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Research article

Transient connection between the vestibular aqueduct and utricle: A study using sagittal sections of human embryonic heads

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ABSTRACT

Background: The aqeductus vestibuli (aqueduct) is believed to connect to the saccule in embryos and adults. However, in embryos, the saccule and utricle are known to communicate widely to provide a common endolymph space “atrium”.
Methods: Using sagittal histological sections from five embryos (crown-rump length or CRL, 14–21 mm), nine early fetuses (CRL 24–35 mm) and 12 midterm and near-term fetuses (CRL 82–272 mm), we revisited the development and growth of the human ear aqueduct.
Results: The atrium took on a thick tube-like appearance as an antero-inferior continuation of the aqueduct, but soon divided into multiple gulfs. Most of the gulfs corresponded to the ampullae of semicircular ducts, while one gulf at the antero-medio-inferior corner corresponded to the future saccule. Notably, in eight of the 14 embryos and early fetuses, the aqueduct ended at the utricle near the primitive ampulla of the anterior (superior) or posterior semicircular duct. Conversely, an embryo of CRL 21 mm was the smallest specimen in which the aqueduct joined the gulf-like saccule. At midterm and near-term, the growing perilymph space separated the aqueduct from the utricle and appeared to push the aqueduct toward the saccule. A topographical change occurred between the embryonic superiorly located utricle and the inferiorly-located saccule to create the antero-posterior arrangement in adults.
Conclusions: Consequently, the vestibular end of the aqueduct was most likely to migrate anteriorly from the utricle to the saccule at 6–8 weeks possibly due to differential growth of the endothelium. Previous reconstructions of the embryonic aqueduct might be biased by the adult morphology.

1. Introduction

The endolymphatic duct of the ear vestibule (aqueductus vestibuli or, simply, aqueduct) originates from an endolymphatic sac near the sigmoid sinus at the posterior cranial fossa and passes through the petrosal bone to end at the saccule of the ear vestibule. Anson (1934) considered the aqueduct as a phylogenetic remnant of the ectoderm invagination although the real invagination is absent in mammalian embryos. The connection of the aqueduct to the saccule was described in embryos and early fetuses according to classical histological studies (Streeter, 1906, 1918; Anson, 1934; O'Rahilly and Müller, 1983). Likewise, recent excellent studies using PC-assisted 3D-reconstruction confirmed the aqueduct-saccule connection at the early stage (Arnold and Lang, 2001; Jeffery and Spoor, 2004; Yasuda et al., 2007; Kagurasho et al., 2012; Toyoda et al., 2015; Ishikawa et al., 2018).

According to Toyoda et al. (2015), the connection became visible in all specimens examined after Carnegie Stage (CS) 22 or approximately 7 weeks of gestation age (GA) despite early differentiation of

Abbreviations: IJV, internal jugular vein; LSD, lateral semicircular duct; MC, Meckel's cartilage; peri, perilymph space; PTT, pharyngotympanic tube; SAC, saccule; SSD, superior semicircular duct; PSD, posterior semicircular duct; TG, trigeminal ganglion; UT, utricle; USD, utriculosaccular duct; VII, facial nerve and its ganglion; VIII, vestibulocochlear nerve and its ganglion; IX, glossopharyngeal nerve and its ganglion; X, vagus nerve and its ganglion

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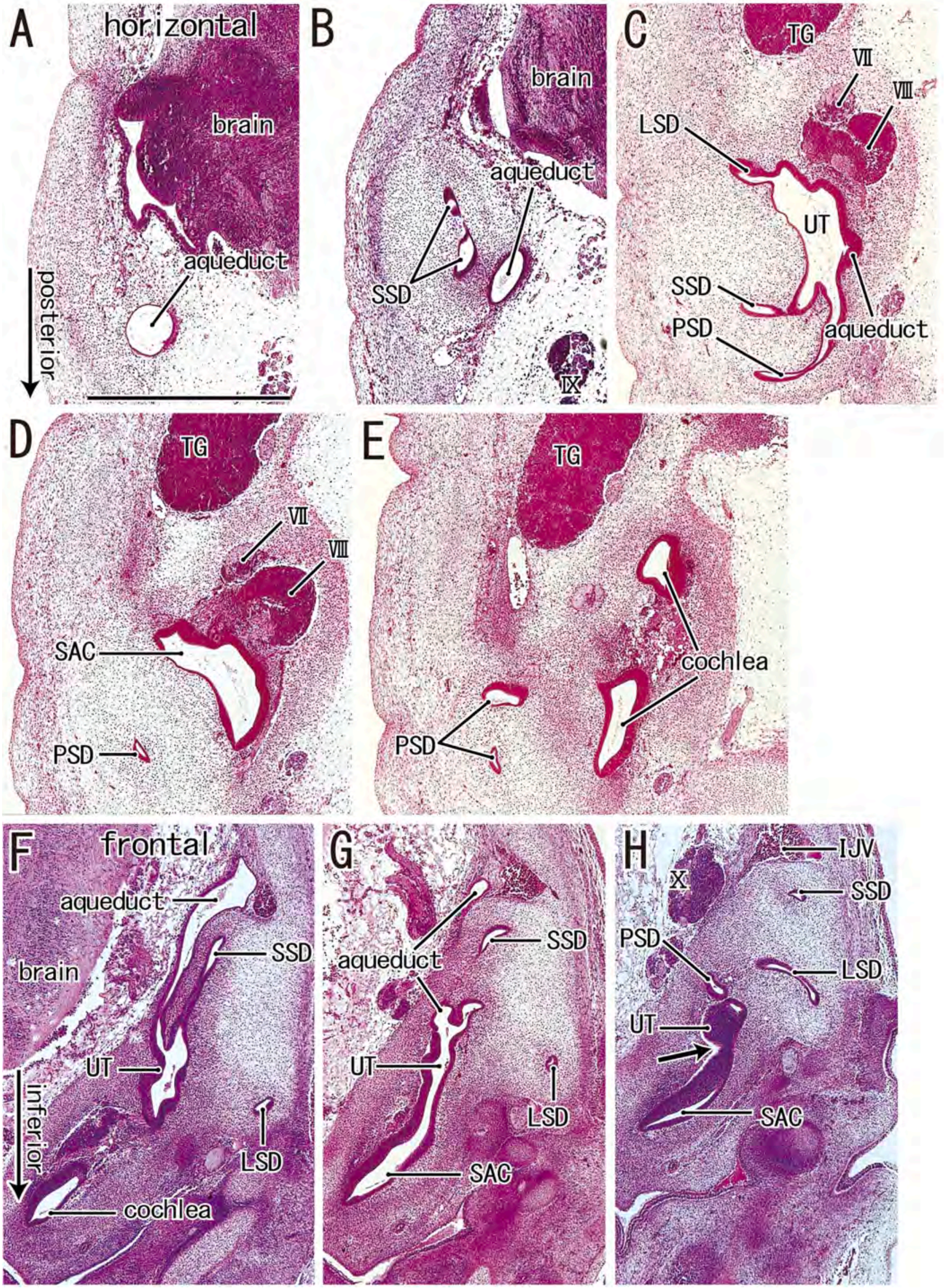
A
horizontal
brain
aqueduct
posterior
B
brain
aqueduct
SSD
IX
C
TG
VII
VIII
LSD
UT
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PSD
aqueduct
D
TG
VII
VIII
SAC
PSD
E
TG
cochlea
PSD
F
frontal
aqueduct
SSD
brain
UT
inferior
LSD
cochlea
G
aqueduct
SSD
UT
LSD
SAC
H
IJV
X
SSD
PSD
LSD
UT
SAC

Fig. 1. Evaluation of sectional planes: horizontal and frontal planes of embryos of 16 mm CRL (CS 19). Panels A-E display horizontal sections containing the vestibule and cochlea, while panels F-H exhibit frontal sections. Intervals between panels are 0.6 mm (A-B), 3.0 mm (B-C), 0.3 mm (C-D), 0.6 mm (D-E) and 0.08 mm (F-G and G-H). Terminals of the aqueduct and semicircular ducts are together shown in a single horizontal section (panel C) or many frontal sections (panels F-H). In horizontal sections, a border between the utricle and saccule was difficult to identify since changes in shape occur in the wall extending almost along the supero-inferior axis. Likewise, also in frontal sections, the utricle and saccule are regarded as a continuous long tube since the border area was restricted within a few sections (arrow in panel H). All panels were prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviation.

the otic vesicle into the aqueduct and cochlear duct at CS17 or GA 5 weeks. However, the question remains as to which structure the aqueduct connects to in the late embryonic period. In fact, Arnold and Lang (2001) demonstrated the aqueduct connecting to a border between the utricle and saccule at CS19 (GA 6 weeks). Yasuda et al. (2007) called a common primordium of the saccule and utricle as the "atrium" and they described that the atrium received the aqueduct. According to them, the atrium differentiated into the saccule and utricle at CS 20 (almost 20 mm CRL; GA 6 weeks). In this context of the saccule-aqueduct connection, to our regret, there is little information about the topographical relation between the aqueduct and differentiating utricle and saccule.

Using histological sections from embryos and early fetuses (GA 6–8 weeks), this study aimed to revisit development and growth of the ear vestibule with special reference to the connecting site of the aqueduct. Another point requiring clarification was in the perilymphatic spaces

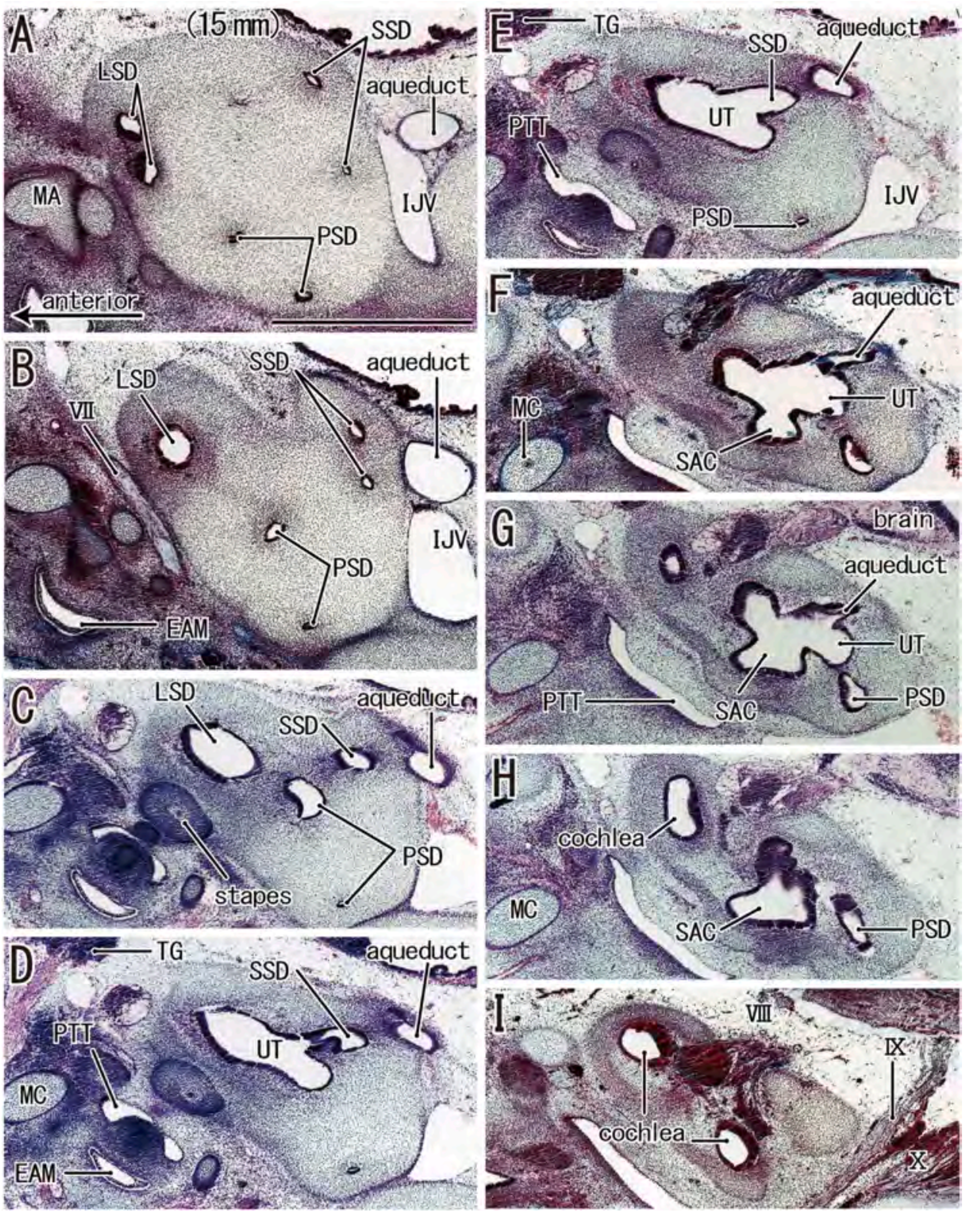

Fig. 2. The saccule as a large gulf of the utricle in an embryo with a CRL of 15 mm (CS 19). Sagittal sections. HE staining. The smallest specimen examined in the present study. The otic capsule is composed of a dense mesenchymal tissue with a clear margin. Panel A (or panel I) displays the most lateral (or medial) plane in the figure. Intervals between panels are 0.1 mm (A-B, B-C), 0.05 mm (C-D, D-E, E-F, F-G, G-H) and 0.2 mm (H-I). The aqueduct origin is identified as a large sack adjacent to the internal jugular vein (IJV; panels A and B). The superior, lateral and posterior semicircular ducts (SSD, LSD, and PSD, respectively) join together to provide a large utricle (UT; panels A-E), with the latter receiving the aqueduct at the posterosuperior angle near the SSD terminal (panels F and G). The saccule (SAC) is identified as a large gulf of the inferior aspect of the utricle (panels F and G) and continues to the cochlea (panels H and I). All panels were prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviations.

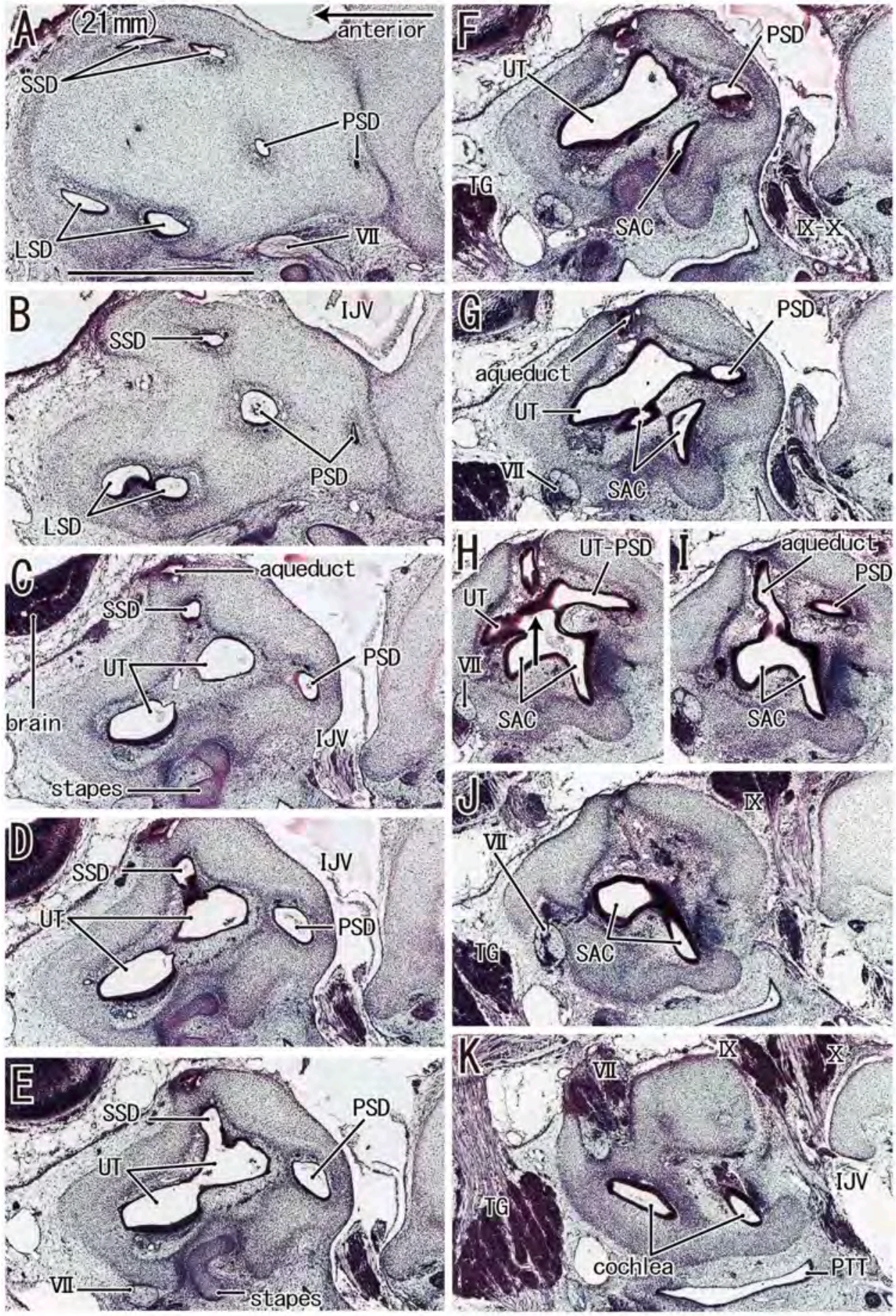

Fig. 3. The smallest specimen in which the saccule receives the aqueduct (CRL, 21 mm; CS 21). Sagittal sections. HE staining. The otic capsule is composed of a dense mesenchymal tissue with a clear margin. Panel A (or panel K) displays the most lateral (or medial) plane in the figure. Intervals between panels are 0.15 mm (A-B, B-C), 0.05 mm (C-D), 0.1 mm (D-E, E-F, F-G), 0.05 mm (G-H, H-I, I-J) and 0.15 mm (J-K). The aqueduct origin is identified as a thin tube extending along the mediolateral axis (panels C-G) and is located on the anterior side of the internal jugular vein (IJV; panel D). The superior, lateral, and posterior semicircular ducts (SSD, LSD, and PSD, respectively) join together to provide a large utricle (UT; panels A-H). The saccule (SAC) is identified as an inferior gulf of the utricle (panels G-H) and, in the immediate medial side of the PSD terminal (arrow in panel H), the saccule receives the aqueduct (panel I). The saccule continues to the cochlea (panels J and H). All panels were prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviations.

expanding along and around the aqueduct after GA 10–11 weeks (Ishikawa et al., 2018). In the adult vestibule, the utricle and saccule are located together in a single bony room and, therein, a huge perilymphatic space separates these two endolymphatic spaces (Merchant and Nadol, 2010; Nomura, 2014). We hypothesized that, in midterm and near-term fetuses, the growing perilymphatic space is likely to change the aqueduct-saccule morphology to provide the utericosaccular duct. Therefore, the second aim was to examine the topographical relation between the aqueduct and perilymphatic space using sections of midterm and near-term fetuses.

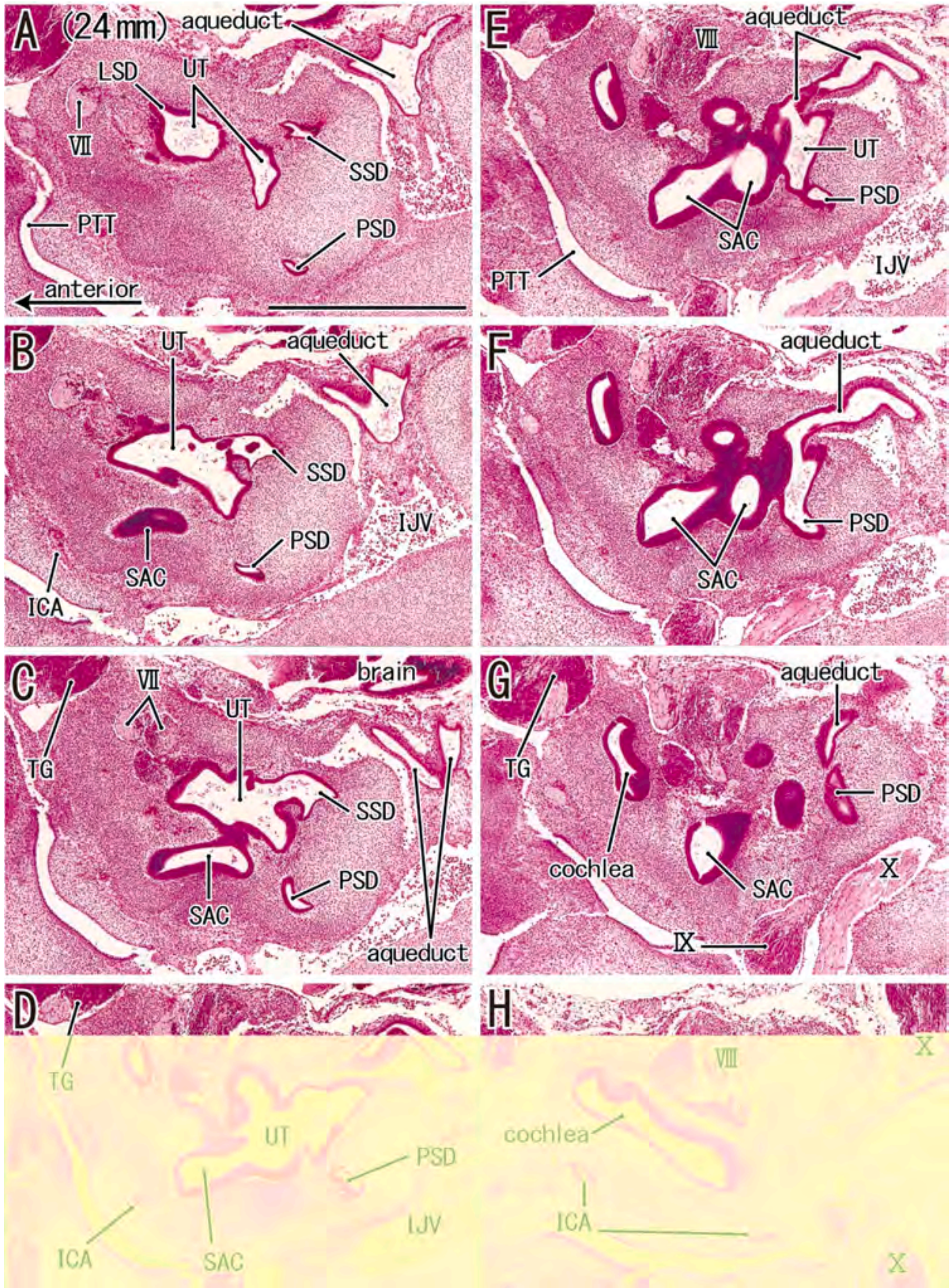

Fig. 4. The saccule as a large gulf at the antero-inferior end of the utricle in an embryo with a CRL of 24 mm (CS 21). Sagittal sections. HE staining. The otic capsule is composed of a dense mesenchymal tissue with a clear margin. Panel A (or panel H) displays the most lateral (or medial) plane in the figure. Intervals between panels are 0.1 mm (A-B, B-C, C-D, D-E), 0.05 mm (E-F), 0.2 mm (F-G) and 0.3 mm (G-H). The aqueduct origin is identified as a thick tube extending along the mediolateral axis (panels A-E) and is located in the lateral side of the internal jugular vein (IJV; panel B). The superior and lateral semicircular ducts (SSD, LSD) meet the large utricle (UT; panels A and B) and the saccule (SAC) is generated by a gulf at the inferior aspect of the utricle (panels C and D). The posterior semicircular duct (PSD) and the aqueduct merge with the posterolateral end of the utricle (panels E and F). The saccule continues to the cochlea (panels G and H). All panels were prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviations.

2. Material and methods

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in 2013). Paraffin-embedded serial histological sections of the human head were obtained from 26 human embryos and fetuses approximately at GA 5–32 weeks (crown-rump length [CRL], 14–272 mm): five of them were embryos at approximately GA 5–6 weeks (CRL 14–21 mm; CS18–21), nine were early fetuses at GA 7–8 weeks (CRL 24–35 mm; CS21–23 or more); seven were midterm fetuses at GA 12–16 weeks (CRL 82–125 mm) and, five were near-term at GA 29–32 weeks (CRL 240–272 mm). The sectional plane was sagittal in all specimens.

The serial sections from specimens at 5–16 weeks GA were part of the large collection kept at the Institute of Embryology, Universidad Complutense Madrid, and were products of miscarriages and ectopic pregnancies managed at the Department of Obstetrics at the university. No information was available for the genetic background of the embryos and/or abortion. These sections in Madrid were stained with hematoxylin and eosin (HE), azan or silver staining. The use of the sections in Madrid was approved by

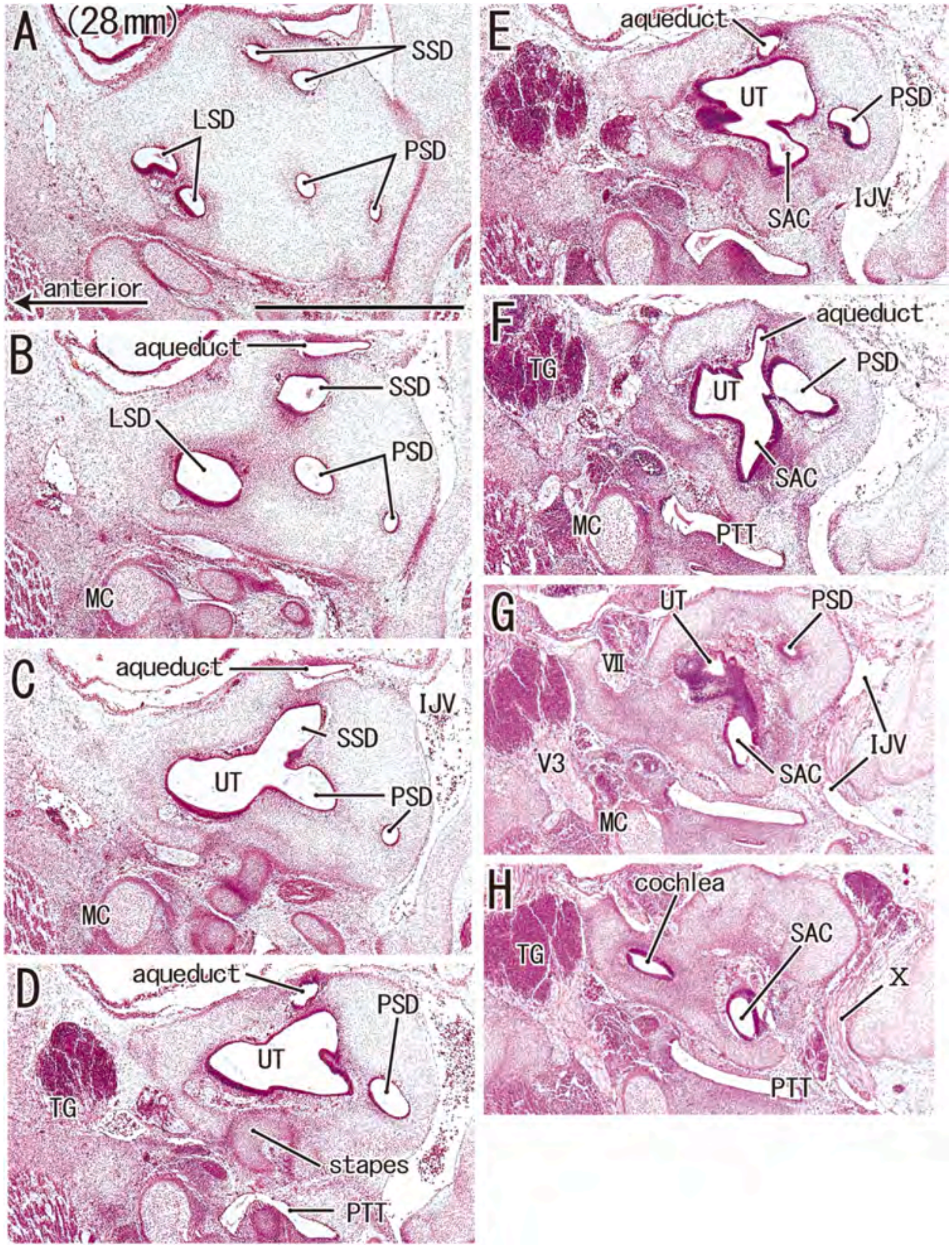

Fig. 5. The largest specimen in which the utricle receives the aqueduct (CRL, 28 mm; CS 23). Sagittal sections. HE staining. Panel A (or panel H) displays the most lateral (or medial) plane in the figure. Intervals between panels are 0.1 mm (A-B, B-C), 0.2 mm (C-D), 0.1 mm (D-E), 0.2 mm (E-F, F-G) and 0.1 mm (G-H). The aqueduct origin is identified as a thin tube adjacent to the internal jugular vein (IJV; panels A-C). The superior, lateral and posterior semicircular ducts (SSD, LSD, and PSD, respectively) join together to provide a large utricle (UT; panels B-D), with the latter receiving the aqueduct at the posterosuperior angle near the PSD terminal (panel F). The saccule (SAC) is identified as a large gulf of the inferior aspect of the utricle (panels E and F) and continues to the cochlea (panels G and H). All panels were prepared at the same magnification (scale bar in panel A, 1 mm). Other abbreviations, see the common abbreviations.

the university ethics committee in Madrid (No. B08/374). The sections were observed and photographed with a Nikon Eclipse 80.

The fetuses at 29–32 weeks GA were part of the collection of the Department of Anatomy, Akita University, Akita, Japan and were donated by their families to the Department in 1975–1985 and preserved in 10% w/w neutral formalin solution for more than 30 years. Data on these specimens included the date of donation and the number of gestational weeks, but did not include the name of the family, obstetrician or hospital or the reason for abortion. The use of these specimens for research was approved by the Akita University Ethics Committee (No. 1428). Before a routine procedure for paraffin embedding, the fetus limb specimens were decalcified by incubating them at room temperature in Plank-Rychlo solution ($AlCl_2/6\ H_2O$, 7.0 w/v%; HCl, 3.6; HCOOH, 4.6) for 3–7 days. We had prepared sagittal sections at 0.1 intervals for our previous studies of the ear (Honkura et al., 2021; Rodríguez-Vázquez et al., 2022). All sections were stained with HE. The sections were observed and photographed with a Nikon Eclipse 80.

In the present description, we will use a term "superior semicircular duct" for the anterior semicircular duct according to previous studies by otologists (e.g., Carey et al., 2000; Takahashi et al., 2012). Prenatally,the anterior duct is located in the superior part of the vestibule. In contrast, the lateral semicircular duct reaches a part much more anterior to the original anterior duct (Kim et al., 2011; Honkura et al., 2021).

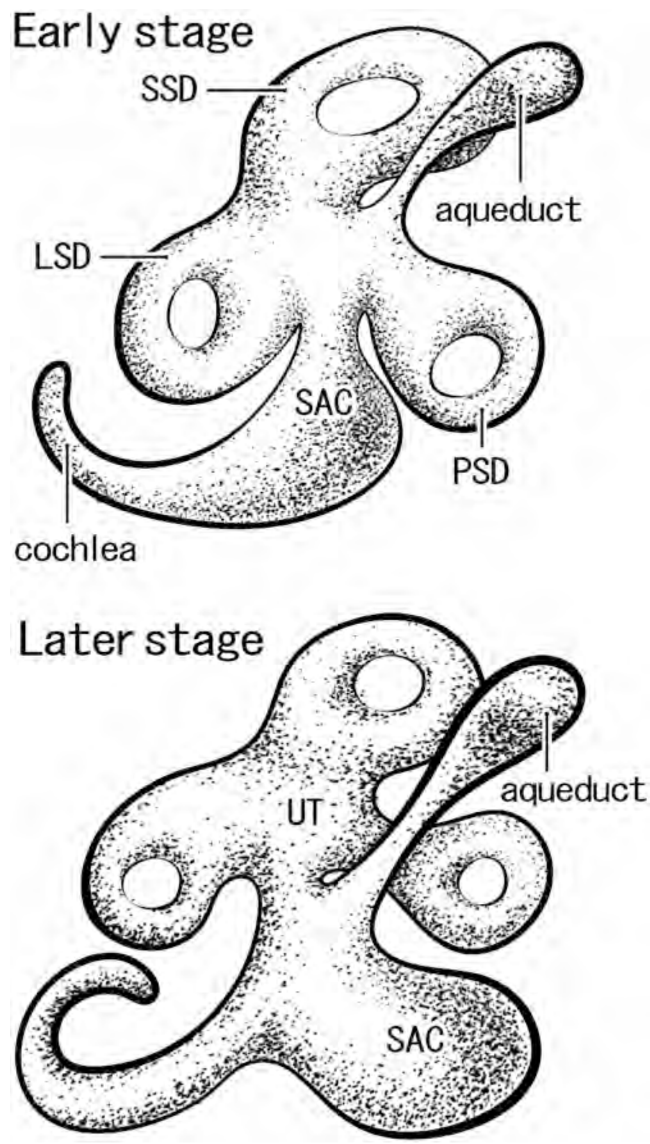

Fig. 6. A change of the vestibular terminal of aqueduct in embryos and early fetuses: a schematic representation. The saccule (SAC), continuous with the cochlea, is identified early as a gulf or protrusion of the utricle (UT). The aqueduct initially ends at the utricle near terminals of the superior and posterior semicircular ducts (SSD, PSD). However, the vestibular terminal of the aqueduct migrates along the epithelial wall to the saccule (SAC). LSD, lateral semicircular duct.

In addition, to make sure of 3D images of the developing vestibule, we observed the other two embryos of 16 mm CRL (CS 19; GA 6 weeks) in Madrid collection: one was cut horizontally, while another for frontal sections. According to observations of these additional sections, we will explain that sagittal sections were the best choice for the present study aim at the first subsection of the Results.

3. Results

3.1. Evaluation of sectional planes

Fig. 1 demonstrates horizontal and frontal sections of embryos of 16 mm CRL for comparison with sagittal sections of the almost same age (Fig. 2; details, see the next subsection). In horizontal sections, the demonstration of a long supero-inferior course of the aqueduct requires many serial sections (0.9 mm in Fig. 1A-C). Terminals of the aqueduct and semicircular ducts could be contained together in a single horizontal section (Fig. 1C). However, the expected changes in shape of the epithelial wall were not evident at a border area between the utricle and saccule (Fig. 1CD) because the changes were most likely to occur at walls along the supero-inferior axis. Likewise, also in frontal sections, the utricle and saccule tended to be regarded as a “continuous long tube” in most sections (Fig. 1G) and this morphology easily connected to a concept of the atrium. A change in shape of the walls at the border area was seen in a limited section (arrow in Fig. 1H). The initial cochlea was seen in the inferior end in horizontal sections (Fig. 1E) and anterior end in frontal sections (Fig. 1F). A scale of the embryonic vestibule was almost 1 mm along the mediolateral and anteroposterior axes and almost 1.5 mm along the supero-inferior axis. Below, to emphasize the border area between the utricle and saccule, we chose sagittal sections (Figs. 3–8).

3.2. Observations of embryos and early fetuses

In two of the five embryos examined (CS18 and 19, CRL 14 mm and 17 mm; Table 1), the atrium of the vestibule, i.e., the initial endolymphatic space for the future utricle and saccule, showed a thick, wavy tube-like shape as an antero-inferior continuation of the

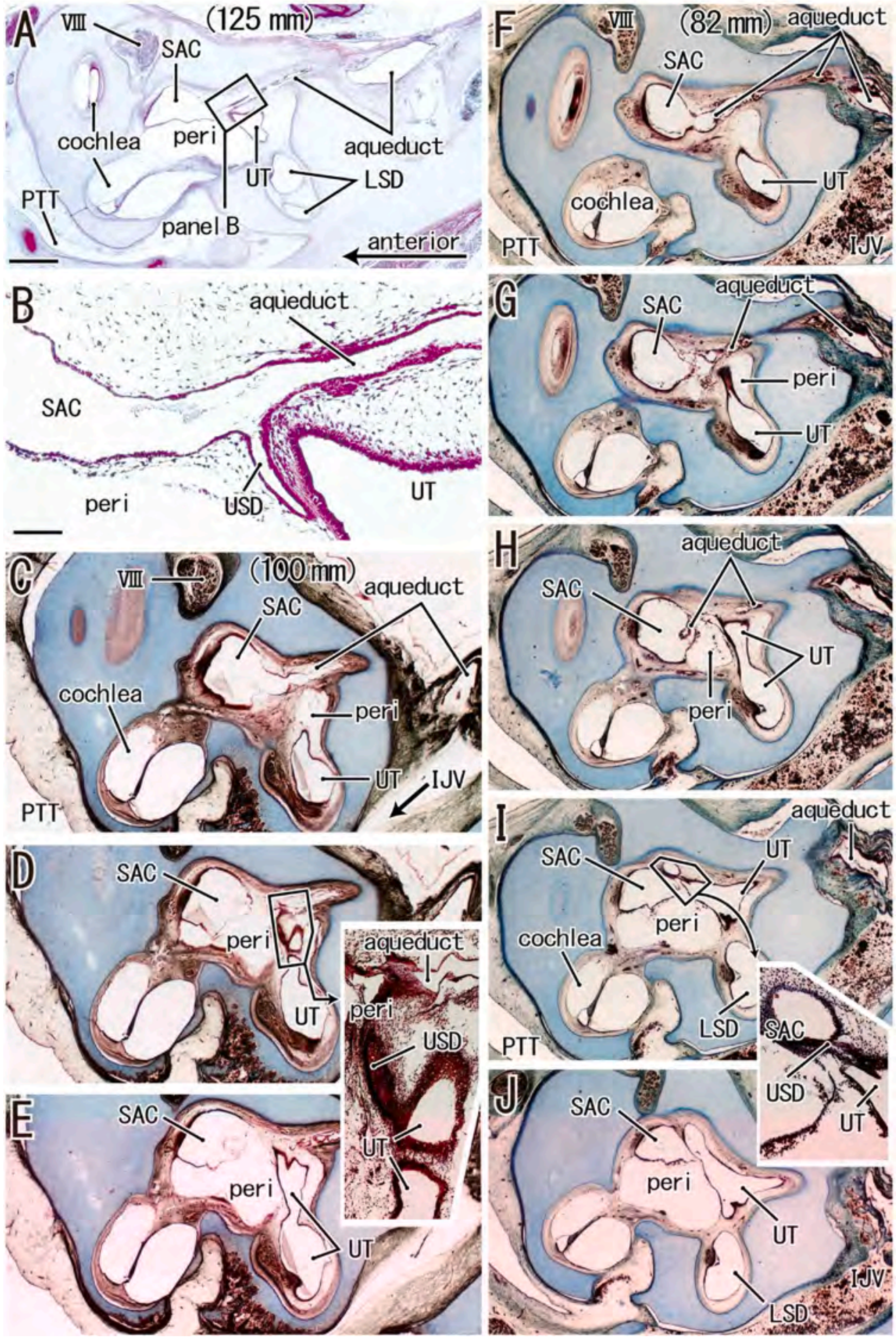

Fig. 7. Aqueduct merging with the saccule and yielding the utriculosaccular duct in three midterm fetuses. Sagittal sections. HE staining (panels A and B) and Azan staining (panels C-J). Panels A and B depict a fetus with a CRL of 125 mm; panels C-E, 100 mm; panels F-J, 82 mm. Intervals between panels are 0.1 mm (C-D), 0.2 mm (D-E, F-G), 0.3 mm (G-H), 0.1 mm (H-I) and 0.2 mm (I-J). In panel A, the aqueduct runs along the utricle (UT) and merges with the saccule (SAC). The utriculosaccular duct (USD) is clearly observed from the aqueduct (panel B). Panel C (or Panel F) displays the most medial plane in panels C-D (or panels F-G). In the three specimens, a perilymph space (peri) appears to push the aqueduct upward to separate the utricle from the saccule (panels A, C and H). The insert between panels D and E (or between panels I and J) is a higher magnification view of the square in panel D (or panel I). In these inserts, the USD is unclear because it was cut along the endothelium. Panels A and C-J were prepared at the same magnification. Two inserts show the same magnification as panel B (scale bars, 1 mm in panel A; 0.1 mm in panel B). Other abbreviations, see the common abbreviations.

aqueduct. The lateral semicircular duct originated from the anterolateral end of the thick tube-like atrium. In contrast, the posterior end of the atrium generated the ampullae of the superior and posterior semicircular ducts. The antero-medio-inferior part of the tube-like atrium continued to the cochlear duct via the undifferentiated saccule. Thus, the saccule simply corresponded to a part of the endolymphatic tube.

In the other three embryos, the atrium took a sac- or balloon-like appearance with multiple gulfs or protrusions (Figs. 2 and 3). Most of the gulfs corresponded to the ampullae of semicircular ducts:

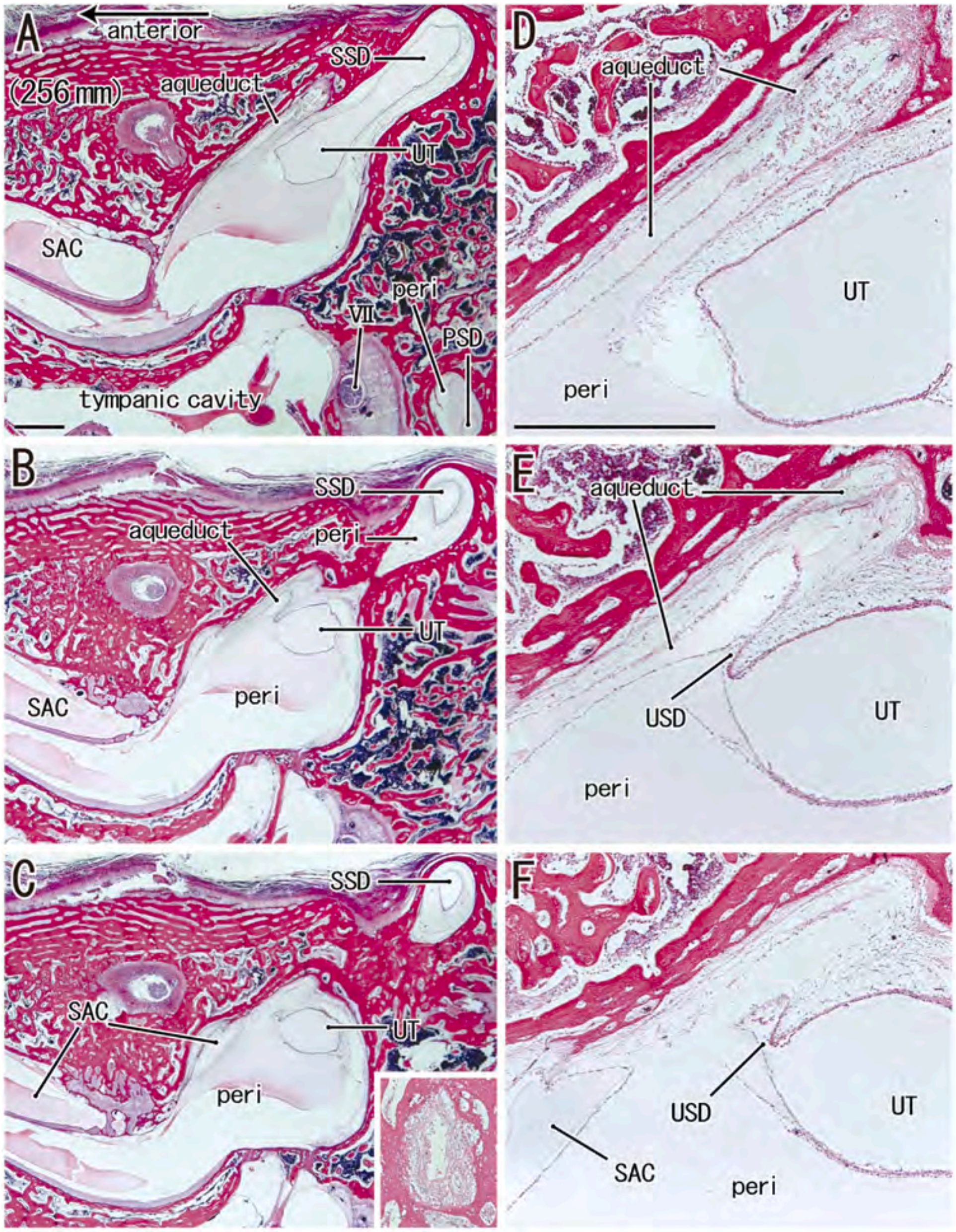

Fig. 8. Aqueduct and enlarged perilymph spaces are observed in a near-term fetus. Sagittal sections. HE staining. Panels D-F are higher magnification views of the centers of panels A-C, respectively. Panel C displays the most lateral plane in the figure. Intervals between panels are 0.5 mm (A-B, B-C). The insert in panel C (magnification, same as panels D-F) pinpointed the aqueduct in a section located 1.6 mm medial to panel A. Note the narrow lumen of the duct. The perilymph spaces (peri) are much larger than the endolymph space of the utricle (UT) and saccule (SAC). The aqueduct enters the bony room near the origin of the superior semicircular duct (SSD; panel A) and extends anteriorly along the bony wall toward the saccule (panels B and C). The utriculosaccular duct (USD) connects the aqueduct with the utricle (panel E). Panels A-C or panels D-F were prepared at the same magnification (scale bars in panels A and D, 1 mm). Other abbreviations, see the common abbreviations.

anterolateral gulfs for the lateral semicircular duct, superior gulfs for the superior duct and inferoposterior gulfs for the posterior duct. Only one gulf at the antero-medio-inferior angle of the balloon-like atrium corresponded to the future saccule and the latter continued to the cochlear duct. Therefore, in these embryos (CS19–21), the atrium had already differentiated into the large utricle and small saccule. Notably, the combination of the large utricle and a gulf-like saccule was also seen in nine early fetuses examined (approximately 7-8 weeks; CRL 24–35 mm).

In the 12 embryos and early fetuses in which the saccule was discriminated from the utricle (Table 1), notably, the aqueduct often ended at the utricle near the primitive ampulla of the superior or posterior semicircular duct (Figs. 2, 4 and 5). The aqueduct was as thick as the ampullae of the superior or posterior semicircular duct. Sometimes, rather than the utricle itself, the aqueduct appeared to merge with the ampulla of the posterior semicircular duct because of a flexion of the utricle at the posterior part (Fig. 4EF). Fig. 4 demonstrated the largest specimen in which the aqueduct joined the utricle. Conversely, an embryo of CRL 21 mm (Fig. 3) was the smallest specimen in which the aqueduct joined the gulf-like saccule. However, even in the specimen of CRL 21 mm, the junction between the aqueduct and saccule was close to and within 0.03 mm medial to the ampulla of the posterior semicircular duct (Fig. 3HI). Fig. 6, a 3D schematic representation, summarizes the topographical change of the vestibular terminal of the aqueduct from the utricle to the saccule.

Table 1
Examination of the aqueduct terminal in embryos and early fetuses.

Specimen	CRL (mm)	CS	Aqueduct terminal (Figures)	Saccule morphology
687	14	18	Atrium near the superior ampulla	Undifferentiated
GV3	15	19	Utricle near the superior ampulla (Fig. 1)	Inferior gulf of UT
#33	17	19	Atrium near the superior ampulla	Undifferentiated
SL	18	19	Utricle	Inferior gulf of UT
Gi20.5	21	21	Saccule (Fig. 2)	Inferior gulf of UT
#43	24	21	Utricle near the posterior ampulla (Fig. 3)	Antero-inferior gulf of UT
#27	25	21	Utricle	Inferior gulf of UT
#41	27	23	Utricle	Antero-inferior gulf of UT
#111	28	23	Utricle near the posterior ampulla (Fig. 4)	Inferior gulf of UT
B32	32	23	Saccule	Anterior gulf of UT
#42	32	30	Saccule	Inferior gulf of UT
#32	35		Saccule	Antero-inferior gulf of UT
C35	35		Saccule	Anterior gulf of UT
H35	35		Saccule	Inferior gulf of UT

UT, urtricle

3.3. Observations of midterm and near-term fetuses

The saccule and utricle were located in a single space surrounded by cartilage at midterm (Fig. 7) and by bones at near-term (Fig. 8). The saccule occupied the anteromedial part of the bony space, while the utricle occupied the posterolateral part. The perilymphatic space had become large as the endolymphatic space of the utricle and saccule at midterm and, it had further increased in size at near-term. At near-term, the perilymphatic space was also well developed in the bony semicircular canal. The aqueduct took a long anterior course along the superior wall of the bony space to join the saccule: this long course was close to the superior wall of the endolymphatic utricle but was separated from the utricle by the perilymphatic space. Moreover, in contrast to a ball-like saccule, the perilymphatic space provided sharp and dull invaginations and flexions of the utricle wall (Fig. 7EI). Therefore, the perilymphatic space appeared to push the aqueduct anterosuperiorly and positioned the endolymphatic utricle away from the aqueduct.

Along the long anterior course, the aqueduct created the thin utericosaccular duct posteriorly or inferiorly (Figs. 7B and 8E). A topographical change occurred between the initially superior utricle and the inferior saccule to create the antero-posterior arrangement in adults. At midterm, the utericosaccular duct was usually unclear because of the relatively thick endothelium cut tangentially (two insets in Fig. 7) in combination with the highly flexed, dentated or caved endolymphatic space of the utricle. The aqueduct continued to the cranial base at midterm, but it was difficult to trace superiorly at near-term: the duct appeared to lose the endothelium (insert in Fig. 8) and the duct space was sometimes obliterated. At midterm and near-term, the perilymphatic space usually contained mesh-like tissues in the vestibule, but the scala of the cochlea was vacant.

4. Discussion

The present study demonstrated that, in embryos and early fetuses, the aqueduct connects to the utricle near the primitive ampulla of the superior or posterior semicircular duct. In fact, a common endolymphatic space “atrium” was identified as a thickened part of the aqueduct. However, the period or stage with the atrium seemed to be very limited. When gulfs or protrusions formed at the atrial wall, the initial saccule was discriminated from the utricle. In an embryo of CRL 21 mm, the smallest specimen in which the aqueduct joined the gulf-like saccule, the meeting site between the aqueduct and saccule was very near the junction between the utricle and posterior semicircular duct. The positional change of the vestibular terminal of the aqueduct (Fig. 6) seemed to occur at a short period (possibly 2–3 days) within 6–8 weeks GA, but the timing varied significantly between specimens. Yasuda et al. (2007) described that the atrium is seen at late CS19 and, at CS20, the saccule and utricle were identifiable: the timing and sequence was consistent with our observations. A previous drawing or 3D reconstruction of embryos in which the aqueduct connects to the saccule might be biased by the adult morphology (see also the Study limitation).

Toyoda et al. (2015) estimated the movement of the external ear using their defined morphometrical parameters, while Kagurasho et al. (2012) pointed out other movements of the inner ear vestibule. They hypothesized that these movements are caused by differential growth (Gasser, 2006). The aqueduct was also most likely to migrate anteriorly from the utricle to the saccule due to differential growth of the endothelium. The semicircular ducts are separated from each other with the movement of the epithelium into the vestibular pouch (Martin and Swanson, 1993). The separated space near the thin epithelium suggests that this movement proceeds independently without interference by the underlying mesenchymal cells (Blechschmidt, 2004). Moreover, during the development of the semicircular duct, the apoptotic body-like fragments are seen in the mesenchyme near the disappearing basement membrane at late CS18 (Represa et al., 1990; Palva et al., 2003; Yasuda et al., 2007). After 8–9 weeks, the growing semicircular ducts seems to provide a change in topographical relation between the utricle and saccule. A gliding in combination with apoptosis might occur between the bony wall and the anteriorly elongated aqueduct.

The long anterior course of the aqueduct was evident along the superior wall of the bony room at midterm and near-term. The growing perilymph space was likely to push the aqueduct anteriorly toward the saccule to force the separation between the aqueduct and utricle. Simultaneously, within a single bony space, the initially superiorly located utricle moves posteriorly, while the inferiorly-located saccule moves anteriorly. During the separation and movement, the initial connection between the aqueduct and utricle remains but elongates as a thin returning duct, i.e., the utericosaccular duct. Therefore, the growing perilymphatic space changed the aqueduct-saccule morphology and provided the utericosaccular duct. Recent beautiful 3D-reconstructions of perilymphatic spaces (e.g., Ishikawa et al., 2018) did not demonstrate the topographical changes especially of the utericosaccular duct possibly due to a limitation of their methods.

Haugas et al. (2010) demonstrated that knockout mice of the transcriptional factor *Gata2* do not carry the perilymphatic space of the ear vestibule. However, according to them, the cochlear perilymphatic space (i.e., the scala) develops in the knockout mice. Therefore, the cascade of perilymphatic space development is different between the cochlea and vestibule. In fact, in the present midterm and near-term fetuses, the perilymphatic space often contained mesh-like tissues in the vestibule, but the scala was vacant. Based on this context, our previous study suggested that a leakage of the cochlear duct endolymph with high osmolarity is likely to cause cavitation of the scala (Kim et al., 2011).

Lastly, the aqueduct at near term was thin and sometimes obliterated. Likewise, in references and textbooks, it was difficult to find a histological demonstration of the fully expanded duct in adults: Figs. 12–36 in Merchant and Nadol (2010) might be a rare exception. We doubt the functional significance of the aqueduct after birth.

Conclusively, the vestibular end of the aqueduct was most likely to migrate antero-inferiorly from the utricle to the saccule at 6–8 weeks possibly due to differential growth of the endothelium. Previous reconstructions of the embryonic aqueduct might be biased by the adult morphology.

Study limitation: Although we evaluated that sagittal sections were the best choice for the research aim, frontal sections had an advantage to show a long tube-like atrium (an initial form of the utricle and saccule; see the Introduction). Likewise, we emphasized "anterior" migration of the aqueduct terminal according to observations of sagittal sections, but frontal sections let us know the migration directed inferomedial rather than anterior (Fig. 1F-H). People may consider a lack of 3D reconstruction as a major limitation of the present study. However, at each step of the scan work of histological sections for PC-assisted 3D reconstruction, a researcher usually gives a "color" to the utricle and another color to the saccule. In this process, the identification is not based on a "result" of reconstruction but a working hypothesis. Therefore, the reliability of reconstruction depends on the initial identification. When they do not find a possibility that the early aqueduct is likely to join the utricle, there is no way to identify a large vestibular room receiving the aqueduct as the utricle even on excellent 3D images.

Ethical statement

The study was performed in accordance with the provisions of the Declaration of Helsinki 1995 (as revised in 2013). The use of the sections in Madrid was approved by the university ethics committee in Madrid (No. B08/374).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Anson, B.J., 1934. The early relation of the auditory vesicle to the ectoderm in human embryos. Anat. Rec. 58, 127–137.
Arnold, W.H., Lang, T., 2001. Development of the membranous labyrinth of human embryos and fetuses using computer aided 3D-reconstruction. Ann. Anat. 183, 61–66.
Blechschmidt, E., 2004. The Ontogenetic Basis of Human Anatomy: Biodynamic Approach to Developments from Conception to Adulthood. North Atlantic Book's, Berkeley, CA, USA.
Carey, J.P., Minor, L.B., Nager, G.T., 2000. Dehiscence or thinning of bone overlying the superior semicircular canal in a temporal bone surgery. Arch. Otolaryngol. Head. Neck Surg. 126, 137–147.
Gasser, R.F., 2006. Evidence that some events of mammalian embryogenesis can result from differential growth, making migration unnecessary. Anat. Rec. 289, 53–63.
Haugas, M., Lilleväli, Hakanen, J., Salminen, M., 2010. *Gata2* is required for the development of inner ear semicircular ducts and the surrounding perilymphatic spaces. Dev. Dyn. 239, 2452–2469.
Honkura, Y., Hayashi, S., Abe, H., Murakami, G., Rodríguez-Vázquez, J.F., Katori, Y., 2021. The third vascular route of the inner ear or the canal of Cotugno: its topographical anatomy, fetal development and contribution to ossification of the otic capsule cartilage. Anat. Rec. 304, 872–882.
Ishikawa, A., Ohtsuki, S., Yamada, S., Uwabe, C., Imai, H., Matsuda, T., Takakuwa, T., 2018. Formation of the periotic space during the early fetal period in humans. Anat. Rec. 301, 563–570.
Jeffery, N., Spoor, F., 2004. Prenatal growth and development of the modern human labyrinth. J. Anat. 204, 71–92.
Kagurasho, M., Yamada, S., Uwabe, C., Kose, K., Takakuwa, T., 2012. Movement of the external ear in human embryos. Head. Face Med. 8, 2.
Kim, J.H., Rodríguez-Vázquez, J.F., Verdugo-López, S., Cho, K.H., Murakami, G., Cho, B.H., 2011. Early fetal development of the human cochlea. Anat. Rec. 294, 996–1002.
Martin, P., Swanson, G.J., 1993. Description and experimental analysis of the epithelial remodellings that control semicircular canal formation in the developing mouse inner ear. Dev. Biol. 158, 549–558.
Merchant, S.N., Nadol Jr., J.B., 2010. Schuknecht's Pathology of the Ear, third ed..,. People's Medical Publishing House,, Shelton, CT, US, pp. 572–599.
Nomura, Y., 2014. Morphological Aspects of Inner Ear Disease. Springer, Tokyo, pp. 67–69.
O'Rahilly, R., Müller, F., 1983. The timing and sequence of events in the development of the human eye and ear during the embryonic period proper. Anat. Embryol. 168, 87–99.
Palva, T., Pääkkö, P., Ramsay, H., Chrobok, V., Šimáková, E., 2003. Apoptosis and regression of embryonic mesenchyme in the development of the middle ear spaces. Acta Otolaryngol. 123, 209–214.
Represa, J.J., Moro, J.A., Gato, A., Pastor, F., Barbosa, E., 1990. Patterns of epithelial cell death during early development of the human inner ear. Ann. Otol. Rhinol. Laryngol. 99, 482–488.
Rodríguez-Vázquez, JF, Iglesias-Moreno, MC, Poch, A, Murakami, G, Abe, H, Honkura, Y., 2022. Fetal development and growth of the fissula ante fenestram in the human ear. Anat Rec (Hoboken) 305, 424–435.
Streeter, G.L., 1906. On the developmental of the membranous labyrinth and the acoustic and facial nerve in the human embryos. Am. J. Anat. 6, 139–165.
Streeter, G.L., 1918. The histogenesis and growth of the otic capsule and its contained periotic tissue-spaces in the human embryo. Contrib. Embryol. 7, 5–54.
Takahashi, N., Tsunoda, A., Shirakura, S., Kitamura, K., 2012. Anatomical feature of the middle cranial fossa in fetal period: possible etiology of superior canal dehiscence syndrome. Acta Oto-Laryngol. 132, 385–390.
Toyoda, S., Shiraki, N., Yamada, S., Uwabe, C., Imai, H., Matsuda, T., Yoneyama, A., Takeda, T., Takakuwa, T., 2015. Morphogenesis of the inner ear at different stages of normal human development. Anat. Rec. 298, 2081–2090.
Yasuda, M., Yamada, S., Uwabe, C., Shiota, K., Yasuda, Y., 2007. Three-dimensional analysis of inner ear development in human embryos. Anat. Sci. Int. 82, 156–163.