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Anatomic and morphometric examination of auditory ossicles in sheep

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Abstract

It is important to know the morphometry of the auditory ossicles for middle ear surgical applications. The present study aims to investigate the morphometric measurements of sheep auditory ossicles and the relationship between these ossicles. In this study, 100 malleus, incus and stapes of 50 Akkaraman sheep were examined using at trinocular stereo microscope and a total of 19 morphometric measurements were taken. The mean values of the morphometric measurements were obtained, and correlation analysis was performed between each part of each auditory ossicle. Similarities were found between the measurements of the lengths of the malleus, manubrium mallei and incus, and the width of the base of the stapes with morphometric measurements in human auditory ossicles. A significant positive correlation was found between the length of the malleus with the length of the manubrium mallei, between the length of the incus with the lengths of the long crus and corpus incudis, between the length of the stapes with the lengths of the rostral crus, caudal crus, caput stapedis, and the length and width of the intercrural foramen. Due to the anatomic similarities between sheep and human auditory ossicles, it was concluded that the auditory ossicles of sheep are suitable for use in the training of human ear surgery applications.

KEYWORDS
auditory ossicles, incus, malleus, morphometry, stapes

1 | INTRODUCTION

The ear is one of the most important sensory organs for humans. Hearing loss may occur due to any disease in the ear. Along with hearing loss, middle ear diseases also affect millions of people every year (Peus et al., 2020).

Various animal models are used to reveal basic information about hearing and to investigate the clinical aspect of hearing (Peus et al., 2020). Due to the fact that sheep ears reflect the human ear anatomy better, can be easily obtained and are similar to human ear dimensions, this research was conducted on sheep (Cordero et al., 2011; Peus et al., 2020).

The auditory ossicles known as the malleus, incus and stapes are located in the petrosus part of the temporal bone and dorsally middle ear cavity. In young animals, another ossicles called 'os lenticulare' can be found between the incus and stapes (Gürbüz et al., 2019). These ossicles are responsible for increasing the vibration of the sound coming from the eardrum and transmitting it to the inner

ear (Dalga & Aslan, 2019). The human malleus consists of the head (caput mallei), neck (collum mallei), anterior process, lateral process and handle (manubrium mallei), and the sheep malleus consists of the caput mallei, collum mallei, manubrium mallei and muscular process (Saha et al., 2017). The human incus consists of the body (corpus incudis), long crus and short crus. At the tip of the long crus, there is a small limb called the lenticular process (Graboyes et al., 2011). The lenticular process is a part of the long crus in humans, whereas it is a separate part of the incus in sheep (Gürbüz et al., 2019). Human stapes consist of a head (caput stapedis), neck (collum stapedis), base of the stapes, anterior crus and posterior crus, and sheep stapes comprise the caput stapedis, rostral crus, caudale crus and base of the stapes (Gürbüz et al., 2019; Saha et al., 2017). The hole located between the rostral crus and the caudal crus in the sheep stapes is called the intercrural foramen (Erdoğan & Kilinc, 2012).

Sheep ears have been used for the evaluation of hearing aids, stapedotomy and cochlear implantation with new surgical techniques because there is a ratio of approximately 2/3 between the dimensions of sheep and human inner and middle ears (Cordero et al., 2011; Gocer et al., 2007; Mantokoudis et al., 2016; Peus et al., 2020; Schnabl et al., 2012). Lavinsky and Goycoolea (1997) explained how to use sheep to test the experimental surgical procedure of utriculostomy, stating that the middle ear of sheep shows significant anatomic and histologic similarities with that of humans. The anatomic similarity between human and sheep ears is especially useful for training and applications related to tympanotomy, tympanoplasty, ossiculoplasty, stapedectomy and myringotomy (Seibel et al., 2006).

This study was carried out to investigate the morphometric measurements of sheep auditory ossicles and the relationship between these ossicles.

2 | MATERIALS AND METHODS

In this study, 100 malleus, incus and stapes extracted from the skulls of 50 Akkaraman sheep obtained from different slaughterhouses were used (Figure 1).

A total of 19 morphometric measurements were taken using a trinocular stereo microscope (OPTIKA SZX-T + SZ-A1 + SZ-ST8), two measurements were taken to reduce the error rate, and the average of these measurements was considered. Five different measurement points were determined for the malleus (Figure 2), six for the incus (Figure 3) and eight for the stapes (Figure 4) (Table 1).

3 | STATISTICAL ANALYSIS

The conformity of the data to the normal distribution was evaluated using Histograms, Q-Q plots and the Shapiro–Wilk test. The homogeneity of variance was tested using Levene's test. The explanatory statistics of the variables are given as mean ± SD and median (first quartile-third quartile) according to the type of variable and the normality assumption. The independent *t*-test and Mann–Whitney *U* test were used for variables in intergroup comparisons. The relationship between the variables was calculated using Pearson's correlation analysis. Data analysis was performed using the SPSS 22.0 package program. The significance level was accepted as $p < 0.05$.

4 | RESULTS

The measurements of the malleus, incus and stapes are shown in Table 2.

A significant positive correlation (*r*: 0.417, *p*: <0.01) was found between the length of the malleus and the length of the manubrium mallei. A significant positive correlation (*r*: 0.619; *p*: <0.01) was found between the width of the caput mallei and the length of between caput mallei and muscular process. The result of the correlation analysis of other measurements of the malleus is shown in Table 3.

A significant positive correlation (*r*: 0.747, *p*: <0.01) was found between the length of the incus and the length of the long crus. A significant positive correlation (*r*: 0.575, *p*: <0.01) was found between the length of the incus and the length of the corpus incudis. A significant positive correlation was found between the lengths of the long crus with short crus and length of the corpus incudis (*r*:

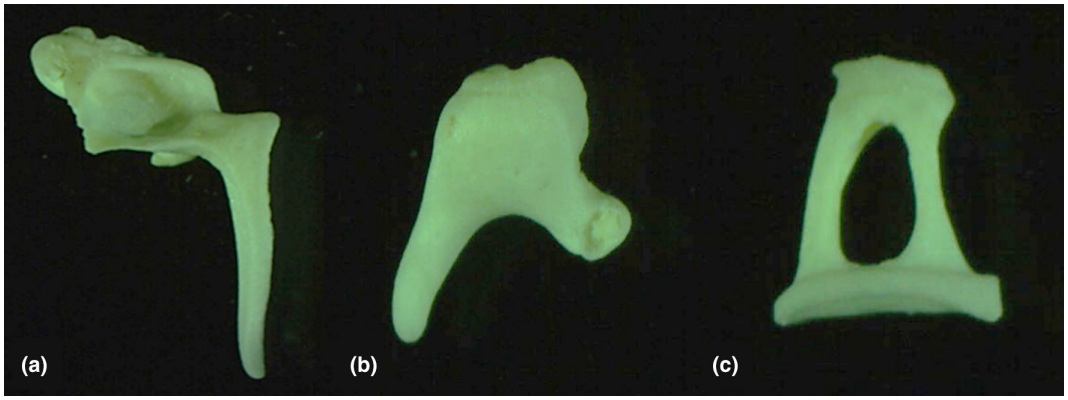

FIGURE 1 Malleus, incus, stapes (Magnification × 40).

0.498, *p*: <0.01; *r*: 0.673, *p*: <0.01). A significant positive correlation was found between length of the short crus with length of the corpus incudis and the width of the corpus incudis (*r*: 0.536, *p*: <0.01; *r*: 0.444, *p*: <0.01). The results of correlation analyses of other measurements of the incus are shown in Table 4.

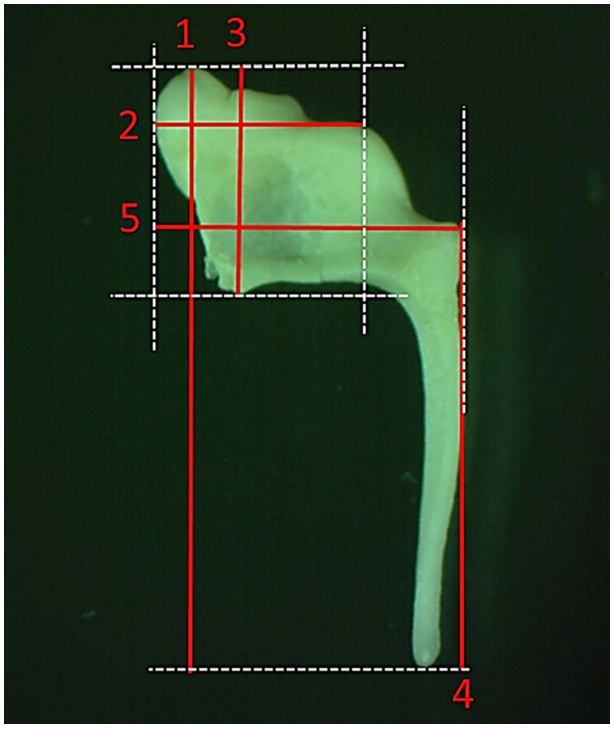

FIGURE 2 Measurements of the malleus (Magnification×40).

A significant positive correlation was found between the length of the stapes with length of the rostral crus, length of the caudal crus, length of the caput stapes, length of the intercrural foramen and width of the intercrural foramen (Table 5). A significant positive correlation was found between the width of the caput stapes and the width of the intercrural foramen (*r*: 0.321, *p*: <0.01).

A significant positive correlation was found between the length of the rostral crus with length of the caudal crus, length of the caput stapes and the width of the intercrural foramen. A positive and significant correlation was found between the length of the caudal crus with length of the caput stapes, length of the intercrural foramen and the width of the intercrural foramen (Table 5). A significant negative correlation (*r*: −0.299, *p*: <0.01) was found between length of the caput stapes and length of the intercrural foramen. A significant positive correlation (*r*: 0.662, *p*: <0.01) was found between the length of the intercrural foramen and the width of the intercrural foramen.

5 | DISCUSSION

The human ear is essential for communication because it allows contributions to language, cognition and intelligibility of the spoken message (Kesser et al., 2013). The difficulty of obtaining human material necessitates the use of animal models, especially in studies aimed at microscopic examination of sensitive structures such as the middle auditory ossicles (Albuquerque et al., 2009; Reis et al., 2017). In this context, studies on the use of sheep as an anatomic model for experimental and otologic surgery training are insufficient (Cordero et al., 2011).

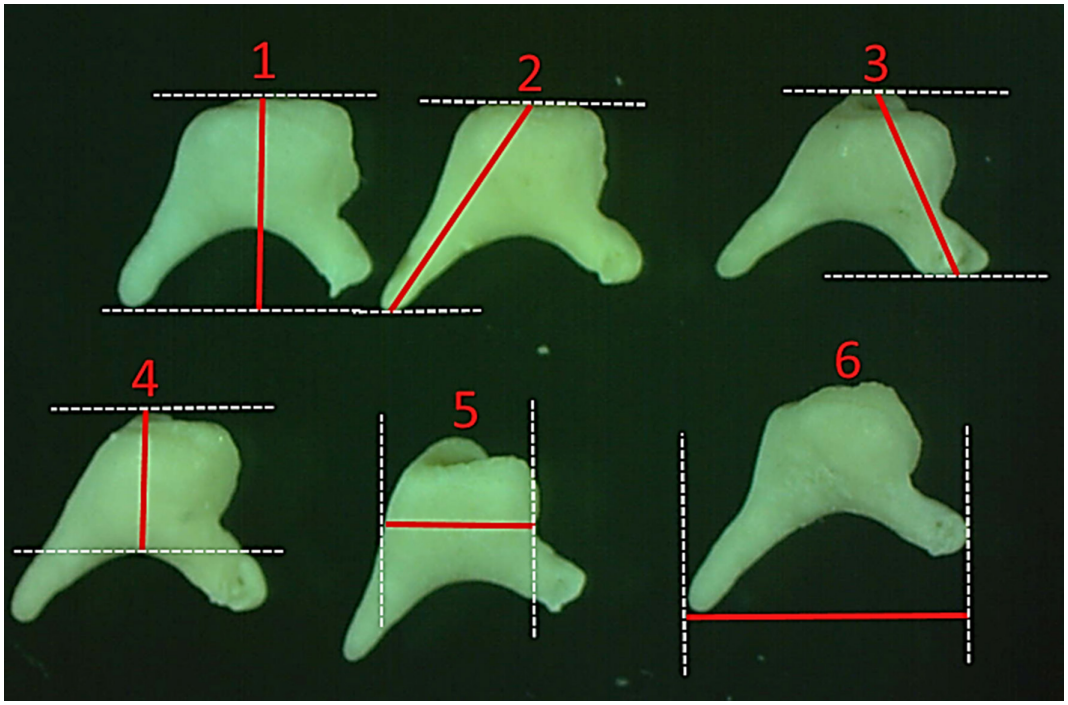

FIGURE 3 Measurements of the incus (Magnification×40).

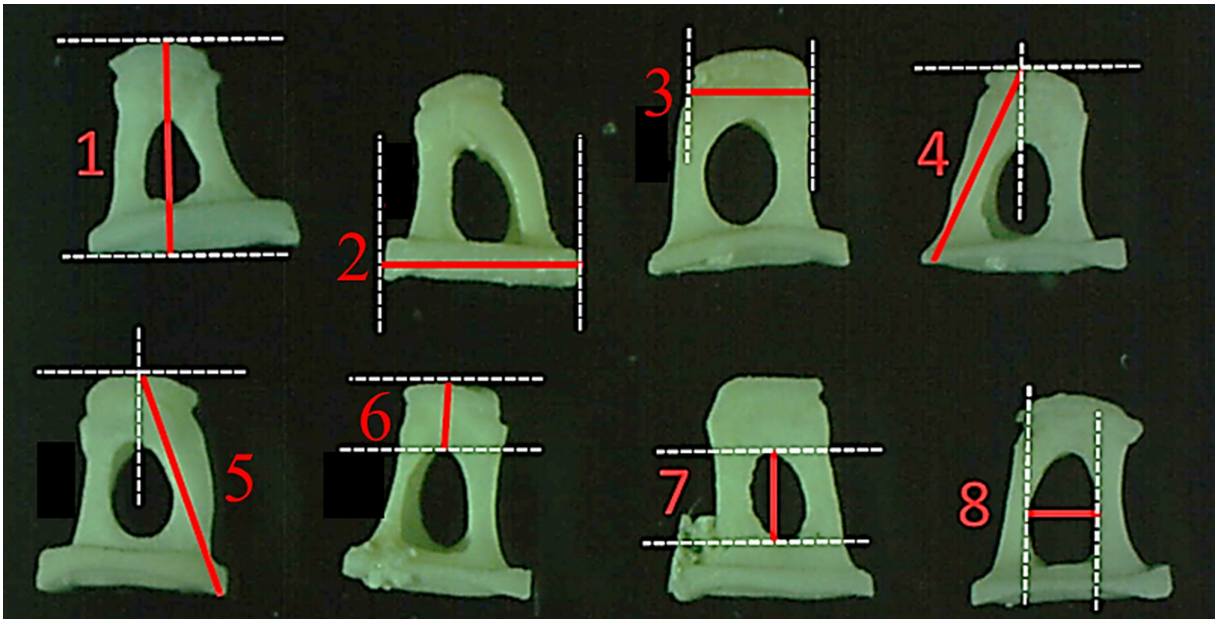

FIGURE 4 Measurements of the stapes (Magnification×40).

TABLE 1 The descriptions of anthropometric measurements of malleus, incus and stapes.

Anthropometric measurements	Descriptions
Length of the malleus	The length between the manubrium mallei and the top of the malleus
Width of the caput mallei	The length between the far right and the far left of the caput mallei
Length of the caput mallei	The length between the lowest point of the caput mallei and top of the malleus
Length of the manubrium mallei	The distance between muscular process and the lowest point of the manubrium mallei
Length of between caput mallei and muscular process	The length between the far left point of caput mallei and muscular process
Length of the incus	The length of the perpendicular descended from the highest point of the incus to the midpoint between the two crus for this point
Length of the long crus	The length between the top of the incus and the lowest end of the long crus
Length of the short crus	The length between the highest point of the incus and the lowest end of the short crus
Length of the corpus incudis	The length between the lowest and highest point of the corpus incudis
Width of the corpus incudis	The length from the midpoint of the corpus incudis between its far right and far left point
Distance between cruses	The distance between the lowest points of the long crus and the short crus
Length of the stapes	The length between the top point of the caput stapedis and the bottom point of the basis stapedis.
Width of base of the stapes	The length between the far right and the far left of the midpoint of the basis stapedis
Width of the caput stapedis	The length between the far right and the far left of the midpoint of the caput stapedis
Length of the rostral crus	The length between the highest point of the caput stapedis and the lowest point of the basis stapedis on the rostral
Length of the caudale crus	The length between the highest point of the caput stapedis and the lowest point of the basis stapedis in the caudal
Length of the caput stapedis	The length between the apex of the caput stapedis and the apex of the foramen intercrurale
Length of the intercrural foramen	The length between the highest point and the lowest point of the foramen intercrurale
Width of the intercrural foramen	The length from the far right to the far left of the midpoint of the foramen intercrurale

There are studies on malleus length in humans, New Zealand rabbits, hamsters, Malakan horses, buffaloes, cattles and sheeps (Arensburg et al., 1981; Fatahian Dehkordi et al., 2022; Gürbüz et al., 2016; Kurtul et al., 2003; Mohammadpour, 2011; Nourinezhad et al., 2021; Unur et al., 2002). In our study, the length of the malleus (7.40±0.42 mm) was similar to the measurement results in humans (Table 6).

TABLE 2 Measurements of the malleus, incus, stapes.

Measurements	*n* = 100
Length of the malleus	7.40 (5.82–8.20)
Width of the caput mallei	3.27 ± 0.26
Length of the caput mallei	2.78 ± 0.23
Length of the manubrium mallei	5.62 ± 0.36
Length of between caput mallei and muscular process	4.49 ± 0.29
Length of the incus	3.03 ± 0.24
Length of the long crus	3.39 ± 0.19
Length of the short crus	2.69 ± 0.17
Length of the corpus incudis	1.86 ± 0.13
Width of the corpus incudis	2.19 ± 0.13
Distance between cruses	3.39 (2.93–3.90)
Length of the stapes	2.25 ± 0.12
Width of base of the stapes	2.08 (1.12–2.35)
Width of the caput stapedis	1.08 (0.83–2.09)
Length of the rostral crus	2.12 ± 0.21
Length of the caudale crus	2.06 ± 0.23
Length of the caput stapedis	0.71 ± 0.10
Length of the intercrural foramen	1.02 ± 0.15
Width of the intercrural foramen	0.67 ± 0.10

Note: Values are expressed as mean ± SD and the median (first-third quartiles).

There are studies on the length of the manubrium mallei in both humans and animals (Arensburg et al., 1981; Gürbüz et al., 2016; Hadziomerovic et al., 2023; Martonos et al., 2021; Mohammadpour, 2011; Peus et al., 2020; Unur et al., 2002). The manubrium mallei length measured in our study (5.62 ± 0.36 mm) was similar to the results of Gürbüz et al. (2016), Hadziomerovic et al. (2023) and Peus et al. (2020), but was longer than in other studies (Table 6).

The caput mallei length and width measurements in our study were found to be greater than in studies conducted on Malakan horses, hamsters, red foxes, goats, New Zealand rabbits, cattles and sheeps (Fatahian Dehkordi et al., 2022; Gürbüz et al., 2016; Hadziomerovic et al., 2023; Kurtul et al., 2003; Martonos et al., 2021; Mohammadpour, 2011) (Table 6).

The data of our study regarding the length of the incus were calculated lower than those of studies conducted on humans and other animals (Arensburg et al., 1981; Fatahian Dehkordi et al., 2022; Gürbüz et al., 2016; Unur et al., 2002), except for the study conducted on hamsters (Mohammadpour, 2011) (Table 6).

The mean length of the long crus of the incus in humans was 6.5 mm (Arensburg et al., 1981), and in buffalos, it was 2.78 mm (Nourinezhad et al., 2021). The length of the long crus (3.39 ± 0.19 mm) measured in our study was found to be longer than in other studies, except for the study conducted on humans. The mean length of the short crus of the incus has been reported as 2.74 mm in buffalos (Nourinezhad et al., 2021). The length of the short crus in our study (2.69 ± 0.17 mm) was similar to the result of Nourinezhad et al. (2021). In the study conducted by Fatahian Dehkordi et al. (2022), the distance between the long crus and short crus was calculated as 3.32 mm in sheeps and 4.79 mm in cattles. This distance was determined as 3.33 ± 0.41 mm in our study.

The length of the corpus incudis was measured as 2.39 ± 0.40 mm in Dalga and Aslan's (2019) study in Hemshin sheep but was shorter in our study at 1.86 ± 0.13 mm. Although the data of our study regarding the average width of the corpus incudis are similar to those of Hadziomerovic et al. (2023), they differ from other studies conducted on animals (Gürbüz et al., 2016; Hadziomerovic et al., 2023; Kurtul et al., 2003; Mohammadpour, 2011) (Table 6).

In our study, the length of the stapes was determined as 2.25 ± 0.12 mm. There is no similarity between the results of our study and other related studies (Fatahian Dehkordi et al., 2022; Gürbüz et al., 2016; Kurtul et al., 2003; Mohammadpour, 2011; Nourinezhad et al., 2021). In the present study, the width of the base of the stapes (2.07 ± 0.16 mm) was wider than in studies performed in both humans and animals (Arensburg et al., 1981; Kurtul et al., 2003; Mohammadpour, 2011; Unur et al., 2002) (Table 6).

The length of the caput stapedis was reported as 1.44 ± 0.33 mm in water buffalo (Nourinezhad et al., 2021). In our study, this measurement was shorter than in the study of Nourinezhad et al. (2021). The caput stapedis width in our study (1.10 ± 0.17 mm) was similar to the results of Martonos et al. (2021), but wider than the results in other studies (Hadziomerovic et al., 2023; Kurtul et al., 2003; Mohammadpour, 2011).

The length of the caudal crus (2.06 ± 0.23 mm) in present study was similar to the results of Hadziomerovic et al.'s (2023)

TABLE 3 Correlation analysis of measurements of the malleus.

	Length of the malleus	Width of the caput mallei	Length of the caput mallei	Length of the manubrium mallei	Length of between caput mallei and muscular process
Length of the malleus	1	**−0.228***	0.143	**0.417****	−0.160
Width of the caput mallei		1	0.086	−0.106	**0.619****
Length of the caput mallei			1	−0.120	−0.122
Length of the manubrium mallei				1	0.137
Length of between caput mallei and muscular process					1

Note: $^{**}p < 0.01$; $^{*}p < 0.05$. Bold indicates significance values.

TABLE 4 Correlation analysis of measurements of incus.

	Length of the incus	Length of the long crus	Length of the short crus	Length of the corpus incudis	Width of the corpus incudis	Distance between cruses
Length of the incus	1	**0.747****	0.157	**0.575****	−0.060	−0.32
Length of the long crus		1	**0.498****	**0.673****	0.213*	−0.57
Length of the short crus			1	**0.536****	**0.444****	0.012
Length of the corpus incudis				1	0.202*	0.105
Width of the corpus incudis					1	0.158
Distance between cruses						1

Note: $^{**}p < 0.01$; $^{*}p < 0.05$. Bold indicates significance values.

TABLE 5 Correlation analysis of measurements of stapes.

	Length of the stapes	Width of base of the stapes	Width of the caput stapedis	Length of the rostral crus	Length of the caudale crus	Length of the caput stapedis	Length of the intercrural foramen	Width of the intercrural foramen
Length of the stapes	1	0.164	0.171	**0.556****	**0.588****	**0.369****	**0.401****	**0.309****
Width of base of the stapes		1	−0.228*	0.230*	0.197*	0.190	−0.062	0.125
Width of the caput stapedis			1	0.196	0.216*	−0.063	0.230*	**0.321****
Length of the rostral crus				1	**0.895****	**0.424****	0.219*	**0.316****
Length of the caudale crus					1	**0.447****	**0.261****	**0.321****
Length of the caput stapedis						1	**−0.299****	−0.036
Length of the intercrural foramen							1	**0.662****
Width of the intercrural foramen								1

Note: $^{**}p < 0.01$; $^{*}p < 0.05$. Bold indicates significance values.

TABLE 6 Comparison of measurements of auditory ossicles with the previous reports (in mm).

Measurements other study	Length of the malleus	Width of the caput mallei	Length of the caput mallei	Length of the manubrium mallei	Length of the incus	Width of the corpus incudis	Length of the stapes	Width of base of the stapes	Width of the caput stapedis	Length of the rostral crus
Unur et al. (2002) (human)	7.69 ± 0.60	–	–	4.70 ± 0.45	6.47 ± 0.55	–	–	1.29 ± 0.22	–	–
Arensburg et al. (1981) (human)	7.8 ± 0.35	–	–	4.4 ± 0.47	6.4 ± 0.24	–	–	1.3 ± 0.07	–	–
Kurtul et al. (2003) (rabbit)	5.28	1.28	–	–	–	1.03	1.95	1.47	0.61	0.79
Gürbüz et al. (2016) (Malakan horse)	9.97	2.94	2.63	5.63	4.11	4.05	3.25	–	1.41	3.06
Mohammadpour (2011) (hamster)	2.86	0.49	1.16	1.50	1.18	0.46	0.76	0.89	0.20	0.39
Nourinezhad et al. (2021) (buffalo)	6.54 ± 0.6	–	–	–	–	–	3.6 ± 0.57	–	–	–
Fatahian Dehkordi et al. (2022) (sheep)	8.97	2.01	–	–	4.9	–	3.44	–	–	–
Fatahian Dehkordi et al. (2022) (cattle)	10.22	2.2	–	–	5.95	–	3.62	–	–	–
Hadziomerovic et al. (2023) (red fox)	–	1.75 ± 0.07	1.15 ± 0.10	5.19 ± 0.53	–	2.19 ± 0.41	–	–	0.61 ± 0.05	2.11 ± 0.10
Martonos et al. (2021) (goat)	–	1.87	1.77	4.49	–	–	–	–	1.21	–
Peus et al. (2020) (sheep)	–	–	–	5.26 ± 0.39	–	–	–	–	–	–
Present Study (2023) (sheep)	7.40 ± 0.42	3.27 ± 0.26	2.78 ± 0.23	5.62 ± 0.36	3.03 ± 0.24	2.19 ± 0.13	2.25 ± 0.12	2.07 ± 0.16	1.10 ± 0.17	2.12 ± 0.21

study on the red fox but was longer than the results of Kurtul et al. (2003), and Mohammadpour (2011), and shorter than reported by Gürbüz et al. (2016). The average length of the rostral crus was 0.79 mm in the New Zealand rabbit (Kurtul et al., 2003), 3.06 mm in the Malakan horse (Gürbüz et al., 2016), 0.39 mm in the hamster (Mohammadpour, 2011) and 2.11 ± 0.10 mm in the red fox (Hadziomerovic et al., 2023). The length of the rostral crus in our study (2.12 ± 0.21 mm) was similar to the result of Hadziomerovic et al. (2023).

No study has been found regarding measurements of the length of between the caput mallei and muscular process, and intercrural foramen length and width. Our data were measured by considering the importance of auditory ossicle anatomy for the surgical approaches in audiometric studies and comparative anatomy.

Santos et al. (2021) detected a significant correlation between the length of the malleus with the height of the malleus and width of the stapes, between the length of the incus with height of the incus, length and height of the stapes, between length of the stapes with height of the stapes in goats. In our study, a strong correlation was found between the length of the malleus and the length of the manubrium mallei, between the length of the incus with the lengths of the long crus and corpus incudis, between the length of the stapes with the lengths of the rostral crus, caudal crus, caput stapedis, length and width of the intercrural foramen. The correlation between ossicles was investigated in our study because variations in the sizes of auditory ossicles and correlations between them are important for ossiculoplasty and implantable hearing aids.

According to the results of our study, the morphometric characteristics of sheep ears are similar to human ears. Sheep ears are excellent models in specialist training for middle ear surgery. They will also assist in the development of surgical skills and experimental otologic studies such as surgical interventions. We believe that our results will be a reference for studies performed on other animal models and will contribute to the literature in terms of morphometrics.

AUTHOR CONTRIBUTIONS

Conception or design: Burcu Kamaşak Arpaçay, Kenan Aycan. The acquisition of data: Berat Yağmur. The data analysis and interpretation: Burcu Kamaşak Arpaçay, Berat Yağmur, Emre Uğuz. Drafting the work: Elif Çömlekçi, Ruken Öncü. Revising it critically for important intellectual content: Tufan Ulcay. All authors have read and final approved of the manuscript being submitted.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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