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Comparative Investigation of Tongue and Esophagus Morphometry in Swiss Albino and Balb-c Mice

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ABSTRACT

This study was carried out to obtain morphometric measurement values of tongue and esophagus in Swiss Albino and Balb-c mice, to examine the biometric differences of these measurements between sexes and groups. Thirty-two mice, 16 Swiss Albino (8 males, 8 females) and 16 Balb-c (8 males, 8 females), were used for this study. Mice anesthetized with the ketamine-xylazine combination were fixed by the technique. The tongue and esophagus of the mice were carefully dissected and removed. Then, morphometric measurements of the dissected organs were taken, and statistical analysis was performed. When the morphometric measurement values were examined, it was seen that all of the measurement values obtained from both Swiss Albino and Balb-c mice were higher in males than in females. Statistically significant differences were observed between the groups in all morphometric measurement values of tongue and esophagus in both mouse races ($p<0.05$). Additionally, positive and significant correlations were found between morphometric measurement values of tongue and esophagus in both groups according to gender. Differences in morphometric measurement values of the tongue and esophagus were determined between genders and groups in Swiss Albino and Balb-c mice. Furthermore, this study provided basic morphometric data that will benefit various scientific fields related to the tongue and esophagus in these animals, especially anatomical studies.

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Introduction

Experimental animals are frequently used in many fields of biomedical, experimental, and behavioral research, including neuroscience, oncology, teratology, immunology, toxicology, pathology, and anatomy (Sengupta, 2013; Elsayed & EL-Gammal, 2024). The house mouse, identified as *Mus musculus Linnaeus*, 1758, has been a member of humanity's close circle throughout the centuries. Swiss Albino and BALB/c mice are a series of common subspecies formed by breeding and rearing house mice in the laboratory environment, and they are also small albino mammal species from the order Rodentia that are widely used in scientific studies (Van Loo et al., 2000; Živković et al., 2016; Ayırtır Başdinç, 2023).

In the present day, house mice (*Mus musculus*) are an excellent mammalian model that is widely used to study the characteristics and diseases of various pathological conditions that disrupt the anatomical structure in systems such as immunity, metabolism, development, and neurological disorders (Morse, 2007; Phifer-Rixey & Nachman, 2015). Moreover, in recent years, various studies have focused on revealing the morphometric characteristics of various anatomical structures, particularly in pet animals, exotic species, as well as in experimental animals (Koca et al., 2023a, 2023b, Yilmaz et al., 2023, Yilmaz et al., 2024).

In mice, the tongue extends from the epiglottis to the lower incisors. The distal part of the tongue is attached to the floor of the mouth, the dorsal part is rough-looking, while the ventral and lateral parts are smooth-looking. There is also a dorsal median groove at the tip of the rodent tongue, which ends shortly before a prominent elevation at the intermolars, known as the intermolar eminence (Treuting et al., 2017). The esophagus is a muscular, tube-shaped organ that stretches from the pharynx to the ostium cardia of the gaster. Its primary function is to transport chewed and swallowed food from the pharynx to the stomach (Çalışlar, 1978).

In this study, our objectives were threefold: (1) to obtain morphometric measurement values of the tongue and esophagus in Swiss Albino and Balb-c mice, (2) to determine correlations between measurement values based on gender, and (3) to investigate the biometric differences of these measurements between genders and groups.

Material and Method

Experimental Animals

Animal material for this study was obtained from Van Yüzüncü Yıl University (Van YYU) Experimental Animal Unit. In the study, a total of 32 mice, 8 male and 8 female, 16 each of Balb c and Swiss albino mice, aged 5-6 weeks, were used. While the average weight of Swiss Albino male and female mice was determined as 37,000±2,976 g and 29,375±1,408 g, respectively, the average weight of Balb-c male and female mice was detecteded as 33,125±2,748 g and 28,500±1,309 g, respectively. The animals were subjected to a 12-hour light-dark cycle and housed in an environment with a room temperature set at 23±1°C. They were provided with ad libitum access to both drinking water and standard rat pellet feed.

Ethics Committee Approval

This study was conducted after obtaining the requisite permissions from the Van YYU Animal Experiments Local Ethics Committee (VAN YUHADYEK), as per their decision dated 30/11/2023 and numbered 2023/13-15.

Anesthesia

For the anesthesia of the mice involved in the study, a combination of Ketamine (50 mg/kg, intraperitoneal) and Xylazine Hydrochloride (10 mg/kg, intraperitoneal) was administered. After the administration of general anesthesia, the animals were perfused by opening the thoracic cavity in accordance with ethical guidelines. Subsequently, the mice were immersed in a 10% buffered formaldehyde solution for one week for fixation. Following this period, the tongues and esophagus of the mice were carefully dissected and removed.

Measuring Parameters

Weight measurements of Swiss Albino and Balb-c mice were conducted using precision scales (TESS®, RP-LCD, Comak Scale, Istanbul), while length measurements were taken using digital calipers. Volume measurements were determined using Archimedes' Principle. In Archimedes' Principle, the volume of water displaced by an object submerged in water is equal to the volume of the object itself. For this purpose, the volume of each organ, delineated from its anatomical borders, was measured using a graduated cylinder with a precision of 2/1000 cm^3. The quantity of overflowing water was carefully noted and documented. In the study, length measurements of organs were assessed in millimeters (mm), weight measurements in grams (g), and volume measurements in cubic centimeters (cm^3). Photographs of the tongues and esophagus of the study mice were captured using a Sony Digital DSC-W830 camera. The terminology used for anatomical references in the study was Nomina Anatomica Veterinaria (2017). Measurement points and abbreviations of the tongue and esophagus in Swiss Albino and Balb-c mice were presented in Table 1. Moreover, the morphometric measurement points of the tongue and esophagus were given in Figure 1.

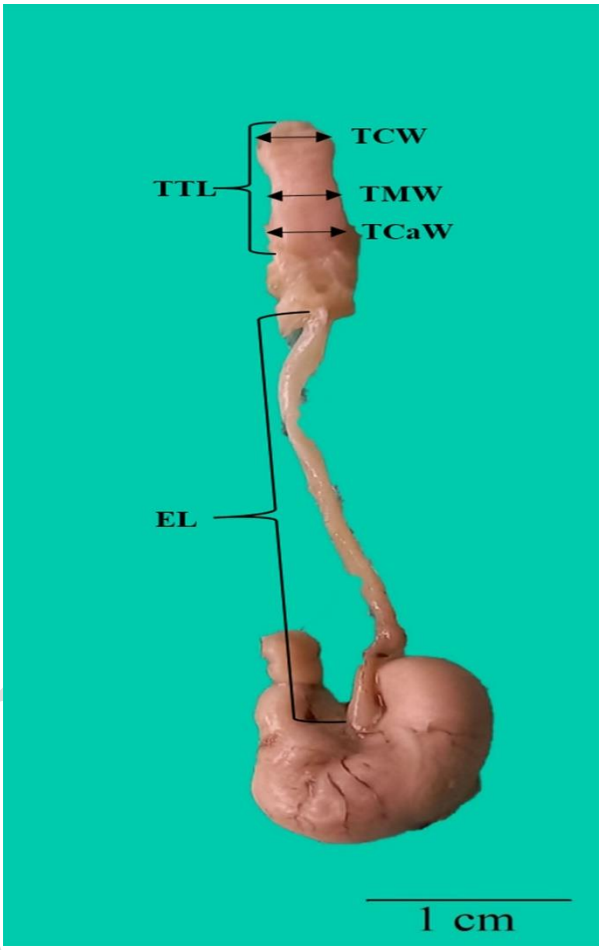

Figure 1. Morphometric measurement points of the tongue and esophagus in Swiss Albino and Balb-c mice

Statistical Analysis

In the study, parametric tests were applied. Descriptive statistics for the variables were presented as mean, standard deviation, median, minimum, and maximum values. The comparison of measurements across groups were conducted using both the Independent T-test and One-Way Analysis of Variance (ANOVA). After conducting the analysis of variance, the "Duncan test" was employed to ascertain any significant differences among the various groups. Pearson correlation coefficients were calculated to determine the relationship between measurements. The statistical analysis was performed using the SPSS software package (IBM SPSS for Windows, version 26).

Results

In the presented study, morphometric measurements of the tongue and esophagus of Swiss Albino and Balb-c mice were taken. The measurements obtained were statistically evaluated for sexual dimorphism, and the analyses conducted were presented in Tables 2-6. Statistically significant differences ($p<0.05$) were observed among the measurement values.

Table 1. Measurement points and abbreviations of the tongue and esophagus in Swiss Albino and Balb-c mice

Parameter	Abbreviation	Definition
1.	W	Weight (g)
2.	TTL	Total length of the tongue in the craniocaudal direction (mm)
3.	TCW	Laterolateral width of the cranial part of the tongue (apex lingua) (mm)
4.	TMW	Laterolateral width of the medial part of the tongue (torus lingua) (mm)
5.	TCaW	Laterolateral width of the caudal part of the tongue (radix lingua) (mm)
6.	TV	Volume of the tongue (cm^3)
7.	TW	Weight of the tongue (g)
8.	EL	Length of the esophagus in the craniocaudal direction (mm)
9.	EV	Volume of the Esophagus (cm^3)
10.	EW	Weight of the Esophagus (g)

Table 2. Descriptive statistics and comparison of morphometric measurement values of the tongue and esophagus of Swiss Albino mice

	Swiss Albino Male					Swiss Albino Female					
	Mean	Std. Dev.	Median	Min.	Max.	Mean	Std. Dev.	Median	Min.	Max.	*p.
W	37.000	2.976	36.000	34.000	43.000	29.375	1.408	29.500	27.000	31.000	0.001
TTL	13.200	1.042	13.550	11.500	14.200	11.913	0.439	11.950	11.200	12.500	0.006
TCW	2.688	0.290	2.750	2.300	3.100	1.850	0.346	1.850	1.200	2.300	0.001
TMW	3.400	0.239	3.400	3.000	3.800	2.863	0.226	2.800	2.600	3.200	0.001
TCaW	4.575	0.381	4.750	3.900	4.900	3.625	0.260	3.650	3.300	4.000	0.001
TV	0.231	0.070	0.250	0.150	0.300	0.181	0.037	0.175	0.150	0.250	0.097
TW	0.127	0.024	0.126	0.098	0.165	0.101	0.016	0.106	0.076	0.117	0.021
EL	30.300	3.818	28.950	26.300	35.400	29.575	1.695	30.100	26.700	31.400	0.631
EW	0.038	0.012	0.042	0.011	0.048	0.033	0.010	0.033	0.024	0.044	0.025
EV	0.156	0.142	0.105	0.080	0.500	0.098	0.015	0.090	0.050	0.150	0.039

*p<0.05; Independent sample T-test

Table 3. Descriptive statistics and comparison of morphometric measurement values of the tongue and esophagus of Balb-c mice

	Balb-c Male					Balb-c Female					
	Mean	Std. Dev.	Median	Min.	Max.	Mean	Std. Dev.	Median	Min.	Max.	*p.
W	33.125	2.748	32.500	30.000	38.000	28.500	1.309	28.500	26.000	30.000	0.001
TTL	12.138	0.558	12.150	11.400	12.900	11.950	0.400	11.850	11.400	12.600	0.453
TCW	2.375	0.266	2.350	1.800	2.600	2.300	0.076	2.300	2.200	2.500	0.802
TMW	2.900	0.278	2.850	2.500	3.300	2.875	0.175	2.800	2.700	3.200	0.833
TCaW	3.263	0.267	3.250	2.800	3.700	3.213	0.318	3.250	2.800	3.600	0.739
TV	0.181	0.026	0.200	0.150	0.200	0.156	0.050	0.175	0.100	0.200	0.227
TW	0.115	0.013	0.122	0.094	0.128	0.104	0.013	0.107	0.081	0.119	0.110
EL	34.200	0.974	34.200	33.100	35.900	31.338	0.969	31.300	29.900	32.900	0.001
EW	0.044	0.010	0.048	0.028	0.056	0.034	0.004	0.035	0.028	0.038	0.017
EV	0.058	0.014	0.050	0.050	0.080	0.050	0.001	0.050	0.050	0.050	0.149

*p<0.05; Independent sample T-test

The descriptive statistics and comparison of morphometric measurement values for the tongues and esophagus in Swiss albino mice are presented in Table 2. According to this, it was observed that all morphometric measurement values of the tongue and esophagus were higher in males compared to females. Additionally, it was noted that the W, TTL, TCW, TMW, TCaW, TW, EW, and EV measurement values were statistically significantly higher in male Swiss albino mice compared to females ($p<0.05$).

The descriptive statistics and comparative analysis of morphometric measurements of the tongue and esophagus in Balb-c mice are presented in Table 3. It was observed that all measured values pertaining to the tongue and esophagus were greater in males compared to females. Additionally, W, EL and EW measurement values were observed to be statistically significantly higher in male Swiss Albino mice than in females ($p<0.05$).

Descriptive statistics and comparison of morphometric measurement values of tongue and esophagus in male-female Swiss Albino and Balb-c mice are determined in Table 4. Upon a general examination of the table data, statistically significant differences were observed between the groups in all morphometric measurement values of the tongues and esophagi for both mice breeds ($p<0.05$). Furthermore, statistically significant differences were observed between male Swiss Albino and male Balb-c mice in W, TTL, TCW, TMW, TCaW, EL, EW and EV measurement values ($p<0.05$). Additionally, statistically significant differences were detected between Swiss albino male and Balb-c female groups in all measurement values except for the EL measurement ($p<0.05$).

Table 4. The descriptive statistics and comparison of morphometric measurement values for the tongues and esophagus in Swiss Albino and Balb-c mice.

	Swiss Albino Male		Swiss Albino Female		Balb-c Male		Balb-c Female		
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.	*p.
W	37.000[a]	2.976	29.375[c]	1.408	33.125[b]	2.748	28.500[c]	1.309	0.001
TTL	13.200[a]	1.042	11.913[b]	0.439	12.138[b]	0.558	11.950[b]	0.400	0.001
TCW	2.688[a]	0.290	1.850[c]	0.346	2.275[b]	0.266	2.300[b]	0.076	0.001
TMW	3.400[a]	0.239	2.863[b]	0.226	2.900[b]	0.278	2.875[b]	0.175	0.001
TCaW	4.575[a]	0.381	3.625[b]	0.260	3.263[c]	0.267	3.213[c]	0.318	0.001
TV	0.231[a]	0.070	0.181[ab]	0.037	0.181[ab]	0.026	0.156[b]	0.050	0.033
TW	0.127[a]	0.024	0.101[b]	0.016	0.115[ab]	0.013	0.104[b]	0.013	0.018
EL	30.300[b]	3.818	29.575[b]	1.695	34.200[a]	0.974	31.338b	0.969	0.001
EW	0.038[b]	0.012	0.033[c]	0.010	0.044[a]	0.010	0.034[c]	0.004	0.018
EV	0.156[a]	0.142	0.098[b]	0.015	0.058[c]	0.014	0.050[c]	0.000	0.017

*p<0.05: Significance levels according to one way ANOVA test; a.b.c: Shows the difference between subgroups (Duncan post-hoc test).

Table 5. Correlation between morphometric measurement values of the tongue and esophagus in Swiss Albino mice based on gender.

Male		Female									
		W	TTL	TCW	TMW	TCaW	TV	TW	EL	EW	EV
W	R	1	0.130	-0.395	0.409	0.516	0.153	0.307	-0.127	0.233	0.493
TTL	R	0.465	1	-0.230	0.049	0.122	0.454	0.592	-0.136	-0.373	-0.369
TCW	R	0.513	0.785*	1	0.373	-0.348	0.471	-0.675	0.263	0.047	0.267
TMW	R	0.120	0.688	0.701	1	0.672	0.413	-0.530	-0.420	-0.393	0.102
TCaW	R	0.378	0.526	-0.029	-0.094	1	0.055	-0.164	0.797*	-0.565	-0.178
TV	R	0.409	0.915**	0.827*	0.764*	0.300	1	-0.158	0.127	-0.238	0.311
TW	R	0.422	0.840**	0.440	0.643	0.653	0.786*	1	0.068	0.308	-0.075
EL	R	0.747*	0.439	0.624	0.225	0.053	0.579	0.306	1	0.476	0.337
EW	R	0.579	-0.076	0.095	0-.263	-0.011	0.002	-0.135	0.749*	1	0.725*
EV	R	-0.284	0.536	0.405	0.766*	0.019	0.564	0.541	-0.263	0.791*	1

* Correlation is significant at the p<0.05; ** Correlation is significant at the p<0.01; R: Pearson Correlation Coefficient.

Table 6. Correlation between morphometric measurement values of the tongue and esophagus in Balb-c mice based on gender.

Male		Female									
		W	TTL	TCW	TMW	TCaW	TV	TW	EL	EW	EV
W	R	1	0.764*	0.866**	-0.311	0.463	-0.055	0.693	0.298	0.787*	0.224
TTL	R	0.090	1	0.709*	0.000	0.208	-0.450	0.914**	-0.142	0.716*	0.361
TCW	R	0.220	0.778*	1	-0.539	0.000	-0.191	0.633	-0.019	0.446	0.437
TMW	R	0.636	0.221	0.445	1	0.083	0.267	0.230	-0.212	-0.059	0.215
TCaW	R	0.358	-0.210	0.005	0.809*	1	0.040	0.063	0.813*	0.793*	0.425
TV	R	0.540	0.105	0.026	0.298	-0.013	1	-0.125	0.039	-0.213	0.447
TW	R	0.586	0.192	0.064	0.365	0.101	0.958**	1	-0.330	0.561	0.383
EL	R	0.096	0.063	-0.452	-0.417	-0.429	0.510	0.559	1	0.476	0.193
EW	R	0.815*	-0.234	0.116	0.543	0.416	0.438	0.413	-0.115	1	0.422
EV	R	0.421	0.761*	-0.638	0.222	0.434	0.447	0.389	0.190	0.620	1

* Correlation is significant at the p<0.05; ** Correlation is significant at the p<0.01; R: Pearson Correlation Coefficient.

Moreover, statistically significant differences were observed between Swiss Albino female mice and Balb-c male mice in the measurements of W, TCW, TCaW, EL, EW, and EV (p<0.05). Similarly, statistically significant differences were determined between Swiss Albino female and Balb-c female mice in the measurements of TCW, TCaW, and EV (p<0.05).

In Table 5, the correlation between morphometric measurement values of the tongue and esophagus in Swiss Albino mice was examined based on gender. Accordingly, in male Swiss albino mice, positive significant correlations were observed (p<0.05) between the following morphometric measurement values: W and EL; TTL and TCW, TV, and TW; TCW and TV; TMW and TV, and EV; EL and EW; EW and EV. Additionally, in female Swiss albino mice, positive significant correlations were detected (p<0.05) between the following morphometric measurement values: EL and TCaW; EV and EW.

In Table 6, the correlation between morphometric measurement values of the tongue and esophagus in Balb-c mice was analyzed based on gender. Accordingly, in male Balb-c mice, positive significant correlations were identified (p<0.05) between the following morphometric measurement values: W and EW; TTL and TCW, and EV; TMW and TCaW; TV and TW. Additionally, in female Balb-c mice, positive significant correlations were observed (p<0.05) between the following morphometric measurement values: W and TTL, TCW, and EW; TTL and TW, and EW; TCaW and EL, and EW.

Discussion

Basic data on organ and body morphometrics in living organisms constitute a vital source of information in various fields of veterinary medicine and biological sciences (Gangrade, 2009). Drug interactions in animal experiments conducted in various ways can be inferred from morphometric data of organs (Gırgırı et al., 2015). While morphological changes in organs may sometimes not be visibly apparent (Bailey et al., 2004), deviations from normal morphometric values can indicate an underlying pathological condition in some cases (Tanna et al., 2011). Among mammals, there is significant diversity in morphological and morphometric characteristics of the gastrointestinal system, including shape and structure, in animals fed various diets (Gırgırı et al., 2015). In this study, morphometric measurement values of the tongue and esophagus in Swiss Albino and Balb-c mice were obtained, correlations between body weight and morphometric measurements according to gender were determined, and differences in these measurements regarding sexual dimorphism were evaluated.

In general, numerous studies have been conducted to determine the anatomical, morphological, and morphometric characteristics of the other digestive system organs, including the tongue and esophagus concerning sexual dimorphism in other animals as well as laboratory animals (Yıldız et al., 2001; Walters et al., 2014; Igado et al., 2015; Gırgırı et al., 2015; Sahd et al., 2017; Bełżecki et al., 2018; Jaji et al., 2019; Ayırtır Başdinç, 2023; Andrade et al., 2023). When examining the morphometric measurement values in our study, it was seen that all measurement values obtained from both Swiss Albino and Balb-c mice were higher in males compared to females. From these results, we can conclude that the morphometric measurement values of the tongue and esophagus in both Swiss Albino and Balb-c mice are larger in males compared to females.

In studies aimed at determining the morphometric and morphological characteristics of gastrointestinal system organs, it has been observed that there is predominantly a positive correlation between external measurement values in animals (such as body weight) and morphometric data of the organs (Walters et al., 2014; Sahd et al., 2016; Bełżecki et al., 2018). However, in studies conducted on morphometric measurements of the tongue and esophagus, alongside positive correlations between these measurements and both external body measurements and other gastrointestinal system organs, negative correlations have also been identified (Igado et al., 2015; Andrade et al., 2023). In this study, considering the sexual dimorphism characteristics of both mice breeds, it was observed that the statistically significant measurements between the morphometric data of the tongue and esophagus were mostly positive correlations. It is thought that these correlations may be very useful in revealing sexual dimorphism features in mice and in various clinical application areas.

Andrade et al. (2023) reported in their study on squirrel monkeys (*Saimiri sciureus Linnaeus*, 1758) that the length measurements of the tongue and esophagus were 3.0 cm and 12.7 cm, respectively, in males. Additionally, in the same study, they calculated the weight of the tongue as 2.2 g in males and 1.9 g in females. Adeniyi (2010) found the anteroposterior length and weight of the tongue in hedgehogs to be 3.10 ± 0.51 cm and 1.32 ± 0.09 g, respectively. In Eastern gray squirrels (*Sciurus carolinensis*), the length and weight measurements of the tongue and esophagus were determined as 2.86 ± 0.61 cm, 1.25 ± 0.22 g, and 6.06 ± 0.74 cm, 0.50 ± 0.08 g, respectively (Nazalak et al., 2015). Jaji et al. (2019) reported that in African hedgehogs (*Atelerix albiventris*), the length of the tongue was 2.97 ± 0.83 cm in males and 3.70 ± 0.26 cm in females; while the length measurements of the esophagus were 6.00 ± 0.30 cm in males and 9.50 ± 2.78 cm in females. In fruit bats (*Eidolon helvum*), the weight and length measurements of the tongue were found to be 3.130 ± 0.425 g, 3.763 ± 0.409 cm in males and 3.143 ± 0.542 g, 3.967 ± 0.104 cm in females, respectively (Igado et al., 2015). In African giant rats, the weight and length measurements of the esophagus were calculated as 1.39 ± 0.08 g and 11.78 ± 0.53 cm, respectively (Nazalak et al., 2012). In the conducted study, the weight and length measurements of the tongue in Swiss Albino and Balb-c mice were determined as follows: for males, 0.127 ± 0.024 g and 13.200 ± 1.042 mm in Swiss Albino mice, and 0.115 ± 0.013 g and 12.138 ± 0.558 mm in Balb-c mice; for females, 0.101 ± 0.016 g and 11.913 ± 0.439 mm in Swiss Albino mice, and 0.104 ± 0.013 g and 11.950 ± 0.400 mm in Balb-c mice. The weight and length measurements of the esophagus were determined as follows: for males, 0.038 ± 0.012 g and 30.300 ± 3.818 mm in Swiss Albino mice, and 0.044 ± 0.010 g and 34.200 ± 0.974 mm in Balb-c mice; for females, 0.033 ± 0.010 g and 29.575 ± 1.695 mm in Swiss Albino mice, and 0.034 ± 0.004 g and 31.338 ± 0.969 mm in Balb-c mice.

In hedgehogs, the volume of the tongue was found to be 0.92 ± 0.12 ml (Adeniyi, 2010). Additionally, in a study aimed at determining the morphometric characteristics of the gastrointestinal system in rabbits at 2, 4, and 6 months of age, esophageal volume measurements were calculated as follows: for male rabbits, 0.74 ± 0.09 cm^3 at 2 months, 1.84 ± 0.14 cm^3 at 4 months, and 1.71 ± 0.07 cm^3 at 6 months; for females, 1.04 ± 0.09 cm^3 at 2 months, 1.75 ± 0.14 cm^3 at 4 months, and 1.50 ± 0.21 cm^3 at 6 months (Yıldız et al., 2001). In the current study, the volume measurements of the tongue and esophagus in Swiss Albino and Balb-c mice were determined as follows: for males, 0.231 ± 0.070 cm^3 and 0.181 ± 0.026 cm^3 in Swiss Albino mice, and 0.156 ± 0.142 cm^3 and 0.058 ± 0.014 cm^3 in Balb-c mice; for females, 0.181 ± 0.037 cm^3 and 0.156 ± 0.050 cm^3 in Swiss Albino mice, and 0.098 ± 0.015 cm^3 and 0.050 ± 0.000 cm^3 in Balb-c mice. It is thought that these differences between animals in the length, weight and volume measurements of the tongue and esophagus of Swiss albino and Balb-c mice are presumed to be due to the breed characteristics of the animals and individual differences between measurement methods.

Conclusion

In conclusion, differences in morphometric measurement values of the tongue and esophagus in terms of sexual dimorphism were identified in Balb-c and Swiss

Albino mice. Additionally, the study provided fundamental morphometric data that will be beneficial for various scientific fields such as anatomical studies, as well as surgical, pathological, and laboratory animal sciences related to the tongue and esophagus in these animals.

Declarations

Funding Statement: The author received no specific funding for this study.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

Ethical Statement: Date: 30/11/2023, Decision Number: 2023/13-15

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