the malleus and the incus, still has to develop.

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Introduction

There are two theories about the origin of middle ear ossicles: the first (classical) theory defends that the malleus and the incus derive from the Meckel's cartilage (first pharyngeal arch) and the stapes from the Reichert's cartilage (second pharyngeal arch) (reviewed by Bast and Anson, 1949; Richany et al., 1954). The second (alternative) theory suggests that the head of the malleus and the body of the incus originate in the first arch, whilst the handle of the malleus, the long process of the incus and the major portion of the stapes originate in the second arch (Hough, 1963; reviewed by Ars, 1989 and Whyte et al., 2003). In both theories the labyrinthine side of the stapes footplate originates from the mesenchyme of the auditory capsule.

Studies are currently being conducted on how the origin of the skeletal components of the middle ear derives from the cranial neural crest. The cells migrate in diffuse currents through the cranial mesenchyme to reach the pharyngeal arches. Studies from Mallo (2001) indicate a very marked specificity in the relationship between the origins of the neural crest in the rhombomere, and its final destination within the pharyngeal arches and the gene expression.

The first outline to appear is the stapes in 7 mm embryos, and next those of the malleus and incus appear in 10 mm embryos (Hanson and Anson, 1962; Anson and Bast, 1959a,b; Louryan, 1993). They acquire the cartilaginous form in the 28 mm embryo (Richany et al., 1954).

Masuda et al. (1977, 1978) observed that the primordium of the stapedial lamina forms in the 16 mm embryo and the lamina is fully formed in the 35 mm embryo and fuses with the base of the stapedial annulus.

The ossification of the ossicles starts in the incus at 14 weeks via a primary centre located in the long process of the incus. The endochondral ossification in the malleus begins at 16 weeks via one single primary centre located at times at head level and at other times at the junction of the head and the neck (Hanson and Anson, 1962). The ossification in the stapes is carried out via three ossification centres located respectively in the posterior crus, base of the footplate and anterior crus.

We have observed the appearance of these centres successively in 18-week foetuses (posterior crus) and 19-week foetuses (stapes base and anterior crus), as reported by Dass et al. (1966).

The further development of the ossicular structure was described by Richany et al. (1954), Anson and Bast (1959a,b), Bowden (1977) and Whyte et al. (2003). They observed process of redesign, reabsorption and new bone.

Olszewski (1990) found that the development of auditive ossicles in human is not completed during fetal life. Kosiagina (1979) shows the mathematical relationship of the growth of the ossicles according to age and creates some tables with which it is possible to determine the age of the foetus.

The auditory chain considered as a whole forms a system of levers that transmit vibratory mechanical energy via the force lines, from the auditory membrane to the oval window (periotic liquid).

In any dynamic system, the trabecular architecture is not organised at random as it is arranged to transmit the pressures and tractions better. This fact also occurs in the middle ear ossicles; firstly the cortical fascicles that make the ossicle rigid are organised and then the trabeculae system, which will provide maximum tensile strength as it acts as a complex system of internal props. Sarrat et al. (1992) provide striking data about the conventional histology of the adult's middle ear ossicles, classifying them as compact type bones. These authors also describe the force lines through each ossicle of the chain by means of Martins' trichrome technique.

These authors described the force transmission device of the different elements of the ossicular chain in the adult. They described the malleus as a compact type bone, whose force lines start in the articular facet via a broad fan, and which are concentrated in the neck and continue along the handle via three fascicles, two cortical ones (external and internal) and one central one. The incus is also a compact type bone and its force lines start both in the articular facet and in its body, to descend down the long process via two cortical fascicles, which interweave and end at isthmus level of the lenticular process. The stapes are the most elegant bones of the chain, with a compact head and small medullar cavities on the inside; the branches are very dense and the footplate has a reduced bone component, based on a thin plain lamina on its tympanic side and a broad layer of hyaline cartilage on its vestibular side; the force lines start at the head, go to the neck and from there they divide into equal parts to descend through the two branches to the footplate.

We have continued with this study and in the present manuscript we describe the prenatal development of these force lines.

Materials and methods

46 series of histological preparations of human embryos and foetuses have been studied (Table 1).

The material comes from medical-legal autopsies on foetuses, whose death occurred as a result of miscarriages, intrauterine death or at birth.

To age the foetuses, we have used the O'Rahilly and Müller tables (1996) which are based on different measurements (maximum length, skull-heel length, biparietal diameter, abdominal circumference and cephalic circumference) as well as body weights. These measurements have been compared with the data provided by the clinical history and by ultrasonography, when these data were available.

The whole head was secured in embryos and foetuses of less than 12 weeks' development, whilst in older ones a thorough and meticulous dissection of the complete temporal bones was performed. All the samples were fixed in 10% formol, decalcified with 2% nitric acid at 25°C. The average decalcification time varied between one and fifteen days depending on the size and thickness of the piece. After the decalcification process the acid was eliminated by washing in water.

After dehydrating the samples in increasing concentrations of alcohol, they were embedded in paraffin, cut with a Leitz microtome in series at 7 µm and stained according to the Martins' trichrome technique (1933).

Results

General aspects in the ontogeny of middle ear ossicles

Different stages are observed in the development of the middle ear ossicle structure with typical morphological characteristics that define them. In the first stage, which we have observed in the 36 day embryo, their outlines are formed via condensation of the mesenchyme of the first two pharyngeal arches. In the second precartilaginous stage (41, 42 and 44 day embryos), the mesenchymal cells are transformed into chondroblasts and in the third stage the ossicles present a cartilaginous structure. We have observed this period in the 48 day embryo and this extends until the appearance of the primary ossification centres and the start of endochondral ossification (fourth stage).

The first centre to appear is the incus centre, at 14 weeks' development and, this is located in its long process. Later, the malleus centre appears at 15 weeks at head level and finally the three stapes centres at 18 weeks in the posterior branch, and at 19 weeks in the base and anterior branch.

The exception is the anterior process of the malleus,

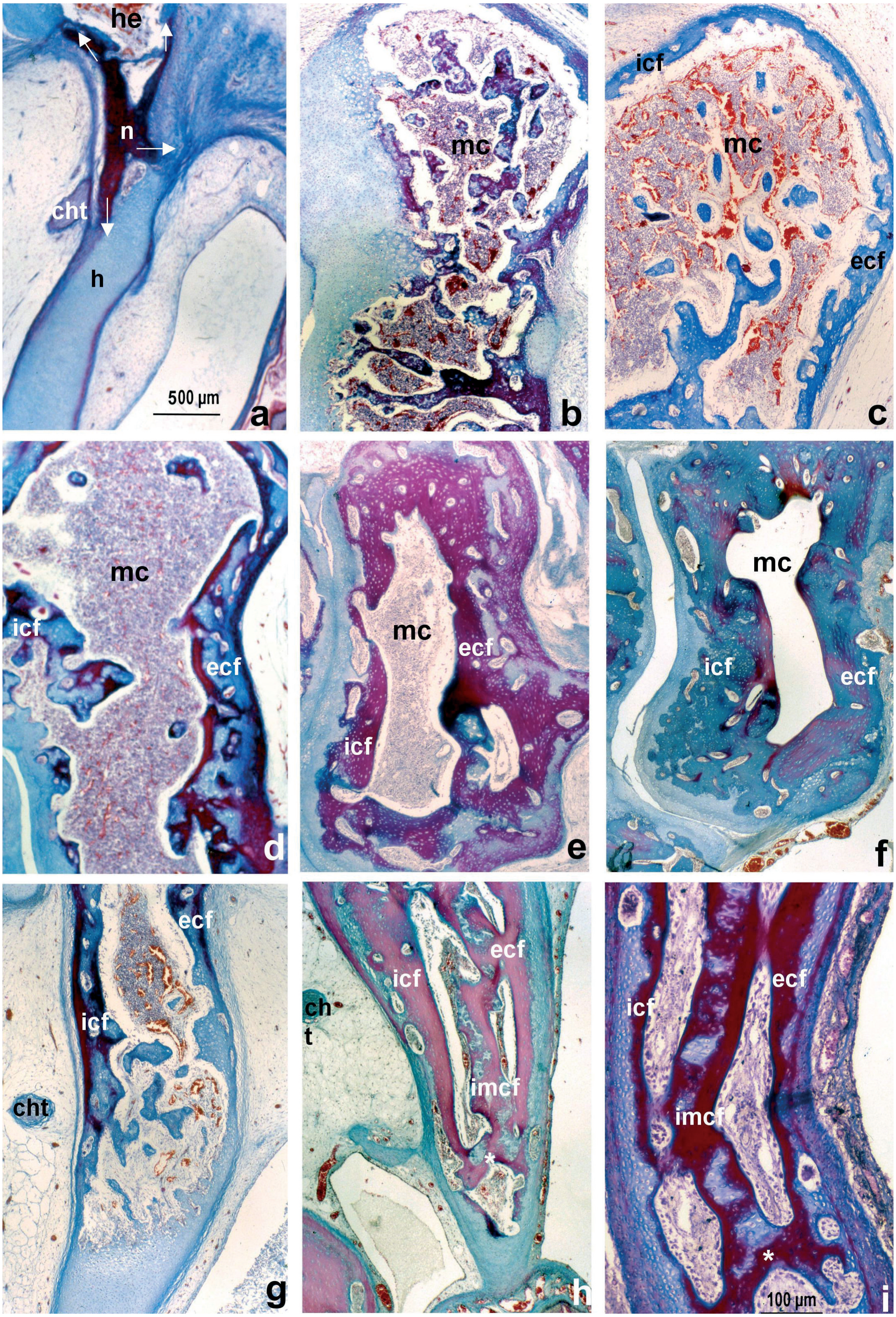

Fig. 1. Development of the dynamic structure of the malleus. **a)** 200 mm (21 weeks), **b)** 220 mm (23 weeks), **c)** 228 mm (24 weeks), **d)** 265 mm (28 weeks), **e)** 280 mm (30 weeks), **f)** 330 mm (37 weeks), **g)** 250 mm (26 weeks) **h)** 290 mm (31weeks) and **i)** 335 mm (38 weeks). HEAD. In **a)** the osseous density of the neck (n) stands out, and also how two rudimentary cortical fascicles (arrows) go from here to the head (he), as well as another two fascicles in a downward direction, which stop at the root of the handle (h)and in the lateral process, respectively (arrows). In **b)** we can see how the ossification progresses in the head of the malleus how the medullary cavity (mc) increases and the endochondral bone on the inside. In **c)** we can see how two cortical fascicles (external and internal) (ecf and icf) are observed in the head, which surround it based on a thin bone lamina. In **d, e and f)** we highlight how the two cortical fascicles are progressively consolidated, due to the apposition and extension mechanisms of the periosteal and endosteal bones, and the reduction of the medullary cavity. HANDLE. In **g)** how the internal cortical of the handle of malleus descends to near its distal end. In **h and i**) the presence of three fascicles (external, intermediate and internal) (ecf, imcf and icf) in the handle, and the establishment of bridges that join them together (*). Cth (chorda tympani). Martins' trichrome technique. Scale bar: a-h, 500 µm; i, 100 µm.

which is ossified by intramembranous mechanisms.

The periosteal collar appears in the ossicles in the fifth stage and inside these the endochondral bone. We have observed the appearance of the endochondral bone in the malleus and incus at 19 weeks and in the stapes at 20 weeks' development.

In the final stage, the bony tissue progressively extends, and the trabecular systems, which transmit the force lines, start to form.

Development of the dynamic organisation of the malleus

The first clear indication of the organisation of the force lines in the malleus has been observed in the 21-week foetus at joint level between head and neck via two not very extensive cortical fascicles (internal and external) that concentrate in the neck and then separate again into two very short fascicles, which stop at the root of the handle (internal) and in the lateral process (external) (Figs. 1a, 2a).

Between weeks 22 and 23 perichondral bony trabeculae start to appear around the medullar cavity, at head of malleus level, which are continuous with the neck cortical fascicles. These trabeculae surround the head except for the incudomalleal articular surface and adjacent areas to this and the amorphous remains of the Meckel's cartilage, which preserve their cartilaginous structure (Fig. 1b). We observe the first signs of ossification in the lateral process (Fig. 2b).

At 24 weeks the whole perimeter of the head circumference has a thin, fine, bony layer except for the articular surface (Fig. 1c). The ossification progresses through the proximal end of the handle of malleus, via two cortical fascicles, which stop halfway. The internal cortical fascicle is more extensive and thicker than the external cortical fascicle.

Between weeks 25 and 28 the endochondral bony trabeculae on the inside of the medullary cavity of the head increase and a bony lamina starts to form immediately below the articular cartilage by means of an apposition mechanism. Large areas are left unossified in this lamina, which, to begin with, is discontinuous, where the cartilage is in direct contact with the medullar cavity (Fig. 1d). In the handle, the internal cortical fascicle descends to near its distal end whilst the external cortical fascicle is still checked at its proximal end. Endochondral bony trabeculae are observed on the inside of the medullary cavity, which are arranged in two groups depending on their direction. The first are transversal to the handle axis and the second are longitudinal (Fig. 1g). Meanwhile, the external cortical fascicle, which originated in the neck, extends towards the lateral process via two small fascicles (upper and lower), which progressively surround the medullary cavity that is being formed on its inside (Fig. 2c).

At 29 and 30 weeks, the head of malleus has two bony fascicles; the first appears at articular surface level (posterior side) and goes vertically downwards, and the second on its upper edge and anterior side. Both converge at neck level (Fig. 1e). In the handle, the endochondral bony trabeculae, which have a transversal layout, are joined by apposition to the external and internal fascicles. This fact means that both fascicles increase in thickness, whilst those with a longitudinal layout fuse and form a new medial fascicle. In the lateral process, both fascicles increase in thickness by means of apposition of the endochondral bone, the upper one being thicker than the lower one represented by a fine bony layer.

At 31 weeks, the dynamic structure of the handle is comprised of three osseous fascicles, internal, medial and external, which join together. The existence of an

Table 1.

CR Length mm	Date development	Embryo - foetus	Sex
6 mm	32 days	Collection Dr Vera	-
9mm	36 days	N 4433	-
14mm	41 days	N 5279	-
15mm	42 days	N 18500	-
16mm	44 days	N 7751	-
21mm	6 weeks	N 26338	-
24mm	7 weeks	N 7484	-
30mm	8 weeks	N 6394	-
34mm	8 weeks	A/45-05	-
36mm	8 weeks	N 14562	-
42mm	9 weeks	N 4517	-
50mm	10 weeks	N 2994	-
60mm	11 weeks	N 26310	female
80mm	12 weeks	A/87-02	female
83mm	12 weeks	N 18964	male
95mm	13 weeks	N 13298	female
120mm	14 weeks	A/21-04	male
130mm	15 weeks	A/22-01	female
132mm	15 weeks	A/12-01	male
140mm	16 weeks	A/9-01	female
150mm	17 weeks	A/15-01	female
170mm	18 weeks	A/16-01	male
176mm	18 weeks	A/14-01	female
181mm	19 weeks	A/27-01	male
183mm	19 weeks	A/33-01	male
191mm	20 weeks	A/28-01	female
200mm	21 weeks	A/34-01	female
212mm	22 weeks	A/31-01	male
220mm	23 weeks	A/25-01	male
228mm	24 weeks	A/5-02	male
242mm	25 weeks	A/7-02	female
250mm	26 weeks	A/67-01	male
255mm	27 weeks	A/24-02	female
265mm	28 weeks	A/8-02	female
270mm	29 weeks	A/36-04	female
280mm	30 weeks	A/59-02	male
290mm	31 weeks	A/17-03	female
300mm	32 weeks	A/21-02	female
310mm	34 weeks	A/4-02	male
320mm	35 weeks	A/53-02	female
325mm	36 weeks	A/54-02	male
330mm	37 weeks	A/65-01	female
335mm	38 weeks	A/32-02	male
-	NB	A/38-01	male
-	NB	A/36-03	female
-	NB	A/59-05	female

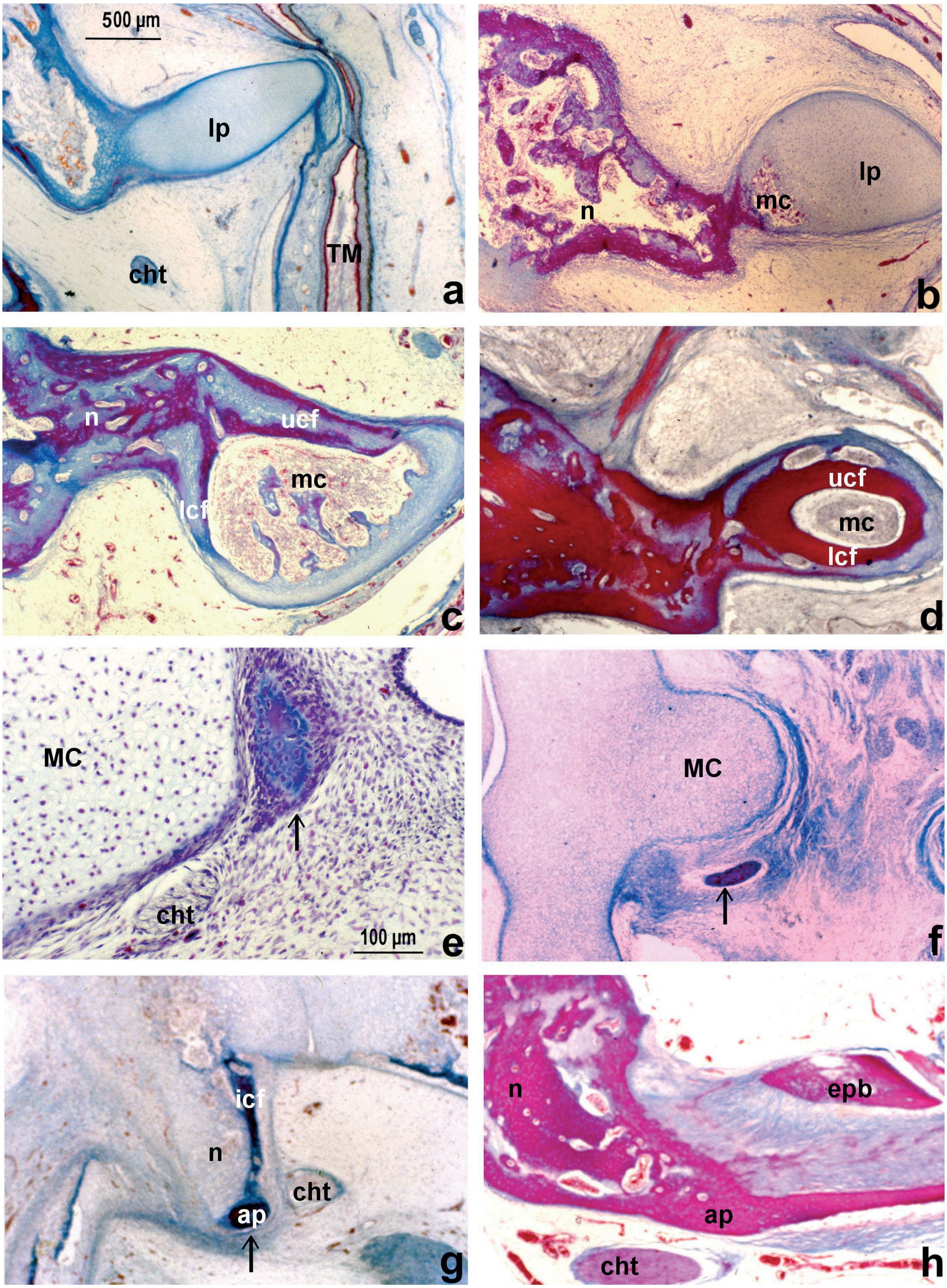

Fig. 2. Development of the dynamic structure of the malleus. **a)** 200 mm (21weeks), **b)** 220 mm (23 weeks), **c)** 255 mm (27 weeks), **d)** 310 mm (34 weeks), **e)** 34 mm (8 weeks), **f)** 50 mm (10 weeks), **g)** 176 mm (18 weeks) and **h)** 325 mm (36 weeks). LATERAL PROCESS. In **a, b, c and d,** the lateral process (lp) of the malleus is ossified via a replacement mechanism by means of two cortical fascicles (upper and lower) (ucf and lcf) which progressively encompass the entire medullary cavity (mc) to later be consolidated. Cht (chorda tympani), TM (tympani membrane), n (neck). ANTERIOR PROCESS. In **e, f, g and h**) the anterior process of the malleus (arrows) is ossified via membranous ossification independent from the rest of the ossicle, it fuses with the neck via the internal cortical (arrow) and it is the first segment of the bone to complete its ossification. MC (Meckel's cartilage), icf (internal cortical fascicle), ap (anterior process), epb (epitympanic bone), n (neck). Martins' trichrome technique. Scale bar: a-d, f-h, 500 µm; e, 100 µm.

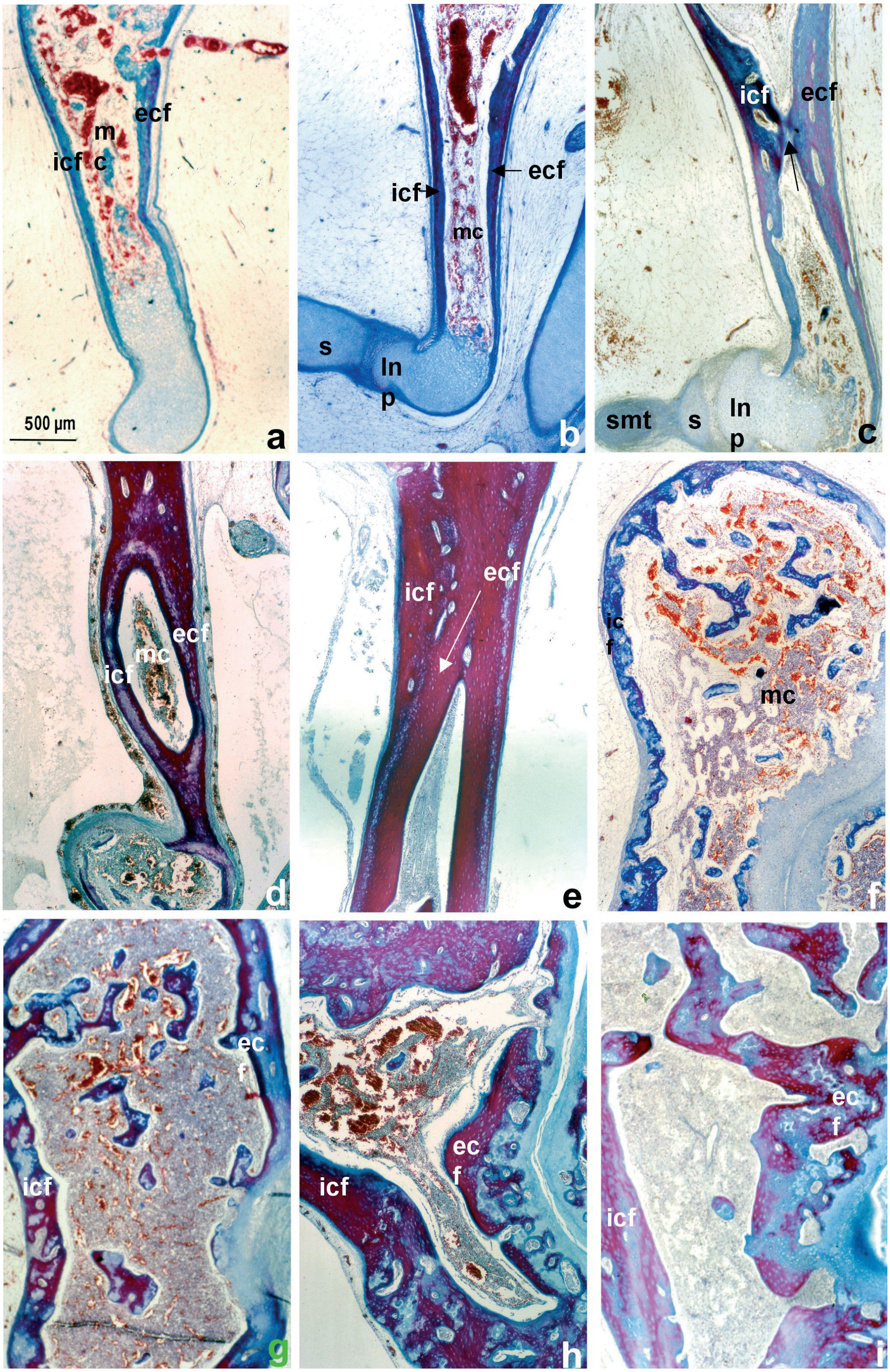

Fig. 3. Development of the dynamic structure of the incus. **a)** 183 mm (19 weeks), **b)** 200 mm (21 weeks), **c)** 212 mm (22 weeks), **d)** 250 mm (26 weeks), **e)** newborn, **f)** 228 mm (24 weeks), **g)** 265 mm (28 weeks), **h)** 325 mm (36 weeks) and **i)** newborn. LONG PROCESS. In **a, b, c, d** and e the development of the architecture in the long process as the cortical fascicles extend in rostral-caudal direction and stop at isthmus level of the lenticular process (lnp), the increase in its thickness and the interweaving of the bone trabeculae forming loops between both cortical fascicles (ecf and icf) (arrow). S (stapes), smt (stapedial muscle tendon), mc (medullary cavity). BODY. In **f, g, h and i** the development of the architecture in the body, the formation of the two cortical fascicles (ecf and icf) that surround the body, the increase in their thickness and the progressive disappearance of the medullary cavity (mc). Martins' trichrome technique. Scale bar: 500 µm.

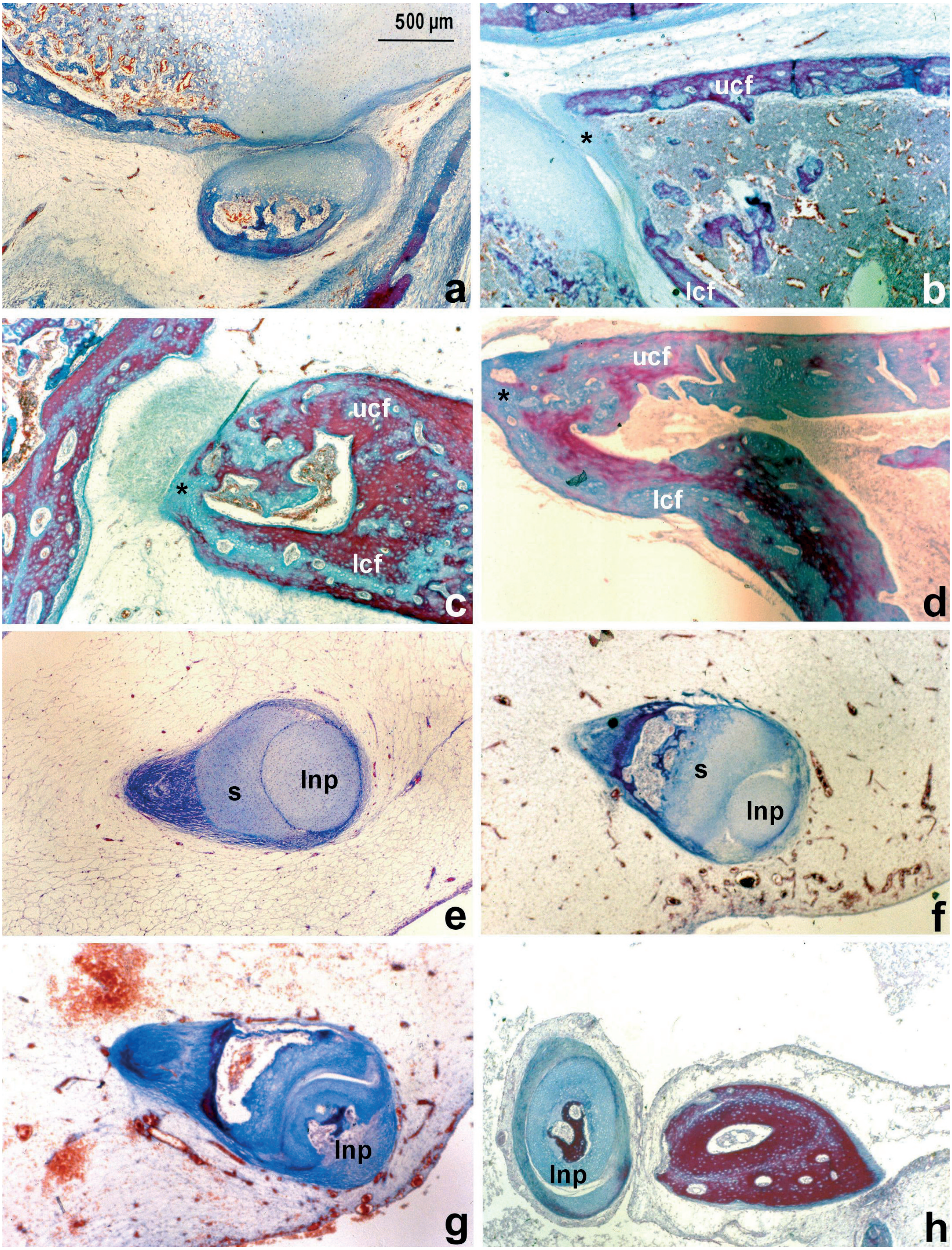

Fig. 4. Development of the dynamic structure of the incus. **a)** 228 mm (24 weeks), **b)** 242 mm (25 weeks), **c)** 290 mm (31 weeks), **d)** newborn, **e)** 220 mm (23 weeks), **f)** 255 mm (27 weeks), **g)** 270 mm (29 weeks) and **h)** 290 mm (31 weeks). SHORT PROCESS. In **a, b, c and d** the development of the architecture in the short process via two upper and lower cortical fascicles (ucf and lcf), and how these become consolidated with time, with hyaline variety cartilage persisting at the end (*). LENTICULAR PROCESS. In **e, f, g and h** the development of the architecture in the lenticular process (lnp) of the incus. The lenticular process continues to have a cartilaginous structure until 29 weeks and after this time a bony core is formed, surrounded by hyaline cartilage. This is a sign that its ossification has been carried out via a replacement mechanism. S (stapes) Martins' trichrome technique. Scale bar: 500 µm.

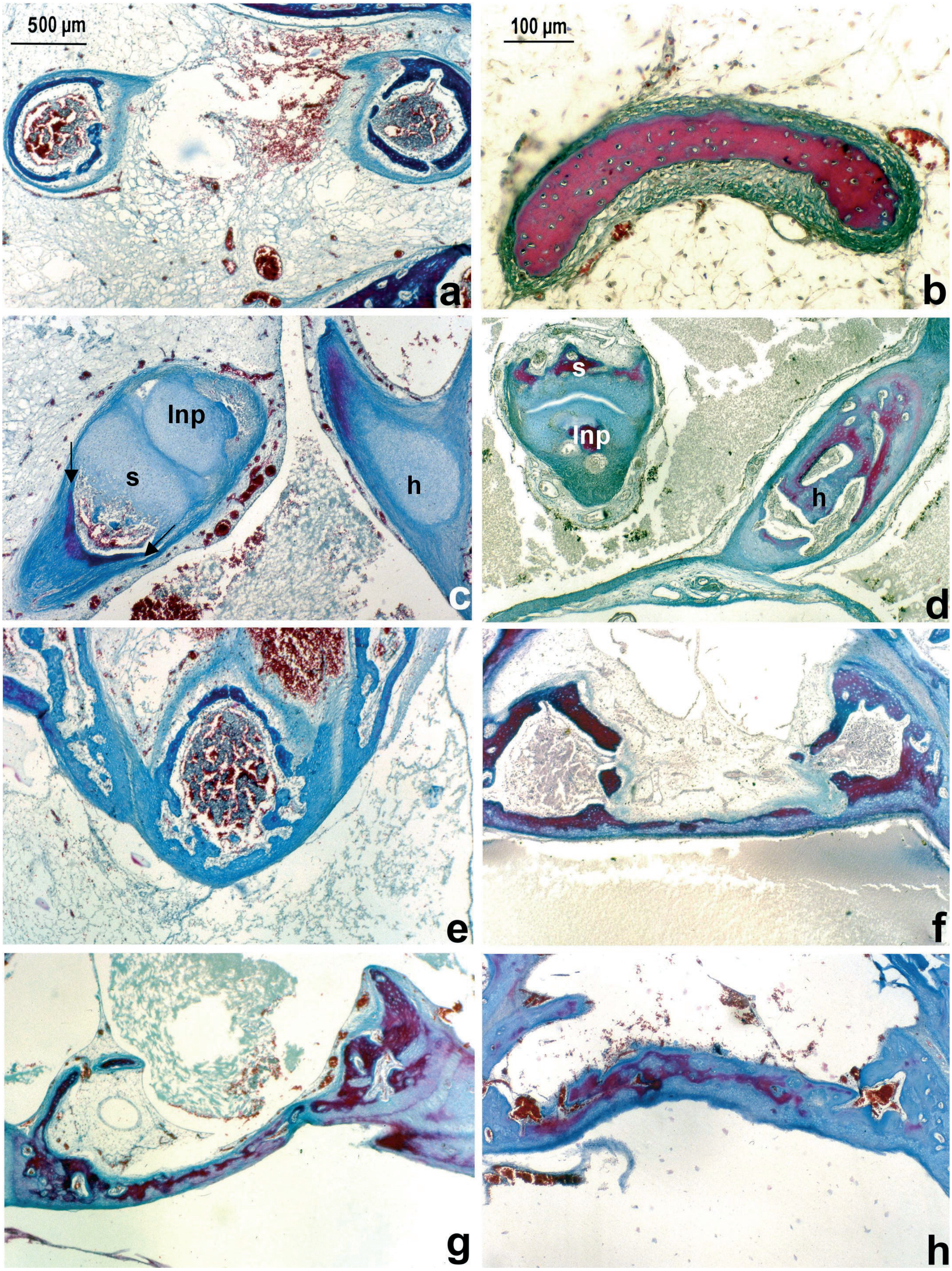

Fig. 5. Development of the dynamic structure of the stapes. **a)** 255 mm (27 weeks), **b)** 280 mm (30 weeks), **c)** 265 mm (28 weeks), **d)** 310 mm (34 weeks), **e)** 255 mm (27 weeks), **f)** 280 mm (30 weeks), **g)** 325 mm (36 weeks) and **h)** 335 mm (38 weeks). BRANCHES. In **a and b** the branches undergo a remodelling, erosion and dissolution process which leads to the disappearance of the original internal cortical fascicle, attaining its typical half-moon morphology at 31 weeks' development. HEAD. In **c and d** we highlight how after 27 weeks the head of the stapes starts to ossify via two fascicles that originate in the neck and go towards the articular surface. At 34 weeks they cover the whole articular surface. Lnp (lenticular process), s (stapes), h (handle). FOOTPLATE. In **e, f, g and h** we highlight the remodelling process and appearance of bony trabeculae in the stapes footplate until a continuous bony lamina is formed. Martins' trichrome technique. Scale bar: a, c-h, 500 µm; b, 100 µm.

intermediate fascicle and their joints with the cortical fascicles mean that the medullary cavity is divided into smaller compartments (Fig. 1h).

In 32 week foetuses, the increase in thickness of the two fascicles that surround the medullary cavities of the head and lateral process stands out. This is caused by apposition and extension mechanisms of the periosteal and endosteal bones, and the subsequent reduction of the latter. In the handle, both cortical fascicles reach the area surrounding the terminal end that maintains its cartilaginous structure.

At 34 weeks, the head contains more and more compact bone surrounding the medullary cavity. The handle has three different fascicles, two thin cortical ones that have not yet consolidated and a central fascicle. The lateral process has organised its dynamic structure consisting of two compact bony fascicles that surround the medullary cavity, all of which is covered with a cartilaginous outer layer (Fig. 2d).

In 35, 36 and 37-week foetuses, all the fascicles increase in thickness due to apposition and extension mechanisms of the periosteal and endosteal bones, and we must highlight how the intermediate fascicle becomes more robust in the handle (Fig. 1f).

In newborns (38 and 40 weeks) the force transmission starts in the handle via two cortical facicles (external and internal) and one central fascicle, which are concentrated in the neck (Fig. 1i). The lines that originate in the lateral process follow two different paths. The first, which includes the transmission of the upper part, goes to the neck and the second, which originates in the lower part, combines with the internal cortical of the handle. The forces are transmitted from the neck to the head via two cortical fascicles, which go to the articular surface (thickest) and to the upper edge and rostral side, respectively.

The development of the anterior process, which is formed via intramembranous type ossification, is the first part of the ossicle to be ossified.

We have observed the appearance of the future anterior process in the 30 mm embryo, independently from the Meckel´s cartilage. This process is located in a caudo-medial position with respect to the Meckel´s cartilage and to the chorda tympani. See in Fig. 2e how the mesenchyme is forming membranes, called “osteoids” where organic matrix filaments deposit. These then ramify and unite to form bony trabeculae.

From 10 weeks' development onwards, the process is completely ossified and is still independent from the rest of the ossicle (Fig. 2f). Between 16 and 18 weeks' development, the process fuses with the neck at the internal cortical level of the neck (Fig. 2g), and after 19 weeks the process is completely ossified and joined to the rest of the ossicle (Fig. 2h).

Development of the dynamic organisation of the incus

We have observed the start of the consolidation of the organisation of the force lines in the incus between 16 and 20 weeks’ development at long process level via two fine perichondral bone cortical fascicles, which extend both in cranial and caudal direction, towards the body and the lenticular process, progressively increasing in thickness (Fig. 3a).

At 21 weeks, the cortical fascicles occupy the whole extension of the process, stopping within the vicinity of the lenticular process, (Fig. 3b) and at 22 weeks both cortical fascicles start to fuse together (Fig. 3c). This fusion becomes more obvious between 23 and 29 weeks. The fine bone lamellae concentrate together, forming loops due to the interweaving of the force lines that end up concentrating in the isthmus of the lenticular process (Fig. 3d).

From 30 weeks until birth, the force lines consolidate via the crossing of bony trabeculae from one cortical to another, reinforcing the fascicles that run through both cortical fascicles (Fig. 3e).

The development of the force lines is delayed in the incus body with respect to the long process and it is not until 24 weeks that we observe the formation of a large medullary cavity surrounded by perichondral bone, with the exception of its articular surface, which is made up of hyaline cartilage (Fig. 3f).

At 26 weeks, the bone that surrounds the cavity becomes more and more compact and two fascicles can be seen. The first, the anterior one, comes from the vicinity of the articular surface and the second, the posterior one, from the rest of the body. Both fascicles continue along the cortical fascicles of the long process. Endochondral bone can be seen on the inside of the medullary cavity, and this occasionally joins the anterior and posterior fascicles.

From 28 weeks until birth, the incus body is of the cavitary morphological variety, with the consistency and thickness of both fascicles increasing progressively. These are divided and separated by the medullary cavity due to apposition and extension mechanisms of the periosteal and endosteal bones (Fig. 3g,h,i).

The short or short process presents a very lax bone structure. During the first stages of development, it has a totally cartilaginous structure. We start to observe the trabecular organisation as from 24 weeks' development, (Fig. 4a) and at 25 weeks it presents two cortical fascicles, of reduced bone density, which intermingle with extensive areas of cartilaginous tissue islands that continue with those of the body. These fascicles are suddenly interrupted at the rear end of the branch, which continues to have a cartilaginous structure (Fig. 4b,c). At the time of birth, the posterior end of the short process of the incus maintains a cartilaginous structure (Fig. 4d).

The lenticular process has a cartilaginous structure, (Fig. 4e,f), until 29 weeks when the first signs of ossification appear, consistent with the presence of a central core of endochondral bone, inside which small medullary cavities can be seen. This is all surrounded by hyaline cartilage (Fig. 4g). This bone core increases with age (Fig. 4h).

In newborns, the force lines start in the articular facet, and are transmitted to the body by means of two cortical fascicles separated by the medullar cavity, and they later descend through the long process via two cortical fascicles, which interweave and end at isthmus level of the lenticular process.

Development of the dynamic organisation of the stapes.

Of the three ossicles, the stapes are the last to start their ossification and their architectural development is the most complicated, as three primary ossification centres, which appear in succession and which fuse together with time, are involved in their formation. Furthermore, certain parts of the stapes undergo constant remodelling consisting of the erosion and dissolution of some of its bone components. This leads to a reduction in its volume.

The ossification of the anterior and posterior crura progressively spreads from the ossification centre located in distal areas to proximal areas and they do not fuse with the neck until 27 weeks' development.

In the cross sections of the branches it can be seen how, at 22 weeks, these present a circular morphology; the areas close to the ossification centre present a bone structure, whilst in the areas further away, the structure still has typical cartilage characteristics. At 24 weeks, both branches are totally ossified, preserving their spherical morphology and presenting a large medullary cavity surrounded by perichondral bone. From 24 weeks and until 30 weeks, a small interruption appears in its internal cortical via an erosion and dissolution process and we can see how the medullary cavity content comes out through this aperture onto the exterior to establish contact with the proximal mesenchyme (Fig. 5a).

Between weeks 31 and 32 of development, the branches take on their classical half-moon morphology, convex on the outside and concave on the inside, and their compact bone constitution permits following the force lines descending through the crura to the footplate (Fig. 5b).

The stapes head continues to have a cartilaginous structure until 26 weeks' development when its ossification begins, and after 27 weeks, two fine cortical fascicles start to develop from the neck, forming a primitive medullar cavity on the interior (Fig. 5c). At 29 weeks, both cortical fascicles have reached the ends of the articular surface. Between weeks 30 and 31, the cortical fascicles start to thicken and two bone columns start to form from both ends, which progressively cover the articular surface. Both columns fuse together at 34 weeks (Fig. 5d). In the stapes head of a newborn the force transmission lines originate on the articular surface and descend through the crura to the bone footplate forming a real Gothic ogive.

The ossification of the footplate has a dual origin, the auditory side of the Reichert cartilage and the vestibular side of the auditory capsule.

On the auditory side, at 20 weeks, we have observed the presence of a primary ossification centre situated on the base, and how from this moment on and until 30 weeks' development this side undergoes a continuous remodelling process, which leads to the disappearance of the majority of it, and resulting in a very fine lamella at the end of the process where bone trabeculae start to appear (Fig. 5e,f).

After 30 weeks' development, the auditory lamina increases in thickness based on the apposition of bone trabeculae. During this whole process, the vestibular lamina maintains its cartilaginous structure (Fig. 5g,h).

Finally, we wish to point out that, with the exception of the vestibular side of the footplate, the areas close to the stapedial-vestibular joint preserve a cartilaginous covering at least until birth.

Discussion

Our observations reflect how the middle ear ossicles present a cavity type structure in the foetal period and in the newborn, with a large medullar centre on the inside surrounded by compact bone. These data confirm the observations of Olszewski (1989) and Yokoyama et al. (1999), who describe how the ossicles are not completely developed until four years of post-natal life.

The architecture of the ossicles progressively consolidates with two different ossification processes intervening in their formation. The first process involves the appearance of the periostic bone cortical fascicles via membranous type ossification and the second involves endochondral ossification via two bone formation mechanisms. The first is a replacement mechanism as occurs in the lateral process and handle of the malleus and lenticular process of the incus, and the second is an apposition and re-absorption mechanism as occurs in the rest of the ossicles.

The structure organisation starts with the formation of the cortical fascicles in the perichondral bone, which extend to the rest of the ossicle and increase in thickness due to apposition and endostic reabsorption, contributing to the formation of the definite bone and consolidating the transmission lines.

The osseous architecture of the incus starts to develop at 16 weeks, at long process level via two fine periostic bone cortical fascicles in the middle third of the process. The cortical fascicles extend both in cranial and caudal direction in order, at 21 weeks, to occupy the whole extension of the process and come to a stop in the vicinity of the isthmus of the lenticular process. Their increase in thickness means that at 22 weeks they both fuse together in the medial area and after 27 weeks they are completely developed based on two cortical fascicles that intertwine.

At the onset, in the incus body, they are represented by bone trabeculae that surround the medullary cavity.

At 26 weeks, these bone trabeculae combine to form two cortical fascicles that continue with those of the long process.

The short process shows a structural weakness, as extensive cartilaginous areas intermingle with isolated bone trabeculae.

The lentiform process maintains a cartilaginous structure until 28 weeks, and after 29 weeks it becomes ossified via an endochondral replacement mechanism.

The malleus starts to organise its osseous structure at 16 weeks' development at neck level via two cortical fascicles, and at 21 weeks the organisation of the architecture presents a compact neck.

The cortical fascicles consolidate slowly in the head of malleus, and at the start, when the articular surface develops and the bone becomes compact, two fascicles are observed that go towards the neck. The first of these arises from the vicinity of the articular surface and the second in the rest of the head.

In the handle it is not until 24 weeks when the two cortical fascicles extend from the neck to the proximal half, and between 26 and 36 weeks when the cortical fascicles extend towards the distal portion. In the same period, endochondral bone trabeculae form in the medullary cavity of the handle, which fuse to form a third central fascicle.

The lateral process maintains its cartilaginous structure until 22 weeks, and after 23 weeks begins to form two upper and lower fascicles that complete their development at 34 weeks.

The stapes begin to organise their structure at 20 weeks' development with respect to both branches, via the formation of two cortical fascicles (external and internal). The internal cortical fascicle undergoes an erosion and dissolution process after 24 weeks, and at 34 weeks it only preserves the external cortical fascicle.

The footplate also undergoes a remodelling process. After 30 weeks, its tympanic side starts to ossify and form a bone lamina that is completely developed in the newborn.

The broad fan that covers the whole extension of the head of the malleus and the body of the incus described by Sarrat et al. (1992) still has to develop in the newborn. We assume that this fascicle will finish its development at around four postnatal years, by which stage Yokoyama et al. (1999) found that the medullary cavity had disappeared and the bone took on a compact structure.

We believe that different mechanical type factors intervene in the organisation of the microarchitecture of the middle ear ossicles, apart from the genetic factors that involve the migration of the cells from the neural crest to the pharyngeal arches and their subsequent differentiation in the different skeletal components of the middle ear (Novacek, 1993; Mallo, 2001). These mechanical factors contribute to establishing the mobility of the ossicles and therefore increase the osteoblastic activity that will increase the bone formation.

Among these factors, we believe that the disappearance of the mesenchyme of the tympanum box and the start of the mobility of the ossicles, are key factors for establishing the architectural pattern of the ossicles.

As we have been able to observe in the histological preparations, it is after 21 weeks' development when the mesenchyme of the tympanum box begins the reabsorption phenomena, completing its pneumatisation around 33 weeks, and four weeks later the pneumatisation of the epitympanum ends. When the pneumatisation of the majority of the tympanum box has occurred, the ossicles are free to move in a liquid medium. These data are very similar to those described by Buch and Jorgensen (1963), Rauchfuss (1989) and Piza et al. (1998).

According to Kasemsuwan et al. (1996) and Takahara et al. (1986) the reabsorption continues during childhood, observing how the quantity of mesenchymatous tissue that remains in the temporal bone decreases as the age of the child increases. With respect to the disappearance of the mesenchyme there are three theories. The first is that it occurs due to reabsorption (Rauchfuss, 1989). The second theory is that it is due to the redistribution and thinning of the mucosa of the tympanum box (Piza et al., 1998). The third theory is that it is caused by the participation of programmed cell death mechanisms, but this apoptosis only occurs around the mesenchyme of the ossicles (Roberts and Miller, 1998).

Coinciding with the start of the disappearance of the mesenchyme of the tympanum box we have observed the presence of contacts between the ossicular bone marrow and the mesenchyme. These contacts are temporary and we believe, as Linthicum et al. (1997) does, that the bone marrow tissue has a haematopoietic function as well as protecting the middle ear from bacterial invasions.

Other references with respect to the mobility of the ossicles are the disappearance of the mesenchymal plug in the meatus and the complete structural maturation of the auditory membrane, as well as of the union with the handle of the malleus. Ars (1984) and Nishimura and Kumoi (1992) observed how at 21 weeks the auditory membrane has completely separated from the external auditory conduct, although it must be specified that at this time the space of the external auditory meatus is still narrow and curved.

The development of the incudomalleal, incudostapedial and stapedial-vestibular joints also have to play an important role in the evolution of the force lines, as these are going to permit the movement of the ossicles. These joints express their own definite characteristics after 20 weeks' development Whyte et al (2002). But the mobility of the ossicular chain is minimal and mostly related to deglutition movements.

Finally, this maturing of the osseous architecture could be related to the maturing of the auditory tract. Moore et al. (1995) observed how the neurons begin to mature at 20 weeks' development, at 30 the neurons in the Corti's ganglion have a similar morphology to that of the adult, and at 35 weeks the cochlear tract reaches its complete morpho-functional maturity.

In newborns (40 weeks), the ossicles' structure is cavitary and has not been completed. The fan-shaped trabecular fascicle, which starts in the articular facets of the malleus and the incus, still has to develop.

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