

Development of synovial membrane in the temporomandibular joint of the human fetus

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Abstract

The development of the synovial membrane was analyzed in serial sections of 21 temporomandibular joints of human fetuses at 9 to 13 weeks of gestation. Sections of two fetuses at 12 weeks of development were used to perform immunohistochemical expression of the markers CD68 and Hsp27 on the synovial lining. Macrophage-like type A and fibroblast-like type B cells, which express CD68 and Hsp27, respectively, were observed at the twelfth week of development. Our results suggest that the development of the synovial membrane is related to the vascularization of the joint and the formation of the articular cavities.

Introduction

Compared with the synovial joints of the limbs, the temporomandibular joint (TMJ) develops relatively late. By the eighth week of development in humans (O'Rahilly's stage 23), the joint cavity of the elbows, hips, and knees are already visible, while the TMJ has only mesenchymal condensation of the mandibular condyle, articular disc, and squamous part of the temporal bone, and no evidence of its cavity.¹⁻⁴

According to Mérida-Velasco *et al.*,^{1,2} by approximately week nine, the condylar chondrification begins in the center of the condylar blastema. The inferior joint cavity starts to develop at the end of the ninth week, as the density of the mesenchymal cells in the central

region decreases because of their differentiation into fibroblasts, constituting the primordium of the articular disc. As the density of the ectomesenchyme decreases, small adjacent spaces between the cells coalesce, forming the inferior joint cavity between the future articular disc and the mandibular condyle. In the 10th week, these newly differentiated fibroblasts become more compact, forming collagen fibers and the articular disc, but there is still no sign of the superior joint cavity.^{1,5-7} In the eleventh week, the organization of the superior joint cavity begins between the squamous part of the temporal bone and the articular disc.^{1,2} More detailed studies of the TMJ development, as well as of their condylar cartilage, articular disc and the matrix proteins on the fetus development were researched through immunohistochemistry and *in situ* hybridization.^{8,9}

The synovial membrane is an important anatomic element of the TMJ. Morphologically, the synovial membrane consists of two layers: an inner cell layer called the intima and a support layer called the vascular subintima, which mixes with the fibrous capsule. The intima consists of cells embedded in an amorphous, fiber-free matrix with an approximate thickness of one to four cells. The subintima consists of loose connective tissue with blood vessels, spread-out fibroblasts, macrophages, mastocytes, adipose cells, and some elastic fibers that prevent membrane folding.¹⁰⁻¹⁵

The intima contains macrophage-like type A cells with phagocytic ability, and fibroblast-like type B cells that synthesize proteins, glycoproteins, and proteoglycans.^{12,13,16-19}

Immunohistochemical techniques allowed to detect macrophage-like type A cells and fibroblast-like type B cells by using anti-macrophage and anti-fibroblast antibodies, respectively. Grabowski *et al.*,²⁰ Nozawa-Inoue *et al.*²¹ and Suzuki *et al.*²² successfully marked macrophage-like type A cells with the antibodies OX6, ED1, CD68, and CD31. On the other hand, the laminin antibodies Mab67, VCAM-1, lumican, fibromodulin, UDPGD, and Hsp25 mark fibroblast-like type B cells, and have been used in studies of the synovial intima.²³⁻³⁰ Although a number of groups have studied about the synovial membrane of the TMJ of children, adults, and animals models, there is little in the literature regarding the development of this structure in human fetuses.¹² Understanding the morphology of fetal development is important not only to better understand the embryological steps that culminate with the individual's anatomic constitution but also to elucidate the intrinsic mechanisms that may be involved in congenital anomalies and postnatal pathologies.³¹

This study aims to improve the anatomic and histologic knowledge of the synovial membrane by determining when the synovial

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memL.O. Carvalho de Moraes begins to form during morphogenesis and the chronological occurrence and dynamics of cell types present in the synovial membrane. It also aims to analyze the morphological differences that appear during the development of the superior and inferior articular cavities.

Materials and Methods

We studied twenty one human fetuses from the Institute of Embryology of the University Complutense of Madrid and Associação de Fundo Incentivo à Pesquisa. The specimens ranged from 40 mm to 100 mm greatest length (GL) with the following ages: three nine-week fetuses; four ten-week fetuses; five eleven-week fetuses; four twelve-week fetuses; five thirteen-week fetuses; post-conception age was determined by measuring GL and external and internal criteria.^{3,32} All specimens were from ectopic pregnancies or spontaneous abortions, and there were no signs of malformation. All fetuses were fixed in 10% formalin and separated into groups studied by light

microscopy and immunohistochemistry.

All samples were decalcified in EDTA for 21 days and rinsed with tap water for 10 min. They were then fixed in 10% formaldehyde for 24 h. Next, they were dehydrated as follows: one 24-h immersion in 50% ethanol; two 6-h immersions in 70% ethanol; and two 6-h immersions in absolute ethanol. Finally, the samples were cleared by a 2-h treatment with xylol and fixed in paraffin. Semi-serial, frontal, and sagittal 4 μ m cuts of the TMJ were made by the microtome Leica, model RM2035[®]. The sections, which included three spatial planes, were stained with hematoxylin-eosin (HE), azocarmine, and Masson's trichrome stain.^{33,34}

Ten samples with gestational ages of 12 weeks were selected for immunohistochemistry. The following antibodies were used: CD68 (Santa Cruz Biotechnology, Santa Cruz, CA, USA); monoclonal anti-human IgG₁ produced in mouse and diluted to 1:100 and Hsp27 (Santa Cruz Biotechnology); monoclonal anti-human IgG₁ produced in mouse and diluted to 1:150 using the indirect immunoenzyme method in three stages and the streptavidin-biotin-peroxidase complex.³⁵ The antibodies CD68 and HSP27 react with the cells macrophage-like type A and fibroblast-like type B, respectively. The positive controls for the antibodies CD68 and Hsp27 were palatine tonsil and breast adenocarcinoma, respectively. The negative controls were the same cases used as positive controls, but a buffer instead of the primary antibody was used for immunohistochemical incubation.

The study was approved by the ethics committee of the Faculty of Medicine of the University Complutense of Madrid and the Federal University of São Paulo Research Ethics Committee.

Results

Week 9-10

All specimens studied during the ninth week showed small spaces or clefts between the primordium of the articular disc and the mandibular condyle that defined the initial formation of the inferior joint cavity. Some vascular branches were located in the periphery of the articular disc primordium (Figure 1A). All specimens studied during the tenth week showed the organization of the inferior joint cavity complete although crossed by a few tracts of connective tissue. There was still no sign of the superior joint cavity (Figure 1B).

Week 11

Blood vessels in the inferior part of the

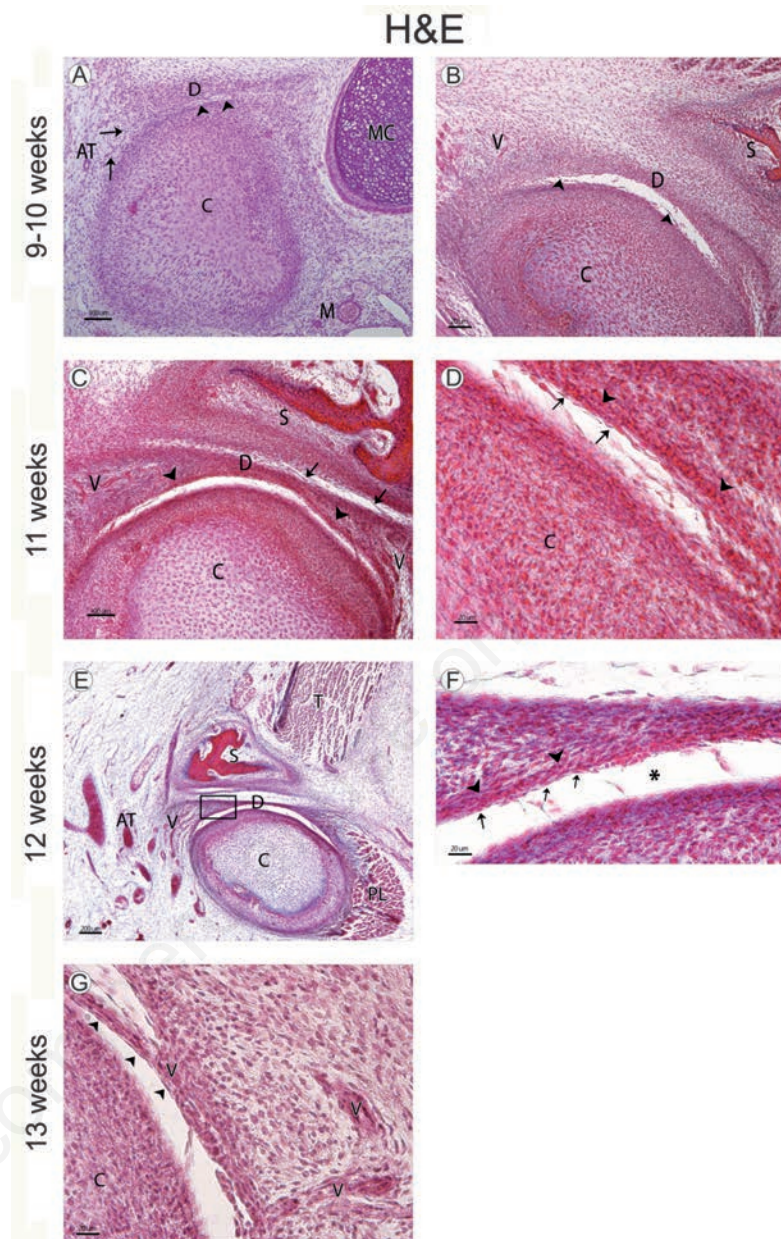


Figure 1. A) Human fetus (38 mm GL; week 9 of development); sagittal section stained with hematoxylin-eosin; the mandibular condyle (C) begins its chondrification; small spaces (arrowheads) show initial inferior joint cavity formation; D, articular disc; C, mandibular condyle; M, maxillary artery; AT, superficial temporal artery; MC, Meckel's cartilage; arrows, vessel. B) Human fetus (48 mm GL; week 10 of development); frontal section stained with hematoxylin-eosin; the inferior joint cavity continues its organization between the articular disc (D) and the mandibular condyle (C); arrowheads, tracts of connective tissue; S, squamous part of the temporal bone; V, vessel. C) Human fetus (65 mm GL; week 11 of development); frontal section stained with hematoxylin-eosin; the organization of the superior joint cavity began between the squamous part of the temporal bone (S) and the articular disc (D); arrowheads, vessels in the inferior part of the primordium of the articular disc; C, mandibular condyle; V, vessels; arrows, tracts of connective tissue. D) Human fetus (65 mm GL; week 11 of development); magnification of panel A; fusiform-like cells (arrows) separate the vessels (arrowheads) of the articular disc from the inferior articular cavity; C, mandibular condyle. E) Human fetus (80 mm GL; week 12 of development); frontal section stained with hematoxylin-eosin; D, articular disc; C, mandibular condyle; PL; lateral pterygoid muscle; T, temporalis muscle; S, squamous part of the temporal bone; V, vessels; AT, superficial temporal artery. F) Human fetus (80 mm GL; week 12 of development); magnification of the squared area in panel A. Vessels (arrowheads), fusiform-like cells (arrows); inferior articular cavity (asterisk). G) Human fetus (97mm GL; week 13 of development) vessels surrounded by fusiform cells (arrowheads) form the primordium of the synovial villi; V, vessels; C, mandibular condyle.

articular disc primordium were seen during the eleventh week. Fusiform-like cells separate the vessels of the inferior articular cavity (Figure 1 C,D).

The organization of the superior joint cavity began between the squamous part of the temporal bone and the primordium of the articular disc. The superior joint cavity was crossed by few tracts of the connective tissue (Figure 1C). Numerous vessels were seen in the lateral and medial parts of the TMJ (Figure 1D).

Week 12

All specimens studied this week showed the joint cavities clearly defined and decrease in the number of septa of connective tissue that crossed them was noted. The vessels in the inferior part of the articular disc did not reach the middle area of the disc (Figure 1 E,F).

Some macrophage-like cells that expressed CD 68 and fibroblast-like type B cells that express Hsp27 (Figure 2 A,C,E) appeared bottoms of the inferior articular cavity.

Week 13

All specimens studied during the thirteenth week showed the vessels located bottoms of the inferior articular cavity. Some vessels surrounded by fusiform cells protruded from the inferior sac bottoms, which corresponds to the primordium of the synovial villi (Figure 1G).

Panels B and D of Figure 2 show the positive control of the antibodies CD68 and Hsp27 in palatine tonsil and breast adenocarcinoma samples, respectively.

Discussion

According to Mérida-Velasco *et al.*,¹² the TMJ joint cavities are not formed synchronously. First, the inferior joint cavity begins to develop in the ninth week as small spaces or fissures appear between the articular disc and the mandibular condyle. Subsequently, the superior joint cavity begins to form approximately in the eleventh week of gestation, between the articular disc and the squamous part of the temporal bone.

The findings by Mérida-Velasco *et al.*¹ contrast with those of Van der Linden *et al.*,³⁶ Sperber,³⁷ Burdi,³⁸ and Ögutcen-Toller,³⁹ who reported that the inferior joint cavity begins to form during the tenth week of gestation, while the superior joint cavity begins to form from the eleventh week of gestation. According to Ohnuki,³⁷ the inferior joint cavity above the mandibular condyle is still being formed during the twelfth week of development, while the superior joint cavity is only visible in the posterior region of the joint.

In humans, buccal movements begin during

weeks 7 and 8 of development⁴¹ at the level of the incudomalleolar joint.⁴² TMJ movements help to form the joint cavity and their absence could result in congenital craniofacial anomalies.³¹ The presence of blood vessels in the articular disc of the TMJ of human fetuses has been described by many authors. Van der Linden *et al.*³⁶ noted that capillaries were distributed in the periphery of the anterior and posterior portions of the articular disc in human fetuses. However, they did not report any vessels in the central region of the articular disc. Wong *et al.*⁴³ observed that in fetuses, 13 to 17.5 weeks of development vessels are

mainly located anteriorly and posteriorly, but no evidence of vascularization was found at the center of articular disc. Thilander *et al.*⁴⁴ studied 61 specimens of articular disc aged 2 days to 26 years and concluded that vascularization only exists in the first year of life, after which it disappears. Interestingly, Sabú *et al.*⁴⁵ observed vessels containing red blood cells in the central portion of the TMJ articular disc of human fetuses.

According to our findings, from the ninth week of development the number of vessels increases around the temporomandibular joint region, sending some vascular branches to the

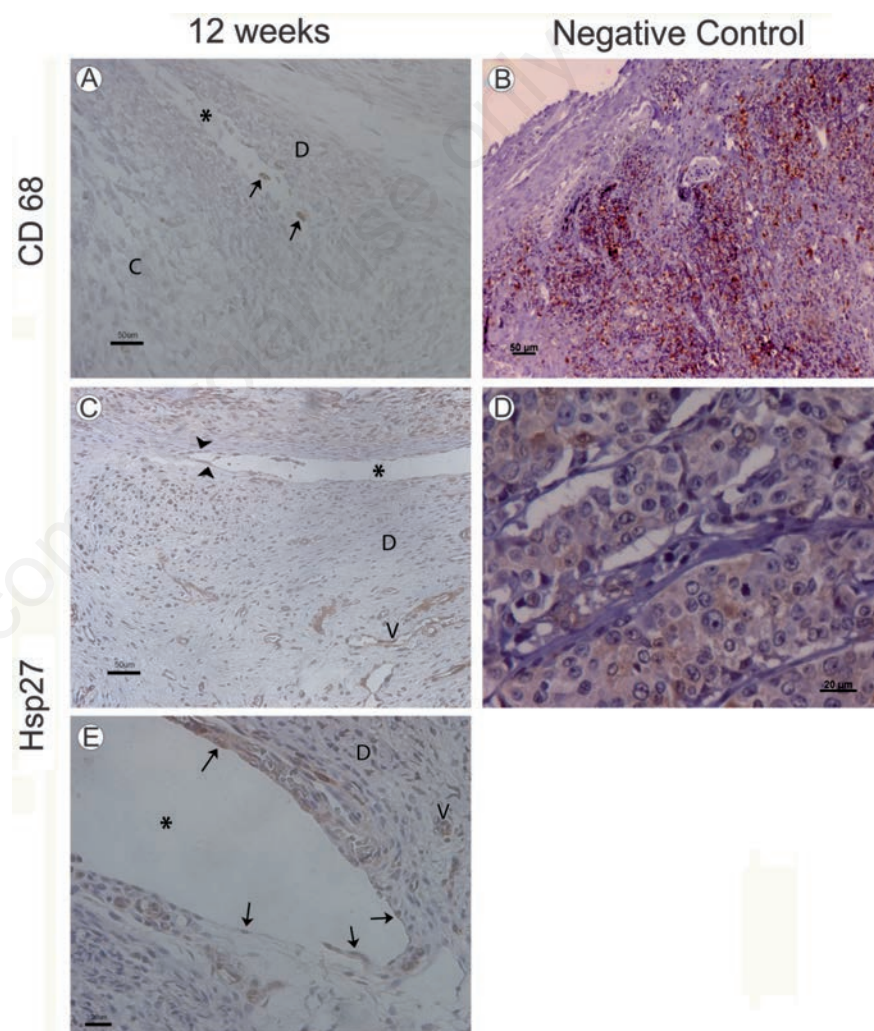


Figure 2. A) Human fetus (75 mm GL; week 12 of development); macrophage-like type A cells marked by CD68 antibodies; C, mandibular condyle; D, articular disc; asterisk, inferior joint cavity. B) Positive control; palatine tonsil; CD68 antibody. C) Human fetus (82 mm GL; week 12 of development); marked fibroblast-like type B cells by Hsp27 antibodies (arrowheads) in the synovial membrane and vessels (V); asterisk, superior joint cavity. D) Positive control; breast adenocarcinoma; Hsp27 antibody. E) Human fetus (82 mm GL; week 12 of development); marked fibroblast-like type B cells by Hsp27 antibodies (arrowheads) in the inferior articular cavity (asterisk). The walls of the vessels (V) are marked with Hsp27 antibodies; D, articular disc.

periphery of the articular disc primordium. In specimens at eleven weeks of development, the vessels reach the central portion of the articular disc and fusiform-like cells separate the disc from the inferior articular cavity. During the twelfth week of development, the articular cavities are well defined and the vessels do not reach the central region of the articular disc. These findings can suggest that the vessels are necessary for the vascularization of the articular disc until the synovial membrane is well developed.

The synovial membrane of the TMJ is areolar and only this type of synovial membrane is characterized by the presence of two cell types in its intima layer: macrophage-like type A cells and fibroblast-like type B cells.¹⁰⁻¹⁷ Macrophage-like type A cells contain numerous vesicles, vacuoles, and lysosomes. Their basic function is to engulf, by phagocytosis, the proteins and carbohydrates present in the synovial.^{14,46,47} Fibroblast-like type B cells are ultrastructurally characterized by the presence of a well-developed rough endoplasmic reticulum with numerous secretory granules.^{12,13,16-19} Macrophage-like type A and fibroblast-like type B cells in the synovial membrane can be identified by immunohistochemistry and transmission electron microscopy in fetuses, adults, and animals before and after birth.^{10-17,30} Ikeda *et al.*⁴⁶ reported that macrophage-like type A cells in the synovial membrane of the TMJ of rats were only visible after birth. However, Sabú *et al.*⁴⁸ found macrophage-like type A and fibroblast-like type B cells only during the gestational period.

Our findings confirm those of other authors, including^{10-14,16,17,19} who found both macrophage-like type A and fibroblast-like type B cells in the synovial membrane before birth. During the 12th week of development of the peripheral parts of the inferior articular cavity, macrophage-like type A and fibroblast-like type B cells were marked by immunohistochemical reaction with the antibodies CD68 and Hsp27, respectively. Fibroblast-like type B cells were also marked in the peripheral portions of the superior articular cavity. These findings could suggest that from the twelfth week of development, with the articular cavities well formed, the synovial membrane is functional and the vessels are only seen in the peripheral areas of the articular disc. The synovial villi form from the thirteenth week of development. There is also a third type of cell not yet fully studied called an intermediate lining cell.⁴⁸⁻⁵¹ More studies are necessary to determine the morphology and ultrastructure of these cells.

This study demonstrates that development of the synovial membrane depends on the fibroblast-like type B cells located on the surface of the synovial membrane and macrophage-like type A cells that supply the

vessels. Our results also suggest that in human specimens at twelve weeks of development, the articular cavities are well formed and the synovial membrane begins to be functional.

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