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ORIGINAL ARTICLE

Hampshire Sheep as a Large-Animal Model for Cochlear Implantation

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Abstract

Background Sheep have been proposed as a large-animal model for studying cochlear implantation. However, prior sheep studies report that the facial nerve (FN) obscures the round window membrane (RWM), requiring FN sacrifice or a retrofacial opening to access the middle-ear cavity posterior to the FN for cochlear implantation. We investigated surgical access to the RWM in Hampshire sheep compared to Suffolk-Dorset sheep and the feasibility of Hampshire sheep for cochlear implantation via a facial recess approach.

Methods Sixteen temporal bones from cadaveric sheep heads (ten Hampshire and six Suffolk-Dorset) were dissected to gain surgical access to the RWM via an extended facial recess approach. RWM visibility was graded using St. Thomas' Hospital (STH) classification. Cochlear implant (CI) electrode array insertion was performed in two Hampshire specimens. Micro-CT scans were obtained for each temporal bone, with confirmation of appropriate electrode array placement and segmentation of the inner ear structures.

Results Visibility of the RWM on average was 83% in Hampshire specimens and 59% in Suffolk-Dorset specimens ($p = 0.0262$). Hampshire RWM visibility was Type I (100% visibility) for three specimens and Type IIa (> 50% visibility) for seven specimens. Suffolk-Dorset RWM visibility was Type IIa for four specimens and Type IIb (< 50% visibility) for two specimens. FN appeared to course more anterolaterally in Suffolk-Dorset specimens. Micro-CT confirmed appropriate CI electrode array placement in the scala tympani without apparent basilar membrane rupture.

Conclusions Hampshire sheep appear to be a suitable large-animal model for CI electrode insertion via an extended facial recess approach without sacrificing the FN. In this small sample, Hampshire specimens had improved RWM visibility compared to Suffolk-Dorset. Thus, Hampshire sheep may be superior to other breeds for ease of cochlear implantation, with FN and facial recess anatomy more similar to humans.

Keywords Sheep · Cochlear implant · Implantable hearing device · Temporal bone · Middle ear · Facial recess · Otology

Introduction

Large-animal models can be used to study cochlear implantation with the same commercial electrode arrays that are used in humans. Cochlear implantation has been studied in several animal models, including non-human primates, cats, mini pigs, piglets, and sheep, each of which has unique advantages and disadvantages. Non-human primates, such as rhesus macaque, have been shown to be suitable for cochlear implantation [1]. However, there are often prohibitive ethical and cost concerns with primate use. Cats have been used in long-term cochlear implant

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studies, for example, to assess residual hearing [2]. However, only partial electrode array insertion is possible in cats due to a shorter cochlea with an average basilar membrane length of 22 mm [3], so custom electrode arrays are used [4].

Pigs and minipigs are another viable option. They have similar cochlear dimensions to humans with a basilar membrane length of approximately 32 mm [3], allowing for full insertion of commercial cochlear implant electrode arrays [5, 6] and study of inner ear drug delivery pharmacokinetics in piglets [7]. Additionally, Yildiz et al. performed an in vivo cochlear implant study of a commercial cochlear implant electrode array in piglets and mini pigs [8]. However, due to substantial differences in porcine temporal bone anatomy [9], the surgical approach is considerably different from humans. Therefore, cochlear implantation studies requiring a similar surgical approach to humans would not be possible in the porcine model. Additionally, due to the different anatomy, it could be difficult to study other middle-ear implantable devices with a porcine model.

Sheep have become popular as a suitable large-animal model for studying cochlear implantation and other implantable hearing devices due to similarities to human temporal bone anatomy [10–17]. The structures of the sheep middle ear and external auditory canal (measured in Corriedale-Texel sheep) are at least two-thirds the size of their equivalent human structures, except for the horizontal diameter of the tympanic membrane, which is slightly less than two-thirds smaller [18]. The cochlear length along the border of the basilar membrane (34.1 mm) and the number of turns (2.25) of the sheep cochlea are similar to these metrics in humans [19]. The round window membrane (RWM) of sheep is also anatomically similar to that of humans [20]. All these observations indicate that sheep would be suitable for studying cochlear implant electrode array insertion. However, the sheep breeds currently reported in the limited literature have had unfavorable anatomy for surgical access to the round window membrane (RWM) via a facial recess approach due to the facial nerve obscuring the round window. This has resulted in difficulty preserving the facial nerve and subsequent problems with feeding difficulties in live-animal survival studies. Mantokoudis et al. describe successful cochlear implantation in cadaveric lamb heads (breed not specified), which required sacrifice of the facial nerve to optimize the visualization of the round window [17]. Their surgical approach also included a canal-wall-down mastoidectomy, which further improves round window visibility. Trinh et al. performed cochlear implantation in cadaveric sheep heads (breed not specified) and similarly found that visualization of the round window membrane required sacrifice of the facial nerve [15].

Kaufmann et al. performed the first to our knowledge in vivo survival cochlear implantation in Suffolk-Dorset sheep [16]. They initially attempted accessing the RWM via a posterior tympanotomy (i.e., a facial recess approach) similar to the approach in humans [16]. However, this reportedly often required decompression and mobilization of the facial nerve to adequately visualize the round window membrane, resulting in facial paralysis in some sheep. Thus, they adopted a retrofacial approach, posterior rather than anterior to the facial nerve, to access the RWM for electrode array insertion. This approach is less generalizable to human cochlear implantation and places the posterior and lateral semicircular canals at greater risk. They also did not achieve complete electrode array insertion depth in their study due to the narrowing of the sheep scala tympani.

A breed of sheep with favorable anatomy for cochlear implantation via a facial recess approach would be more generalizable to cochlear implantation in humans and would also allow for various middle-ear implant studies. We studied the feasibility of the Hampshire breed of sheep (which has not been previously reported on) as a large-animal model for RWM cochlear implantation via an extended facial recess approach. For comparison, we also evaluated this approach in Suffolk-Dorset sheep (which has been used in earlier studies).

Materials and Methods

Sixteen temporal bones were extracted from five fresh-frozen cadaveric Hampshire sheep heads (four female adults and one male lamb) and three female adult Suffolk-Dorset sheep heads. Sheep were pre-screened for *Coxiella burnetii*, the causative organism of Q fever, as the surgical drilling is an aerosolizing procedure. All specimens were post-mortem and came from sheep destined for human consumption. Temporal bones were surgically prepared with a mastoidectomy and extended facial recess approach (including sacrifice of the chorda tympani) to gain access to the RWM [21]. RWM exposure was optimized by thinning the posterior ear canal wall and drilling the round window niche bony overhang. RWM visibility was graded using St. Thomas' Hospital (STH) classification: 100% visible (Type I), greater than 50% visible (Type IIa), less than 50% visible (Type IIb), or none exposed (Type III) [22].

The RWM was lysed with a curved needle (Wullstein, FENTEXmedical GmbH, Neuhausen ob Eck, Germany). Cochlear implant (CI) electrode array insertion was performed in one Hampshire specimen with a Cochlear Nucleus® 24 Contour Advance™ practice electrode and another Hampshire specimen with a MED-EL Flex24 practice electrode. Each temporal bone was imaged using a micro-CT scanner (Quantum FX, PerkinElmer, Waltham, MA) at a voltage of 90 kV and current of 180 µA with a field of view (FOV) diameter of 40 × 40 mm focused on the inner, middle, and external auditory canal and an isotropic voxel size of 80 µm. Micro-CT scans were used to confirm the appropriate placement of the CI electrode arrays in the two implanted specimens, including the calculation of linear and angular insertion depth using the 3D volume rendering in the open-source software 3D Slicer [23].

Two Hampshire temporal bones were also imaged at a voltage of 90 kV and current of 180 µA with a field of view (FOV) diameter of 20 × 20 mm focused on the inner ear and an isotropic voxel size of 40 µm. No fixation was used for the micro-CT scans. The scala tympani, scala vestibuli, and semicircular canals from these two specimens were segmented using the open-source software 3D Slicer. The cochlear basal length (CBL), also known as the A value [24], from these two specimens was measured. The base-to-apex cochlear length along the centerline of the scala vestibuli was also measured from these two specimens.

Results

The RWM was exposed in all Hampshire specimens, and the facial nerve coursed sufficiently posterior to the RWM for adequate exposure (Fig. 1). Under the STH classification, RWM visibility in Hampshire specimens was Type I (100% visibility) for three specimens and Type IIa (> 50% visibility) for seven specimens (Table 1). The mean visibility in Hampshire specimens was 83% (SD 15.8%). As a comparison, RWM visibility in Suffolk-Dorset specimens was Type IIa (> 50% visibility) in four specimens and Type IIb (< 50% visibility) in two specimens (Fig. 2). The mean visibility in

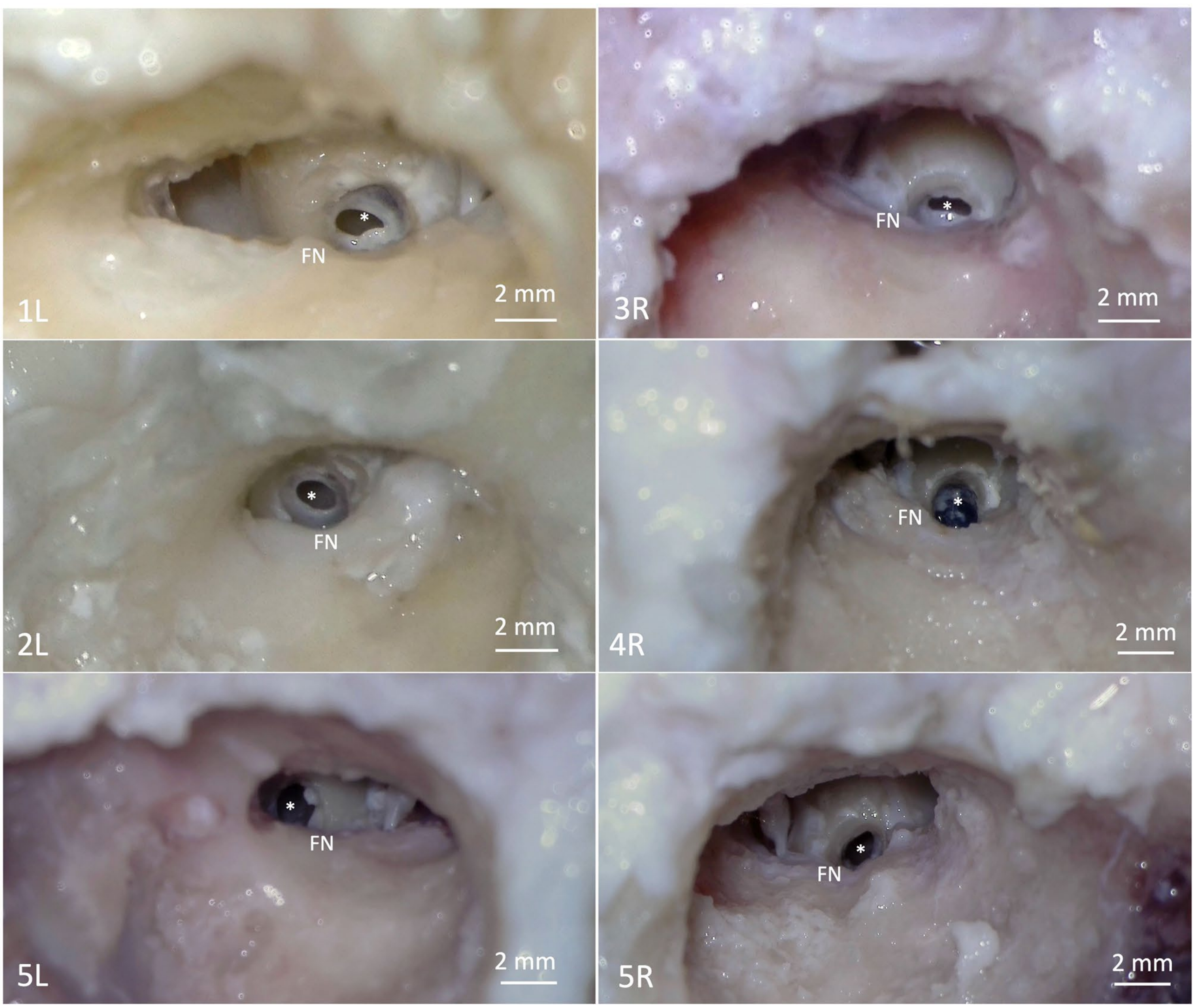

Fig. 1 View of lysed RWM (asterisks) through extended facial recess surgical approach in six different Hampshire specimens. FN, facial nerve

Table 1 Round window visibility of each temporal bone

Temporal bone	Breed	Sex	Age (years)	STH grade	Visibility (%)	FN exposed
1L	Hampshire	F	5	IIa	95	N
1R	Hampshire	F	5	I	100	Y[a]
2L	Hampshire	F	3	I	100	Y
2R	Hampshire	F	3	IIa	60	N
3L	Hampshire	F	8	I	100	Y
3R	Hampshire	F	8	IIa	65	N
4L	Hampshire	M	0.75	IIa	70	Y
4R	Hampshire	M	0.75	IIa	80	Y
5L	Hampshire	F	5	IIa	90	N
5R	Hampshire	F	5	IIa	70	N
6L	Suffolk-Dorset	F	4	IIa	90	Y
6R	Suffolk-Dorset	F	4	IIa	80	Y
7L	Suffolk-Dorset	F	3.5	IIb	35	Y
7R	Suffolk-Dorset	F	3.5	IIa	60	Y
8L	Suffolk-Dorset	F	3.5	IIb	35	Y
8R	Suffolk-Dorset	F	3.5	IIa	55	Y

STH St. Thomas' Hospital, *FN* facial nerve

[a]FN was intentionally decompressed in this specimen to delineate its anatomy

Suffolk-Dorset specimens was 59% (SD 22.7%). The facial nerve was preserved in all specimens. It was exposed in 5/10 Hampshire specimens and in 6/6 Suffolk-Dorset specimens. In the first specimen we dissected (Hampshire specimen 1R), we intentionally decompressed the facial nerve, completely removing its bony covering to delineate its anatomy. The facial nerve appeared to course more anterolaterally relative to the RWM in Suffolk-Dorset specimens than in Hampshire specimens. The chorda tympani nerve was sacrificed in all specimens to optimize exposure of the middle ear cavity, technically making this an extended facial recess surgical approach.

Micro-CT scans confirmed appropriate CI electrode array placement in the scala tympani without apparent rupture of the basilar membrane or organ of Corti in both implanted specimens (1L and 5R from Hampshire sheep). Videos scrolling through the micro-CT scans of 1L and 5R post-implantation are shown in Supplemental Videos 1 and 2. In specimen 1L, implanted with the Contour Advance practice electrode, the linear insertion depth was 12.52 mm, and the angular insertion depth was 241° (Supplemental Fig. 1). In specimen 5R, implanted with the Flex24 practice electrode, the linear insertion depth was 11.57, mm and the angular insertion depth was 244° (Supplemental Fig. 2). Figure 3 depicts segmentations of the CI electrode arrays inserted into the cochleae of the two specimens. Segmentations of the scala tympani, scala vestibuli, and semicircular canals from specimen 3L are shown in Fig. 4A. In specimen 3L, scala tympani volume was 16.83 mm^3, scala vestibuli volume was 34.73 mm^3, and the volume of the semicircular canals was 43.43 mm^3. In specimen 5R, scala tympani volume was 17.47 mm^3, and scala vestibuli volume was 31.70 mm^3. Volume was not calculated for the semicircular canals in specimen 5R, as part of the superior semicircular canal was damaged on the micro-CT scan. As shown in Fig. 4B, there is significant narrowing of the scala tympani in the first turn of the cochlea. The *A* value (cochlear basal length) was 6.89 mm in specimen 3L and 6.93 mm in specimen 5R (Supplemental Figs. 3 and 4). The cochlear length of specimen 3L was 22.44 mm, and the cochlear length of specimen 5R was 21.17 mm.

Discussion

We demonstrated successful cochlear implant electrode array insertion via an extended facial recess approach while preserving the facial nerve and posterior external auditory canal wall in the Hampshire breed of sheep. RWM visibility was > 50% in all ten Hampshire temporal bones and 100% in 3/10 Hampshire temporal bones. In specimens without 100% visibility, visibility was partially limited by the course of the facial nerve. Prior cadaveric studies in sheep of different breeds have described the sacrifice of the facial nerve and/or a canal-wall-down mastoidectomy to access the RWM [15, 17]. We preserved the facial nerve in all Hampshire specimens, exposing it in 5/10 specimens. We intentionally exposed and decompressed the facial nerve in the first specimen that we dissected (Hampshire temporal bone 1R) to better delineate its anatomy.

The STH classification system has been used to describe RWM visibility in the context of human cochlear

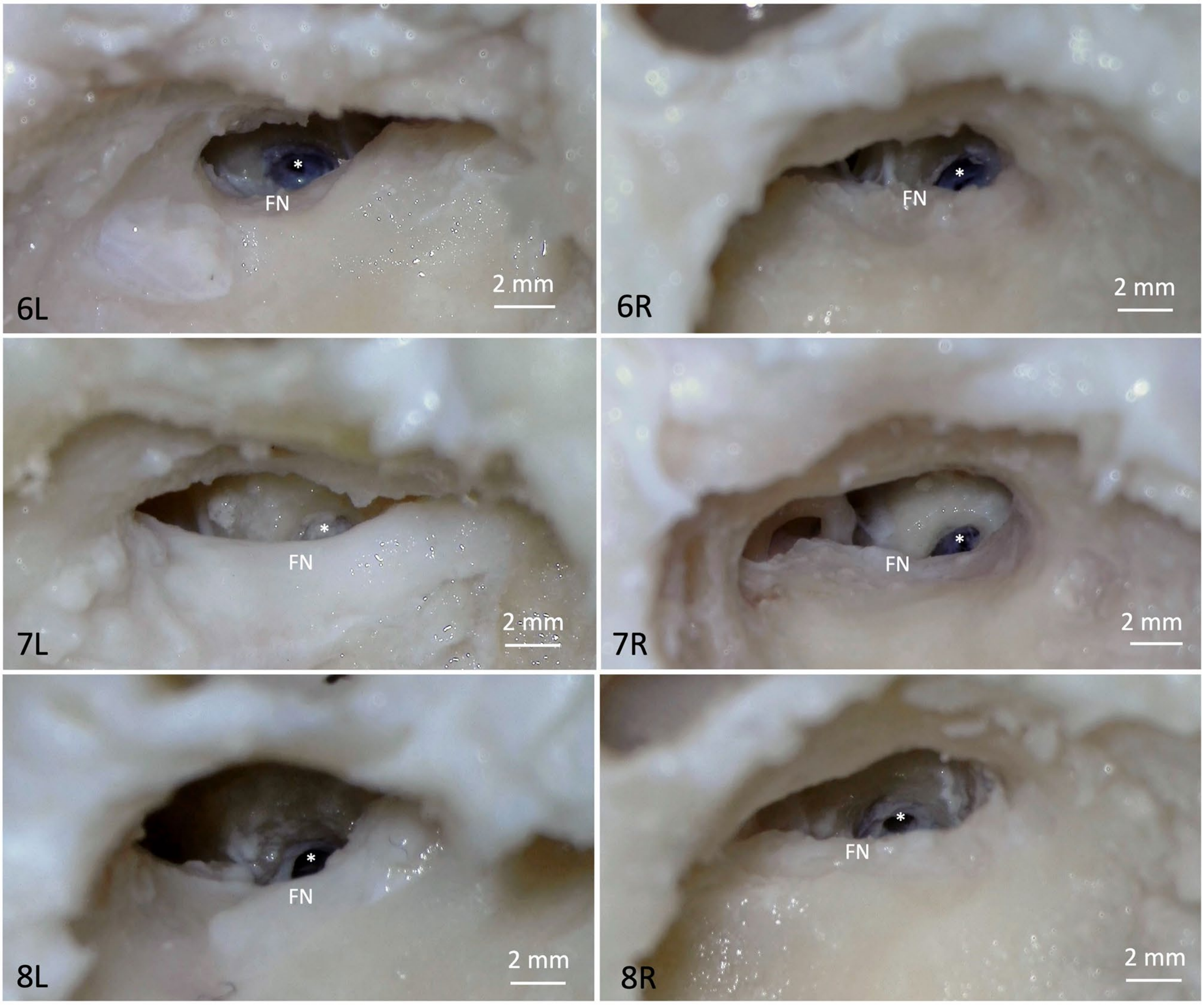

Fig. 2 View of lysed RWM (asterisks) through extended facial recess surgical approach in six different Suffolk-Dorset specimens. FN, facial nerve

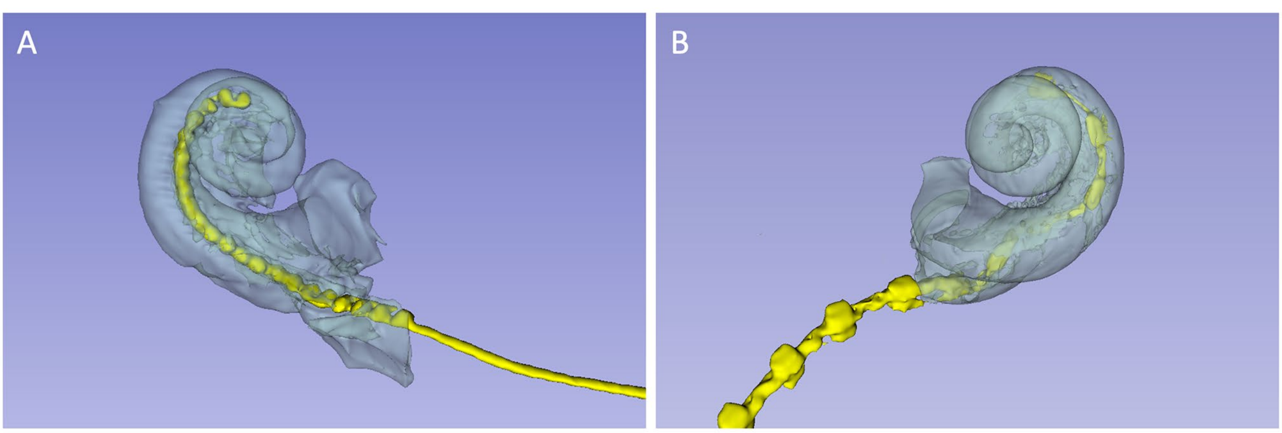

Fig. 3 Three-dimensional volume renderings from micro-CT scans of cochleae inserted with cochlear implant electrode arrays. **A** Cochlear Nucleus 24 Contour Advance practice electrode array inserted in the cochlea of specimen 1L. **B** MED-EL FLEX24 dummy electrode array inserted in cochlea of specimen 5R. Both cochleae were of Hampshire sheep

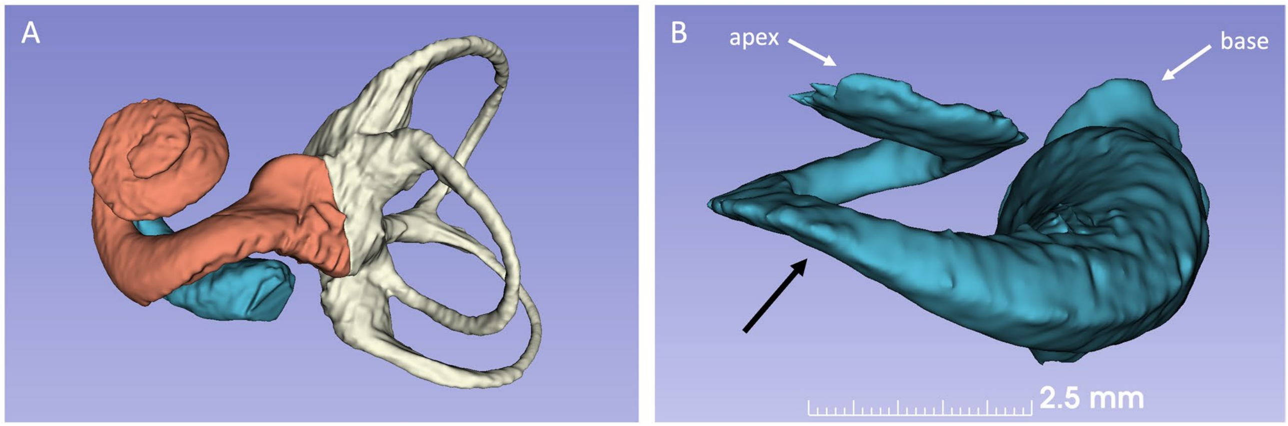

Fig. 4 Three-dimensional volume renderings of segmented inner ear structures from Hampshire temporal bone 3L **A** Segmentation of scala tympani (turquoise), scala vestibuli (orange), and semicircular canals (gray) in specimen 3L. **B** Segmentation of scala tympani showing significant narrowing, estimated to be roughly 0.3 mm, in the first turn (black arrow), with base and apex indicated

implantation [22]. A prospective study of 128 human cochlear implant recipients found that 89% of adult patients and 78% of pediatric patients had RWM visibility > 50% [22]. Although the STH grading system has not previously been used in sheep, our results demonstrate that our surgical approach in the Hampshire breed of sheep achieves comparable visibility of the RWM to human cochlear implantation with preservation of the facial nerve. The two male lamb temporal bones (from the same animal) were the only Hampshire specimens in which we exposed the facial nerve but did not achieve 100% exposure of the RWM. We found the two male lamb specimens (9 months old) to be subjectively more difficult to dissect as compared to the adult female specimens, as the mastoid seemed smaller and less pneumatized. Although these two specimens are not enough to draw conclusions, perhaps adult female sheep have more favorable anatomy.

The Suffolk-Dorset temporal bones were also subjectively more difficult to dissect than the Hampshire due to a more anterolateral facial nerve course, which made visualization of the RWM difficult. Although we did expose the RWM to some extent in all the Suffolk-Dorset temporal bones without sacrificing the facial nerve, the mean visibility was less than that of the Hampshire specimens. Also, to visualize the RWM, we needed to expose the facial nerve in all the Suffolk-Dorset specimens compared to only half of the Hampshire specimens. Additionally, it was difficult to drill the round window niche bony overhang in several of the Suffolk-Dorset specimens without manipulating and traumatizing the facial nerve, which was not the case in any of the Hampshire temporal bones.

We successfully performed cochlear implant electrode array insertion in Hampshire temporal bones with two different human CI electrodes, a Cochlear Nucleus® 24 Contour Advance™ practice electrode, and a MED-EL Flex24 practice electrode. Post-insertion imaging verified appropriate placement in the scala tympani without apparent rupture of the basilar membrane or organ of Corti. Similar to other studies of sheep cochlear implantation, we did not achieve complete electrode insertion. We achieved 12.52 mm and 241° of insertion with the Contour Advance electrode array and 11.57 mm and 244° of insertion with the Flex24 electrode array.

Although Schnabl et al. and Trinh et al. reported full insertion in their cadaveric sheep CI studies [15, 19], Mantokoudis et al., in their attempt to minimize cochlear trauma in 9–12-month-old lamb heads, did not achieve full insertion [17]. Kaufmann et al. similarly reported incomplete insertion depths ranging from 4.6 to 12 mm (average 7.3 ± 2.2 mm) in their live CI study in adult Suffolk-Dorset sheep [16]. They postulated that perhaps the other studies achieving full insertion resulted in elevation or rupture of the basilar membrane. Kaufmann et al. speculated that the incomplete insertion could be explained by the narrowing of the sheep scala tympani. Supporting this theory, we also observed a significant narrowing of the scala tympani in the segmentation of the sheep's inner ear (Fig. 4B) to a height of roughly 0.3 mm. Of note, the contour advance electrode array has an apical diameter of 0.5 mm, and the Flex24 electrode array has apical dimensions of 0.5 × 0.3 mm. In contrast to sheep, the human scala tympani at 270 degrees (roughly where the area of narrowing occurs in sheep) has a mean central height of 0.99 mm (SD 0.16), mean lateral height of 0.81 mm (SD 0.17), and mean perimodiolar height of 0.86 mm (SD 0.11) [25]. The cochlear basal length in the two measured sheep cochleae (Supplemental Figs. 3 and 4) was roughly two-thirds the cochlear basal length in humans (6.89 mm and 6.93 mm in the sheep cochleae compared to a mean length of 9.3 mm in humans) [24]. Our measured cochlear lengths of 22.44 mm in specimen 3L and 21.17 mm in specimen 5R are shorter than the 34.1 mm reported by

Schnabl et al. averaged from five measured lamb cochleae [19]. Perhaps this difference is due to a difference in sheep breed and/or a difference in measurement technique.

This study is limited by a small sample size of ten Hampshire and six Suffolk-Dorset temporal bones. Our sample of Suffolk-Dorset specimens (3.67 years old on average), although all adults, was younger than the Hampshire specimens (4.35 years old on average), which did include two lambs. The STH grading system was devised for humans, so there may be bias in applying it to sheep. Surgeons were not blinded to the breed, which may have introduced bias. However, the first author performed or assisted in all the surgeries. For each specimen, we maximally thinned the posterior ear canal wall and drilled the RWM niche bony overhang to optimize visibility to the best of our ability. Our surgical approach also involves sacrifice of the chorda tympani, because we wanted to investigate the possibility of using the sheep model to study middle ear devices. Preserving the chorda tympani is likely feasible in cochlear implantation experiments in sheep and has already been demonstrated in the porcine model [8]. Regardless, chorda tympani sacrifice is likely acceptable in live-animal trials. Another limitation is that this study was performed in fresh but previously frozen specimens, which is not entirely generalizable to live sheep, as there may have been tissue atrophy, including atrophy of the facial nerve in the cadaveric specimens, and facial nerve functionality could not be assessed. And although Hampshire sheep are available in the United States and United Kingdom, this breed may not be readily available in other parts of the world.

Sheep have some disadvantages relative to other animal models for cochlear implantation research. The narrowing of the sheep scala tympani prevents full electrode array insertion, so only partial insertion is possible. Additionally, sheep are further genetically from humans than other animals such as pigs. Although sheep have been used in a 30-day survival cochlear implantation study [16], longer-term in vivo studies have not yet been demonstrated. Notable advantages in sheep include a very similar RWM to humans, similar surgical access to humans with respect to temporal bone anatomy, and similar cochlear dimensions (length and number of turns) to humans. Depending on the goals and objectives of the study, sheep may be the preferred large-animal model. This study shows that future sheep studies can consider cochlear implantation via an extended facial recess approach in live Hampshire sheep due to similarities to human anatomy for RWM access with a low chance of traumatizing the facial nerve.

Conclusion

We found that the Hampshire breed of sheep appears to be a suitable model for cochlear implant electrode array insertion via an extended facial recess surgical approach, making it potentially more generalizable to human cochlear implantation than other breeds of sheep reported in the literature. In our small comparison to Suffolk-Dorset sheep, the facial nerve in Suffolk-Dorset specimens appeared to course more anterolaterally relative to the RWM, limiting RWM visibility (similar to previous studies' descriptions [16]). Similar to other studies in sheep, we found that only partial electrode insertion was possible due to the narrowing of the sheep scala tympani towards the end of the first turn. Our findings, although not definitive given the small sample size, suggest that researchers seeking a large-animal model for cochlear implantation via a facial recess approach should consider using Hampshire sheep.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10162-024-00946-1.

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