■ ORIGINAL ARTICLES

Ontogenic Development of the Incudostapedial Joint

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Objective: To study the development of the incudostapedial joint in human embryos and foetuses.
Material and method: 46 temporal bones with specimens between 9 mm and new-borns were studied. The preparations were sliced serially and dyed using the Martins trichrome technique.
Results: The incudostapedial joint takes on the characteristics of a spheroidal joint at 16 weeks of development. The cartilage covering the articular surfaces is formed by different strata that develop in succession: the superficial stratum at 19 weeks, the transitional between 20 and 23 weeks, and the radial from 24 weeks on. The subchondral bone develops after 29 weeks by the mechanisms of apposition and extension of the periosteal and endosteal bones, but it is not until week 34 that it completely covers the articular surfaces, following constitution of the bone fascicles transmitting the lines of force. The articular capsule is formed from the inter-zone, the surface zone develops the capsular ligament, and the internal surface develops the synovial membrane.
Conclusions: At the time of birth, the incudostapedial joint is completely developed.

Key words: Development. Human. Incudostapedial joint.

Desarrollo ontogénico de la articulación incudoestapedial
Objetivo: Estudiar el desarrollo de la articulación incudoestapedial en embriones y fetos humanos.
Material y método: Se han estudiado 46 huesos temporales con ejemplares comprendidos entre 9 mm y recién nacidos. Las preparaciones estaban cortadas en serie y teñidas con la técnica de tricrómico de Martins.
Resultados: La articulación incudoestapedial adquiere las características de una articulación sinovial de tipo enartrosis a las 16 semanas de desarrollo. El cartílago que recubre las superficies articulares está formado por diferentes estratos que se desarrollan sucesivamente: el superficial, a las 19 semanas; el de transición, entre las 20 y las 23 semanas, y el radial, a partir de las 24 semanas. El hueso subcondral se desarrolla a partir de las 29 semanas por los mecanismos de aposición y extensión del periostal y el endostal, pero no es hasta la semana 34 cuando recubre por completo las superficies articulares, constituidos los fascículos óseos por los que se transmitirán las líneas de fuerza. La cápsula articular se forma a partir de la interzona, la zona superficial desarrolla el ligamento capsular y la interna, la sinovial.
Conclusiones: En el momento del nacimiento la articulación incudoestapedial está completamente desarrollada.

Palabras clave: Desarrollo. Humano. Articulación incudoestapedial.

INTRODUCTION

The incudostapedial joint has long been known in adults.[1-3] Kirikae[4] (1960), Djeric et al[5] (1987), and Hüttenbrink et al[6] (1987) included the incudostapedial joint in the "enarthrosis" variety of diarthrosis, and its articular surfaces would be the lenticular apophysis of the anvil and the head of the stapes. Djeric et al[5] described details for it such as dense and fibrous cartilage in the lenticular apophysis and characteristically paved hyaline in the head of the stapes, and noted that the articular capsule may completely surround the lenticular apophysis or only its distal part and that it is thicker in the posterior part.

The surface of the lenticular process is spherical in shape, and its length exceeds its width; in the head, the articular surface presents a vault shaped gap.[7]

Being a diarthrosis type joint, it can suffer injuries in adulthood by rheumatoid arthritis.[8,9]

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Received June 19, 2008.
Accepted for publication June 25, 2008.

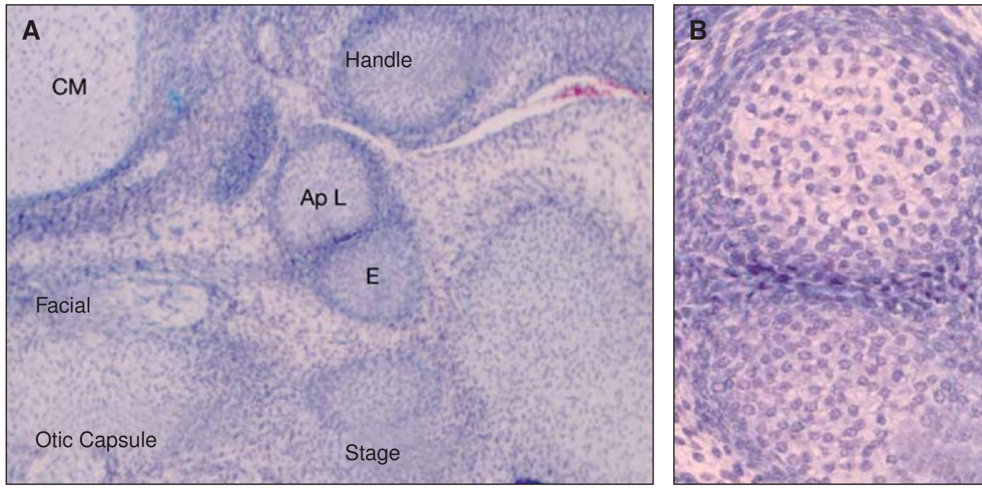

Figure 1. Overview and detail, in an embryo of 36 mm (8 weeks), of the presence of a homogeneous interzone separating the lenticular apophysis (Ap L) of the head of the stapes (E). A: Martins trichrome, ×10. B: Martins trichrome, ×40. CM indicates Meckel cartilage.

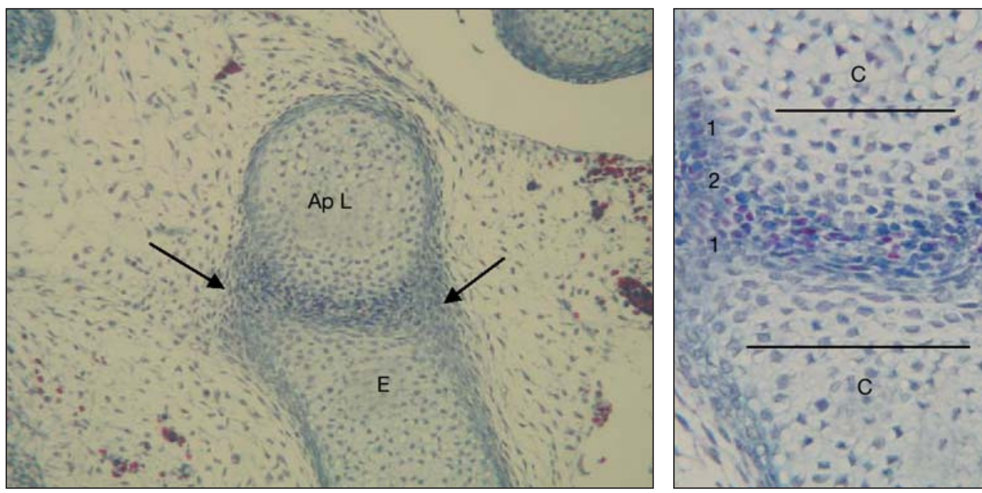

Figure 2. Overview and detail in an embryo of 42 mm (9 weeks). Note that the interzone has differentiated into trilaminar. Left, Martins trichrome, ×20. Right, Martins trichrome, ×60. 1: chondrogenic layer; 2: middle layer; Ap L: lenticular apophysis; C: cartilage; E: head of the stapes.

By contrast, there are few references to the ontogeny and development of the incudostapedial joint, and they are only limited to mentioning the existence of an intermediate zone separating the ossicles and their independence as well as their inclusion in diarthroses of enarthrosis type.[7,10-12]

MATERIAL AND METHOD

We have reviewed 46 temporal bones belonging to embryos and foetuses with samples which ranged between 9 mm and newborns.

The material comes from medico-legal autopsies of foetuses whose death occurred by spontaneous abortion, intrauterine death, or death at the time of birth.

To date we have used the foetal tables of O'Rahilly et al[13] (1996), which are based on linking different measurements (maximum length, skull-heel length, biparietal diameter, abdominal circumference, and cephalic circumference) and body weight. These measurements were compared with data provided by clinical history and ultrasound when available.

In embryos and foetuses with less than 12 weeks of development, all the head was fixed, while in the older ones a careful and meticulous dissection of the temporals as blocks was carried out. All samples were fixed in formalin at 10%, decalcified with nitric acid at 2% and at a temperature of 25°C. The average time of decalcification ranged between 1 and 15 days depending on the size and thickness of the specimen. After the decalcification process, the acid was eliminated by rinsing under running water.

After dehydrating the samples in alcohol of progressively increasing concentrations, they were included in paraffin, cut serially with a Leitz microtome at 7 μm and stained with the Martins trichrome technique.

RESULTS

Formation of the Articular Interline

The outlines of the lenticular apophysis and the head of the stapes are separated by a homogeneous condensed mesenchyme interzone in weeks 7 and 8 of development (Figure 1).

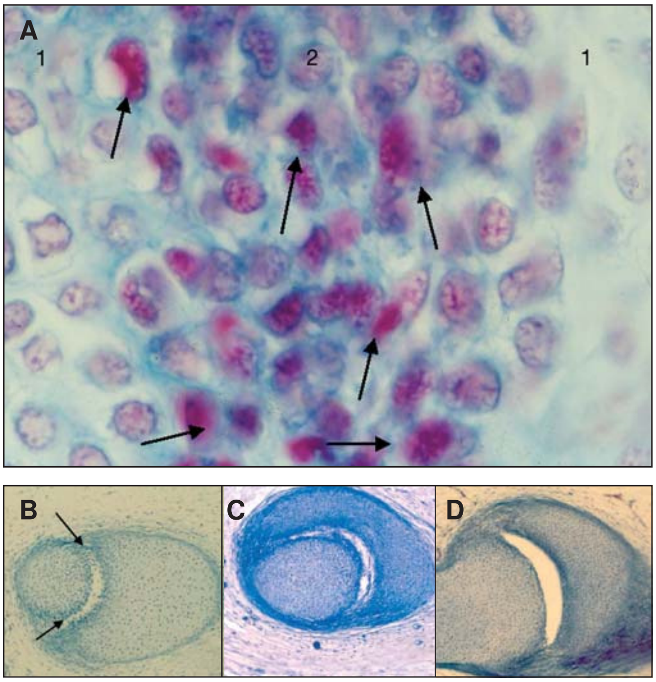

Figure 3. Cavitation phenomena in the interzone and how they are completed at 16 weeks and the incudostapedial articulation acquires the characteristics of an enarthrosis type joint. A: in the foetus at 12 weeks, the presence of cells with apoptotic appearance in the middle layer stands out (Martins trichrome, ×100). B: at 13 weeks (Martins trichrome, ¥20). C: at 14 weeks (Martins trichrome, ×20). D: at 16 weeks (Martins trichrome, ×20). 1: chondrogenic layer; 2: intermediate layer.

This interzone is different at trilaminar, made up of 2 chondrogenic layers separated by an intermediate strip in week 9 of development (Figure 2).

After 12 weeks of development, cavitation phenomena begin in the interzone, appearing at first with signs of apoptosis. The cells appear more stained than surrounding cells, the nuclei become condensed, they shrink and frequently become fragmented and their cytoplasm increases (Figure 3A). The cavitation phenomena consolidate in weeks 13 and 15 of development (Figures 3B and C).

After 16 weeks the incudostapedial joint has completed its cavitation and acquires at that point of development the characteristics of a diarthrosis of enarthrosis type (Figure 3D).

Articular Cartilage

When the ossicles are still in the cartilaginous stage, the cells near the articular interline become differentiated and a (superficial) stratum begins to organize, with small cells arranged in parallel to the surface and bound by collagen fibres (Figure 4A). Between weeks 20 and 23 of development, a new layer (transition) appears consisting of round or oval cells at random (Figure 4B).

The emergence of a (radial) stratum comprising larger round cells arranged in columns perpendicular to the articular surface, and with collagen fibres interposed to them in radial direction, is observed between 24 and 25 weeks.

The mineralization zone appears after 26 weeks of development. After the establishment of the mineralization zone, a series of osteogenic changes begin to take place, leading to the formation of gaps in its interior (Figure 4D) and the appearance of bone layers below the articular cartilage (Figures 4E and F). This transformation is similar in the lenticular apophysis and head of the stapes.

Subchondral Bone

The appearance of a subchondral bone layer immediately below the articular cartilage is observed after 29 weeks of development. At the beginning, this layer is interposed between the gaps carved in the mineralized zone and the marrow cavity (Figure 4E), it does not cover the entire articular surface and there are still wide areas in which the cartilage continues to contact directly with the marrow cavity (Figure 4E).

It is after week 34 that the subchondral bone covers the entire articular surface and the bone fascicles that will transmit the lines of force are created. After 35 weeks, these fascicles increase in thickness by mechanisms of apposition and extension of the periosteal and endosteal bones, and they acquire a more compact aspect. This growth makes the gaps become smaller. These gaps initially contain active osteocytes and haematopoyetic cells but, over time, some empty gaps or cells in process of degeneration are found in their interior.

Capsular Ligament

The primordium of the capsular ligament develops from the surface of the interzone by condensation of the surrounding mesenchyme after 8 weeks of development, and consists of a loose layer in longitudinal orientation (Figure 5A).

At 10 weeks it condenses and forms a layer that is continued with the perichondrium of the ossicles (Figure 5B).

Between 11 and 19 weeks of development, there is an increase of collagen fibres (Figures 5C and D), which are continued with the perichondrium and penetrate into the cartilage, after 21 weeks, to constitute the typical Sharpey fibres (Figure 5E), which after 23 weeks are completely covered by cartilage (Figure 5F).

The appearance of the marrow cavity in the lenticular apophysis and head of the stapes causes the capsular ligament to be related to it (Figure 5G). When the subchondral bone completely covers the joint surface, this ligament is included in it by the action of osteoblasts located between the bundles of fibres and the apposition of the neighbouring bone.

Synovial

The synovial membrane is developed from the inner part of the synovial mesenchyme. In the early stages of development it is made up of cells distributed at random, among which there are peripheral vascular bundles that eventually constitute 2 separate layers, the external one is conjunctive, with abundant capillary vessels, and is in direct contact with the capsular ligament, and the second one is internal and shows scant cellularity, which tends to be concentrated at the free edge.

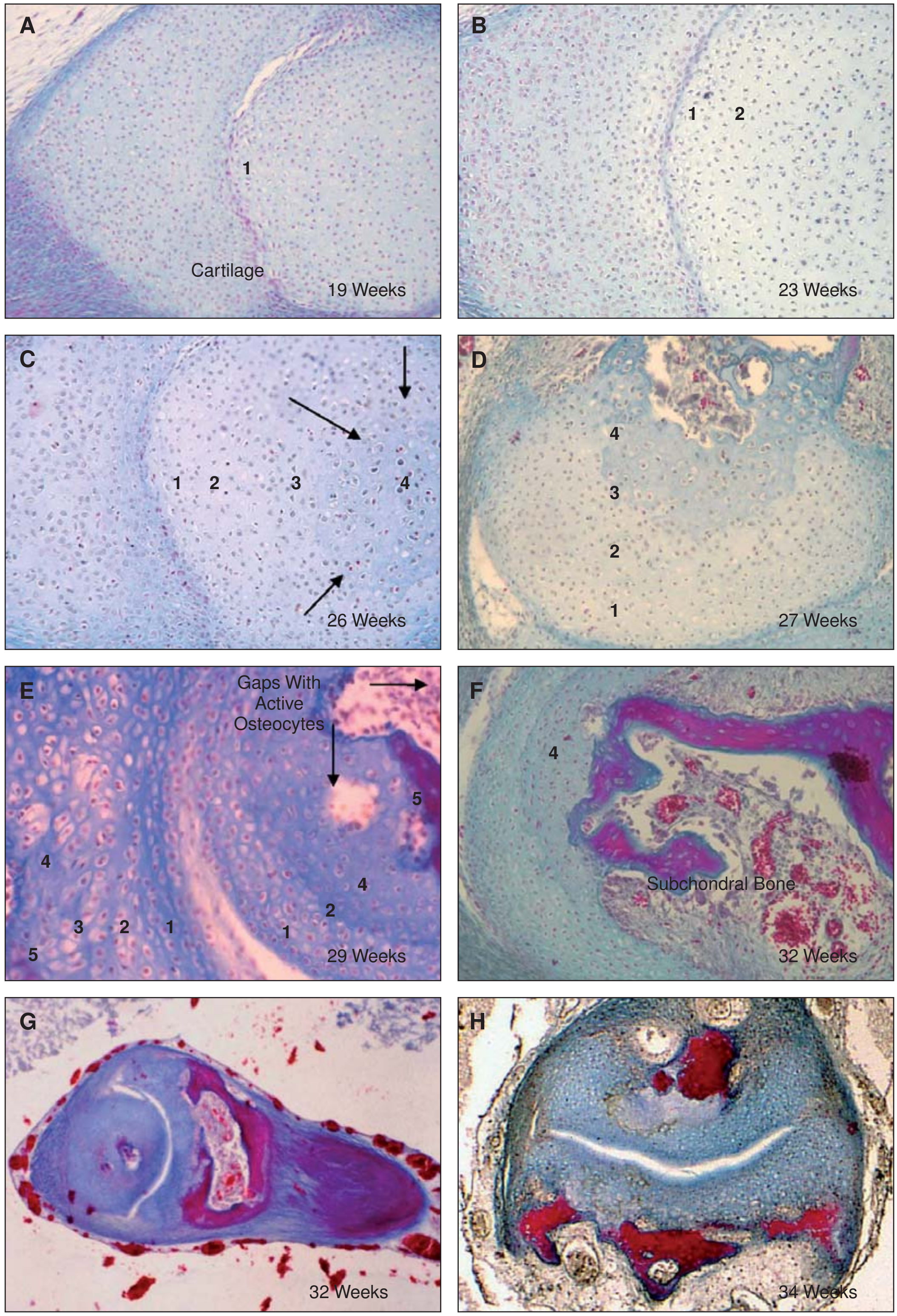

Figure 4. Note the different stages of development of the articular cartilage from the joint surfaces of the lenticular apophysis of the anvil and the head of the stapes. A: formation of the surface layer (1) (Martins trichrome, ×40). B: formation of the transitional layer (2) (Martins trichrome, ×40). C: radial (3) and area of mineralization (4) (Martins trichrome, ×20). D: formation of gaps in the mineralization zone (Martins trichrome, ×20). E: subchondral bone formation (5) (Martins trichrome, ×20). F: the subchondral bone surrounding the gaps (Martins trichrome, ×20). G: the subchondral bone does not completely cover the articular surface (Martins trichrome, ×40). H: the articular surface is completely covered by bone (Martins trichrome, ×40).

DISCUSSION

There are few references on the formation and development of the incudostapedial joint that is the subject of this paper, and they are limited to mentioning the existence of an interzone which separates the ossicles and their independence.[10,11]

From the study of embryonic-foetal material, the participation of this articulation in the diarthrosis of enarthrosis type can be inferred.[7,12]

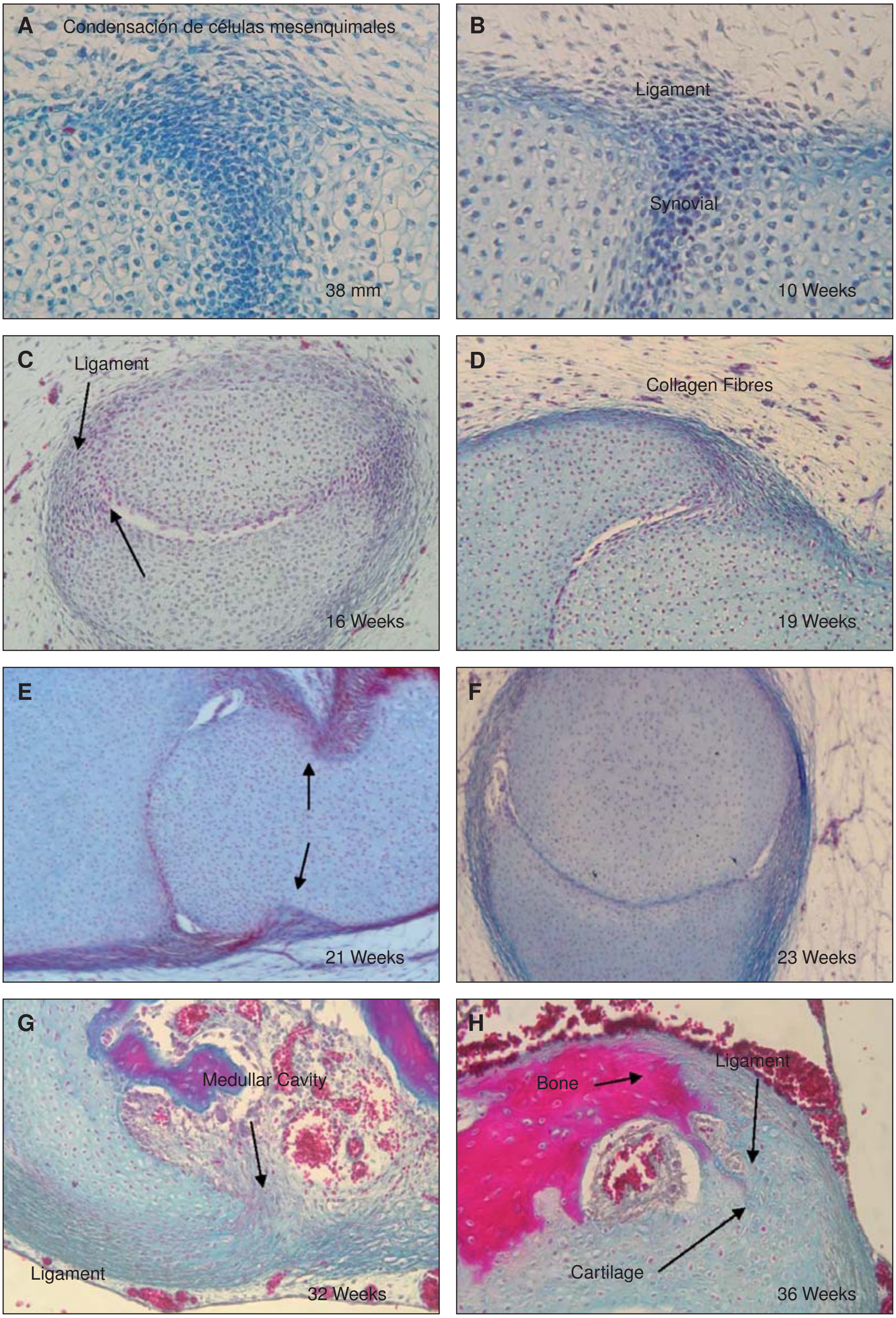

Figura 5. Formation of the capsular ligament. A: the primordium of the capsular ligament develops from the intermediate layer of the articular interzone, which shows a loose structure (Martins trichrome, ×20). B: the ligament is condensed (Martins trichrome, ×40). C: the difference between ligament and synovium can be observed (Martins trichrome, ×40). D: note the appearance of collagen fibres in the interior (Martins trichrome, ×40). E: fibres penetrate into the cartilage (Sharpey fibres) (Martins trichrome, ×40). F: fibres are completely covered in cartilage (Martins trichrome, ×40). G: the appearance of the medullar cavity causes the ligament to be in contact with it (Martins trichrome, ×4). H: the ligament contacts with the bone (Martins trichrome, ×10).

Thus, after 8 weeks of development, a homogeneous interzone appears, which becomes trilaminar in the ninth week. The cavitation mechanisms begin between weeks 12 and 15, and after 16 weeks the articular cavity is fully developed.

We believe that the cavitation mechanism is intrinsic, not extrinsic as proposed by O'Rahilly et al[14] (1972), for large synovial joints, because in them the formation of the cavity is coupled to movement and in that period of development the ossicles are immersed in a mesenchymal matrix that does not allow their mobility. It is not until week 21 of development that resorption phenomena begin in the mesenchyme of the eardrum chamber, and its pneumatization is completed towards week 37.[15-17] This hypothesis is reinforced by the work of Takahara et al[18] (1987), who do not observe movements of the ossicles until 8 months of prenatal development, which for these authors is the time when the mesenchyme disappears from the eardrum chamber.

We believe that the disappearance of the interzone to form the articular cavity could be produced by programmed cell death (apoptosis), a common phenomenon and necessary during embryonic development to remove provisional tissues. The morphology adopted by the interzone cells, characterized by hypereosinophilia and cytoplasmic retraction with nuclear fragmentation (cariorrhexis), leads us to consider this hypothesis and also because it affects only certain cells, not necessarily contiguous, and not the cartilage surrounding the articular surfaces.

Like O'Rahilly et al,[14] we believe that adjacent chondrocytes will be responsible for phagocyting these cells.

Over time, the cartilage covering the ends of the surfaces of the lenticular apophysis and the head of the stapes suffers a series of differentiations until it becomes a hyaline; at the beginning of development the chondroblasts of the interarticular line cannot be differentiated from the rest in the ossicles. After 19 weeks the superficial stratum can be observed; weeks 20 to 23 are of transition, and after 24 weeks, the cartilage acquires three layers. At about 26 weeks the mineralized zone can be observed and at 29 weeks, the subchondral bone layer, and at 34 weeks it is fully developed and covers the entire articular surface.

The bone covering the articular facets of both ossicles consolidates slowly. At first it is represented by bone trabeculae which become increasingly compact and form lines of force that transmit movement to the incudostapedial joint.

A fact we wish to highlight is the formation of bone gaps within the subchondral bone. Initially they contain active bone cells (osteoblasts, osteoclasts, and osteocytes) that eventually degenerate. These observations are consistent with the work of Marotti et al[19] (1998), who observed that 40% of the bone gaps existing in the ossicles in the first 2 years are empty or contain degenerated osteocytes. We believe this is because the tympanic ossicles present very early ossification, reach their adult size in the foetus and end their growth after 4 years of age, going from having a cavity structure to a more compact one.[20-22] To this we must add that the tympanic ossicles are the only long bones that lack epiphyses and metaphyses and thus their growth remains constant, with minimal remodelling.

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