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Cavitation processes in a space filled with loose mesenchymal tissues: a comparison between the retrosternal space and the middle ear tympanic cavity in human fetuses

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Background: *During the expansion of the pleural cavity in early fetuses, a thick sheet of loose mesenchymal tissue (SLMT) appears between the lung bud and body wall. Subsequently, the growing lung bud invades into the SLMT and the latter becomes fragmented to disappear. To compare this with the tympanic cavity filled with loose mesenchymal tissues, the present study aimed to demonstrate the development, establishment, and breaking of the SLMT in the retrosternal space.*

Materials and methods: *Although the retrosternal tissue was almost absent or very thin at 7 weeks, the SLMT appeared behind the manubrium sterni at 8 weeks. Accordingly, at 9–10 weeks, cavitation occurred in the SLMT to expand the pleural cavity. Therefore, the volume of SLMT was not determined by the adjacent structures such as the pericardium and sternum. Likewise, mesenchymal tissues filling the middle ear disappeared after 26 weeks.*

Results: *There were considerable individual variations in the timing of beginning and location of the tympanic cavitation. However, in contrast to the retrosternal SLMT, the volume of the future tympanic cavity is determined by the adjacent hard tissue and tympanic membrane much earlier than the cavitation. The mesenchymal tissue carried abundant vessels in the middle ear but none or few veins in the retrosternal SLMT.*

Conclusions: *The concept that the lung bud invades into the splanchnic mesoderm to expand the pleural cavity seems oversimplified. Mechanical stresses from the pleural cavity might induce retrosternal cavitation, while a loss in blood supply might cause tympanic cavitation.* (Folia Morphol 2023; 82, 2: 332–338)

Key words: cavitation, retrosternal space, pleural cavity, middle ear, tympanic cavity, human fetuses

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INTRODUCTION

According to Gray's Anatomy [4], pleural cavity expansion is related to lung growth and invasion into the splanchnic mesoderm. However, a previous study [7] showed that the process of the pleural expansion is not simple; during the early development of the lung (at 5–6 weeks or stages of 11–23 mm crown-rump length [CRL]), morphology changes drastically in and along the inner aspect of the lateral and posterior thoracic wall. First, the lung bud attaches to the rib, intercostal muscle, and mesonephros with no or little pleural space. A thick sheet of loose mesenchymal tissue (SLMT) then appears between the lung bud and body wall. Finally, the growing lung bud invades into the SLMT, causing fragmentation to disappearance of the SLMT. The coelomic mesothelium or initial pleura lines covers the SLMT in the second phase. Although transient, the SLMT is not a splanchnic mesoderm itself, but a highly derived structure. Furthermore, contrary to literature [11], the bilateral bottoms of the initial pleural cavity alongside the adrenal do not correspond to the pneumatoenteric recess of the lesser sac [7].

Due to the large increase in the adrenal volume at 5–6 weeks, the transient appearance of the SLMT seemed difficult to be identified in the posterolateral thoracic cavity. In contrast, connective tissue growth of the anterior thoracic wall can be easily compared between the different stages. The lung anterior parts (segments III and VIII) extend anteriorly into a thin retrosternal space between the sternum and pericardium in early fetuses [3]. This study first aimed to clearly demonstrate the transient SLMT behind the sternum.

Cavitation in a space filled with loose mesenchymal tissues is not a limited morphology in the SLMT along the growing pleural cavity. It is well known that the ear ossicles and their joints are embedded in loose mesenchymal tissues. However, there might be no clear description of when the mesenchymal tissue disappears in the tympanic cavity. According to Bast and Anson [1, 2], restricted cavitation occurs near the tympanic membrane at 18–21 weeks, and the mesenchymal packing disappears except for recesses of the tympanic cavity by 24 weeks. However, Rodríguez-Vázquez et al. [14] showed that the mesenchymal tissues around the incudomalleolar joint disappear around 34 weeks. This study also aimed to revisit the tympanic cavitation and compare it with the retrosternal space. This comparison would provide better understanding of possible heterogeneity in the cavitation process since, in contrast to the middle ear, the retrosternal space seems to have no definite marginal structures except for the sternum. This comparison would provide better understanding of possible heterogeneity in the cavitation process since, in contrast to the middle ear, the retrosternal space seems to have no definite marginal structures except for the sternum.

MATERIALS AND METHODS

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in 2013). For observations of the retrosternal space, we used histological sections from 24 human fetuses (7–11 weeks; CRL, 25–61 mm); 10 fetuses of CRL 25–35 mm (7–9 weeks) and 14 fetuses of CRL 46–61 mm (10–11 weeks). Most sections (15 fetuses) were sagittal to visualise the entire length of the sternum. For observations of the tympanic cavitation, we used sagittal sections from 14 human fetus heads (16–37 weeks; CRL 125–310 mm) similar to that used in a recent study [12].

All the aforementioned sections (paraffin-embedded) were part of the large collection kept at the Department of Anatomy of the Universidad Complutense, Madrid, and the embryos were obtained from miscarriages and ectopic pregnancies from the Department of Obstetrics of the University. No information was available on the genetic background of the embryos and/or abortion. The sections were stained with haematoxylin and eosin, Azan or silver impregnation. This study was approved by the Ethics Committee of Complutense University (B08/374). Development of the sternum in early fetuses [13] and sagittal section morphology of the thoracic region [8, 9] are previously reported.

Most images of histology were taken with a Nikon Eclipse 80, whereas photographs at ultra-low magnification (objective lens $< 1\times$) were obtained using a high-grade flat scanner with translucent illumination (Epson scanner GTX970).

RESULTS

Observations of the retrosternal space and pleural cavity

The length and width of the retrosternal mesenchymal tissues are shown in sagittal (Figs. 1, 2) and horizontal (Fig. 3) sections, respectively. At 7 weeks, the retrosternal space or loose tissue was very thin and limited to bilateral sites around the

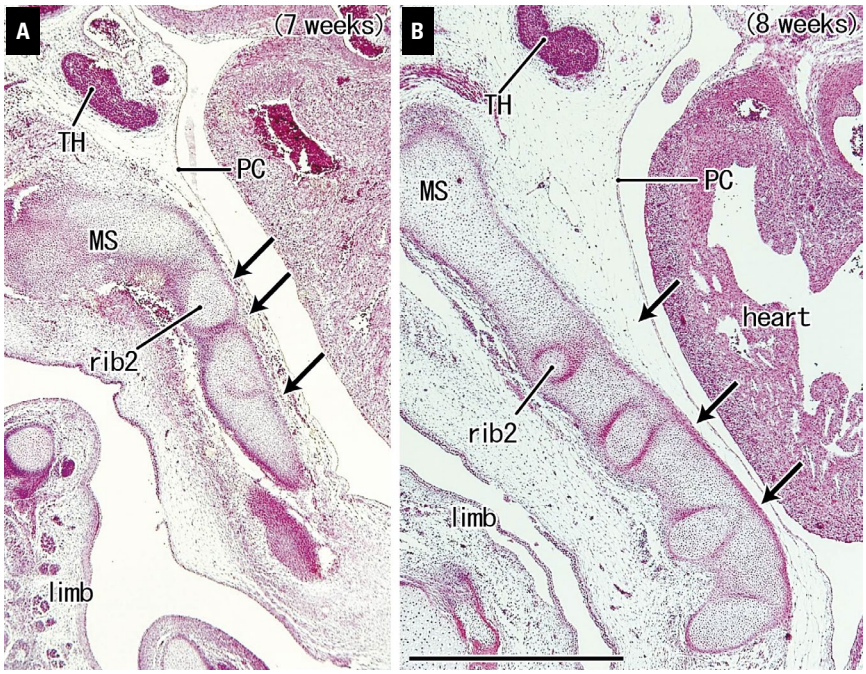

Figure 1. A, B. Retrosternal space observed in sagittal sections. Haematoxylin and eosin staining. Left side of the manubrium sterni (MS) and thymus (TH); **A.** A fetus of 28 mm crown-rump length (7 weeks); **B.** 35 mm crown-rump length (8 weeks). A thin tissue layer (arrows) is compressed between the sternum and pericardium (PC). These sections are medial to the course of the internal thoracic artery. Panels A and B were prepared at the same magnification (scale bar in panel B, 1 mm).

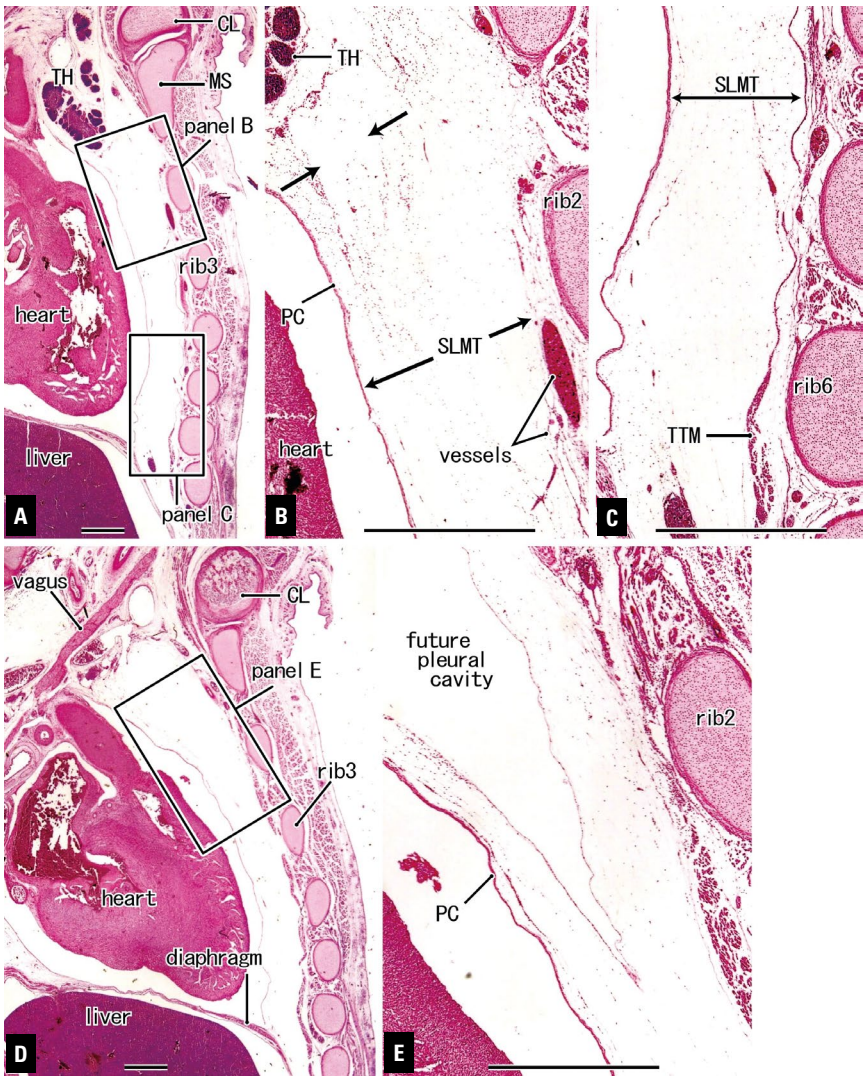

Figure 2. A–E. Thick mesenchymal tissues behind the sternum. Haematoxylin and eosin staining. A fetus of 59 mm crown-rump length (10 weeks). Sagittal sections containing the thymus (TH) and right sternoclavicular joint. Panel A is 0.6 mm medial to panel D. Panels B and C (or panel E) show higher magnification views of squares in panel A (or panel D). A thick sheet-like mesenchymal tissue (SMLT) is sandwiched between the rib and pericardium (PC). Arrows in panel B indicate a small space suggesting the cavitation for the future pleural cavity. Likewise, panel E exhibits a definite space enclosed by a fascia in the anterior side of the right vagus nerve (vagus); CL — clavicle; MS — manubrium sterni; TTM — transversus thoracic muscle. Panels A and D were prepared at the same magnification (all scale bars, 1 mm).

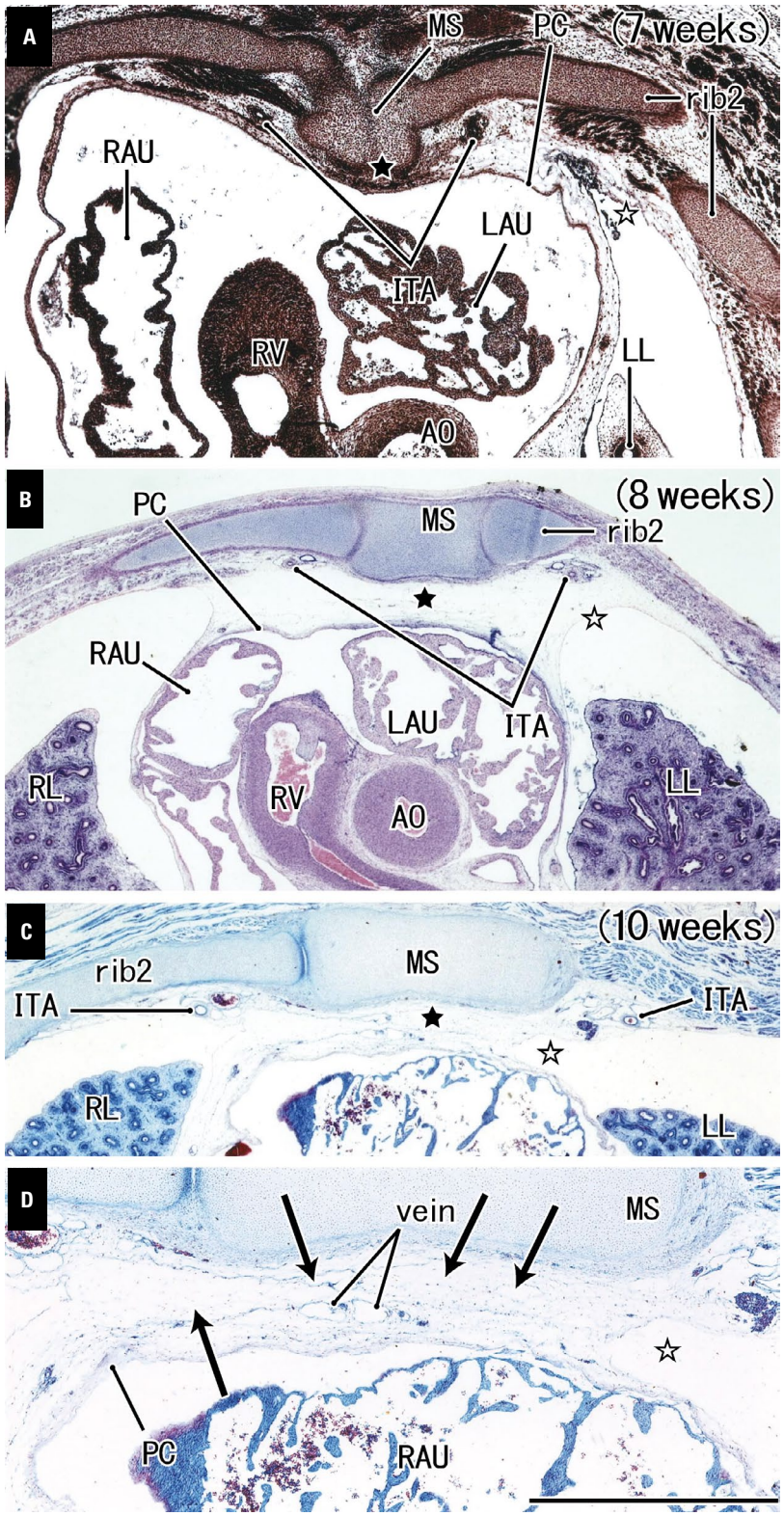

Figure 3. A–D. Change in thickness of the retrosternal mesenchymal tissue observed in horizontal sections at the level of the second rib. Panel A (silver impregnation), a fetus of 26 mm crown-rump length (7 weeks); panel B (haematoxylin and eosin staining), 35 mm crown-rump length (8 weeks); panels C and D (Azan staining), 64 mm crown-rump length (10 weeks). The mesenchymal tissue (black star) behind the manubrium sterni (MS) is thin in panel A, but thick in panels B and C. The anterior end of the left pleural cavity (open star) migrates medially from panel A to panel C. Panel D is a higher magnification view of the central part in panel C. In the retrosternal mesenchymal tissue, fasciae enclose four spaces suggesting the future cavity (arrows in panel D). Panels A–C were prepared at the same magnification (scale bars in panel A and D, 1 mm); AO — ascending aorta; ITA — internal thoracic artery; LL — left lung; PC — pericardium; RAU — right auricle; RL — right lung; RV — right ventricle.

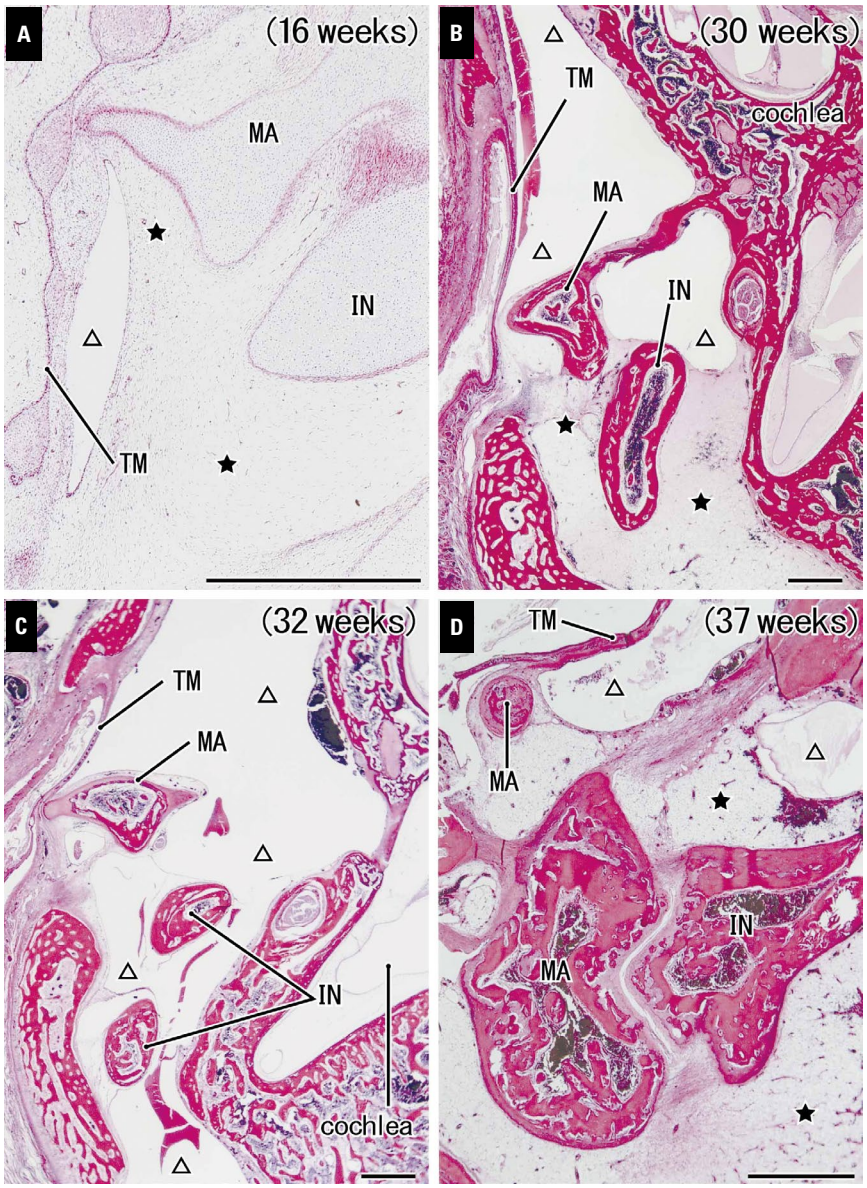

Figure 4. A–D. Cavitation in the middle ear for comparison with the retrosternal space. Haematoxylin and eosin staining. Panel A, a fetus of 125 mm crown-rump length (16 weeks); panel B, 256 mm crown-rump length (30 weeks); panel C, 272 mm crown--rump length (32 weeks); panel D, 310 mm (37 weeks). At 16 weeks, the middle ear is filled with mesenchymal tissues (stars) except for a slender cavity (triangle) near the tympanic membrane (TM). In panel B, the cavitation (triangles) reached half of the middle ear, but the presumed lateral recess is filled with mesenchymal tissues (stars). In panel C, the cavitation (triangles) reached most parts of the middle ear. In panel D showing the largest specimen in this figure, the malleus (MA) and incus (IN) are still embedded in mesenchymal tissues (stars). All scale bars, 1 mm.

internal thoracic vessels (Figs. 1A; 3A). Thus, the pericardium appeared attached to the sternum. The anterior end of the lung did not reach a site in the medial side of the right atrium. However, at 8 weeks (Figs. 1B; 3B), the retrosternal space was identified as the SLMT. The thickening of the SLMT most likely started at the upper site near the thymus and progressed to the lower site near the diaphragm. At 9–10 weeks (Figs. 2; 3C), a cavitation occurred in the upper and lateral parts of the SLMT and connected with the pleural cavity. The cavitation was identified as small or large, multiple fascial spaces (Figs. 2B, E; 3D). Simultaneously, the anterior end of the lung approached the sternum but it did not reach the SLMT or manubrium sterni. Therefore, the lung did not expel mesenchymal tissues of the SLMT from the retrosternal space. The retrosternal SLMT rarely contained vessels except for the internal thoracic artery and vein. The pericardium was thick behind the sternum in contrast to the thin pleura at the anteromedial end.

Observation of a mesenchymal packing in the middle ear

The middle ear was filled with loose mesenchymal tissues except for a site near the tympanic membrane in 5 fetuses at 16–25 weeks (Fig. 4A). In a fetus of 210 mm CRL (26 weeks), the mesenchymal tissue was restricted to the lateral recess of the tympanic cavity. However, the residual 8 fetuses at 27–37 weeks exhibited variations; the cavitation finished at 32 weeks (Fig. 4C), while the incudomalleolar joint was embedded in the loose tissue at 37 weeks (Fig. 4D).

Furthermore, the lateral recess was filled with loose mesenchymal tissues (Fig. 4B), while the incudomalleolar joint tended to be surrounded by the loose tissue. The mesenchymal tissue contained abundant capillaries. The mesenchymal tissues in the middle ear were always delimited by the bones, cartilages, and tympanic membrane. Therefore, a shape of the future tympanic cavity was established much earlier than the cavitation.

DISCUSSION

It is well known that the proliferated epithelial cells temporally obliterate the lumen of the duodenum during cavitation or recanalization [6]. Similarly, even in late-term fetuses, the laryngeal cavity is temporally obliterated by the proliferated epithelial cells before recanalisation for switching of the airway [15]. In the duodenum and larynx, the outer margin of the proliferated epithelia is determined by the duodenal and laryngeal walls, respectively. The definite walls contain muscles and are delimited by the thick basal lamina. In both the middle ear and retrosternal space, the cavitation does not occur in the proliferated epithelial cell mass but occurs in a packing of loose mesenchymal tissues. Moreover, the process seemed to be significantly different between the middle ear and retrosternal space (see below).

Any margins of the future tympanic cavity, i.e., the bones, cartilages, and the tympanic membrane, were fully developed until the cavitation occurs. Thus, the shape of the future tympanic cavity was determined in the middle ear. Indeed, the retrosternal space was also determined by a space between the sternum and pericardium. Since the latter was not a hard tissue, the mesenchymal tissue or SLMT became drastically thick at 7–8 weeks, possibly due to a mechanical stress imposed by the anteriorly expanding lung. The thickness of the SLMT might be adjusted to the future thickness of the retrosternal pleural cavity. The mechanical stress might induce cell death in the SLMT to provide multiple fascial space inside. Therefore, the concept that the lung bud invades into the splanchnic mesoderm to expand the pleural cavity seems an oversimplification. In fact, the growing lung neither invades a thin mesenchymal tissue along the body wall nor the SLMT. Moreover, the interpretation that the lung determines the size of the SLMT also seems erroneous. The SLMT along the base of the lung was not as thick as that in the retrosternal space and the former receives an inferior edge of the pleura. Hence, the size of the SLMT might not depend on the marginal shape and thickness of the pleural cavity.

Overall, before cavitation, the middle ear mesenchymal packing was delimited by hard tissues, whereas the SLMT had a variable size and shape depending on the stage. In contrast to the middle ear mesenchyme, none or few vessels developed in the SLMT; the former contributes to blood supply to the contents (ear ossicles and nerves), but the SLMT does not seem to have a role in blood supply except in the internal thoracic artery and vein. A mechanical stress from the expanding pleural cavity (not from the lung itself) might induce retrosternal cavitation, while blood supply loss might cause tympanic cavitation. The SLMT was somewhat similar to a mass of loose mesenchymal tissues around the developing kidney [5, 10]; however, the loose tissues led to the development of a thick renal fascia that surrounds the kidney. Therefore, the perirenal space was quite different from the pleural cavity in the development. Finally, the present study demonstrates a significant individual variation in the establishment of cavitation in the middle ear; the disappearance of the mesenchymal tissue packing was likely to be delayed after birth.

With or without definite marginal structures like bones or muscular walls, there seemed to be different triggers, processes and end-stage morphologies in the cavitation. Recent techniques to identify cell death would reveal a difference in signal transduction between the suggested two-types of triggers for cavitation: a mechanical stress in the retrosternal space and a possible ischaemia in the middle ear.

CONCLUSIONS

The concept that the lung bud invades into the splanchnic mesoderm to expand the pleural cavity seems oversimplified. Mechanical stresses from the pleural cavity might induce retrosternal cavitation, while a loss in blood supply might cause tympanic cavitation.

Conflict of interest: None declared

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