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Stereological investigation of medulla oblongata volume in Norduz sheep

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Abstract: In this study, it was aimed to stereologically examine the medulla oblongata volume of Norduz sheep in the Gürpınar District of Van Province. Upon receiving news from the sections in the Gürpınar region, we went to the region. A total of 12 Norduz sheep skulls, 6 females and 6 males, were received from the Norduz sheep slaughter in the region. After the brains of Norduz sheep were dissected, they were fixed as a whole in formaldehyde for 1 week. Medulla oblongatas were separated from the brain and kept in formaldehyde for 1 week. Tissues obtained by sequential random sampling from the medulla oblongata were used for texture tracking. After texture tracking, 10–12 sections of 5 µm thickness were taken by sequential random sampling method using a rotary microtome (Leica RM, 2135, Leica Instruments, Nussloch, Germany) using steel disposable microtome blades. These obtained sections were stained with the hematoxylin-eosin staining method and viewed under an Olympus CX31 microscope with Olympus U - TV0, 5XC - 3 Tokyo Japan. Photos taken with a 4× objective were used for volume calculations. Total medulla oblongata volume, medulla volume, and cortex volume values were calculated separately from the obtained images using the Shtereom I program. The Cavalieri's principle was used when applying stereological methods for volume calculations. In the statistical evaluation between the sexes, no difference was found in the values obtained in the comparison between the total volume values, medulla volume values, and cortex volume values between both sexes. In addition, there was no difference between the sexes in terms of medulla/total volume ratio values. However, a value of 0.004 was obtained in terms of cortex/total volume ratio values. In this case, since $p < 0.005$, it is possible to talk about a statistically significant difference in male and female sheep in terms of this ratio value. Again, the medulla/cortex volume ratio value was calculated to be 0.041, and a significant difference was observed in terms of this value in the evaluation between sexes. The volume calculations obtained by stereological methods were evaluated with statistical programs and all the results were presented to the literature.

Key words: Cavalieri's principle, medulla oblongata, Norduz sheep, stereology, volume

1. Introduction

It has been observed that the sheep reared in the region are well adapted to the conditions of the region, and it has been observed that the low level of domestic gene resources in terms of yield characteristics are in danger of extinction. It has become necessary to protect and develop local gene resources because they play a serious role in the development of new types that comply with local breeding conditions at certain times and are more resistant to certain diseases. It has been understood that there are also Norduz type sheep, which has no chance of being identified, although many specific types (Karakaş, Kangal, Güney, Karaman) have been identified within the Akkaraman breed, which constitutes a high part of the sheep population in Türkiye [1,2]. When the morphological and physiological characteristics of Norduz sheep were evaluated, it was revealed that they had some different characteristics from other domestic breeds. In addition, it is known that Norduz sheep have different characteristics in the region. It is assumed that the yield that can be obtained can be increased if the breeders in the region are trained and their conditions are good. Considering that Norduz sheep are in danger of extinction as a domestic gene source, it has emerged that it will be important to develop and increase some urgent measures [3]. The nervous system is the name of the system that connects the living thing with its own body and the external environment [4]. In order for every living organism to survive, it must show appropriate reactions to the differences in its environment. This increases the chances of survival of the species. The regulation of related reactions is under the responsibility of the nervous system, which is arguably the most complex structure among body systems [5]. The nervous system consists of two anatomical parts, the central nervous system (CNS) (systema nervosum centrale) and the peripheral nervous

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system (systema nervosum peripheric um) (PSS) [6]. The central nervous system, on the other hand, consists of two parts, the spinal cord and the encephalon [4]. The medulla oblongata belonging to the brain, the spinal cord located in the ventral part of the rhombencephalon, has an anatomical structure that is broader in cattle and short in horses. The border between the spinal cord and the medulla oblongata gives the transverse plane assumed to pass just in front of the first cervical nerves. Its connection with the pons in front of it is the sulcus pontobulbaris [4,7–9]. The pons is twice as large as the other parts near the medulla oblongata [8]. Stereology is a branch of science that helps to make interpretations concerning the three-dimensional properties of the structure by moving from the two-dimensional images of any structure that is actually thought to be three-dimensional. In stereology, the Cavalieri principle is used as an accepted method for quantitative studies [10]. When the origin of the term stereology is examined, the word "stereos", which means a body containing a three-dimensional axis, is encountered. When evaluated historically, it is concluded that the stereology method was used exactly in the 1960s [11].

The aim of this study is to determine the total volume in the medulla oblongata, and the cortex and medulla volume values that shape the medulla oblongata separately, using stereological methods in Norduz sheep, which is a breed specific to Van Province and bred in the Norduz region of Gürpınar District. The region we live in has given the priority of this study to the Norduz sheep because it is a different breed. Our hope and goal is that with this study planned on the medulla oblongata, which is a part of the nervous system, the medulla oblongata volume values of the Norduz sheep, which is a local breed in the literature, will lead other studies to be carried out in the field of veterinary medicine and be evaluated as a reference study.

2. Materials and methods

2.1. Materials

This study was carried out at the Experimental Medicine Application and Research Center in accordance with the decision of the Animal Experiments Local Ethics Committee of Van Yüzüncü Yıl University, dated 29.04.2021 and numbered 2021/4 - 21. Laboratory studies were carried out in the research laboratories of Van Yüzüncü Yıl University Veterinary Faculty Anatomy and Veterinary Faculty Pathology Departments. In order to obtain the medulla oblongatas used in the study, a total of 12 Norduz sheep skulls, 6 females and 6 males, were obtained under the control of a zootechnician and upon receiving news of the slaughter of Norduz sheep at different times from the Norduz region villages in the Gürpınar District of Van Province.

2.2. Method

2.2.1. Dissection of the medulla oblongata

The skulls of Norduz sheep taken from the Norduz region at different times were dissected and the brains were first removed as a whole. The removed brains as a whole were fixed in formaldehyde for 1 week. Next, the medulla oblongata was carefully separated from the cerebral hemispherium and other structures of the brain. Medulla oblongatas were kept in formaldehyde for 1 week for fixation. The physical dissector method was preferred for the stereological study. It was found convenient to divide the medulla oblongata into three parts in order to fit the tissue samples into the tissue tracking cassettes. The obtained tissues were given for tissue follow-up.

2.2.2. Sampling method

A pilot study was conducted before the study in order to determine the number of samples, the number of sections, and sampling. In stereological studies to be conducted in the literature, it has been reported that at least 5 animals are required to obtain an error coefficient of approximately 0.05 [12]. With the pilot study, it was determined that the number of samples, the number of sections, and the sampling method were appropriate.

2.2.3. Obtaining the sections

In order to obtain the number of sections determined in the pilot study, 5-µm-thick sections were obtained from each of the tissue samples of the medulla oblongata, which was blocked by paraffin after sampling. Sections were taken using a Rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany). An average of 3–5 sections were obtained from each medulla oblongata sample of an animal. Starting from a random one among the first 5 sections, every 80th section following it was taken. Sampling was done systematically and randomly at the rate of 1/80. The sections were stained with hematoxylin-eosin and histological images were obtained (Figures 1–3). Volume counts were calculated with the 4× objective. Sampled sections were then added to the study. After this pilot study, based on the sampling method, the same procedures were applied to the medulla oblongata of the other sheep, and the microtome sectioning processes were continued based on the sampling rates and shape in the pilot study.

2.2.4. Image analysis

Before counting, images were taken under the microscope (Olympus CX31 brand microscope, Olympus U - TV0, 5XC - 3 Tokyo Japan). The Cavalieri Principle was used when applying stereological methods [13,14]. Since the numerical ratios of the points are valid instead of the volume, the number of points was preferred for volume calculations [15,16]. When using a dotted area measurement ruler, calculations were made automatically

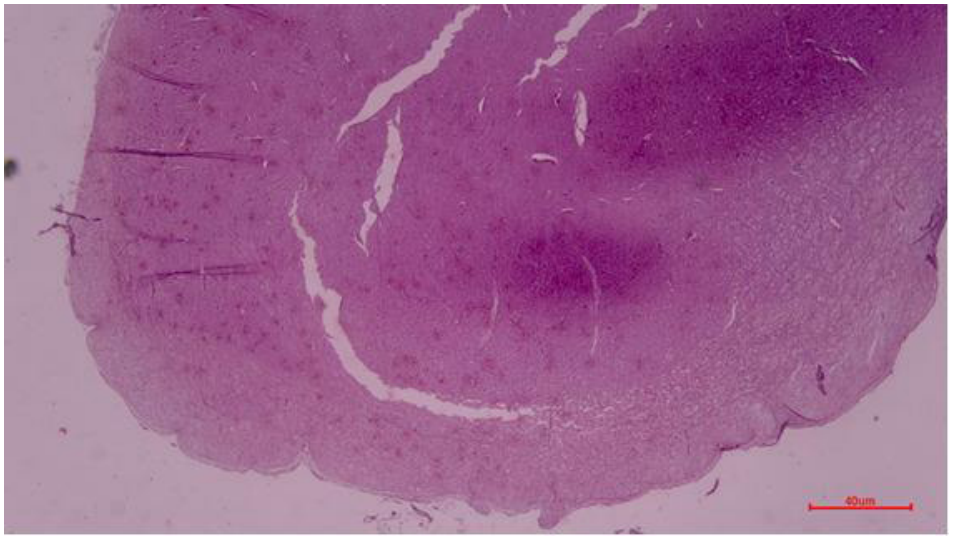

Figure 1. Medulla oblongata in female Norduz sheep (× 4×), hematoxylin-eosin staining

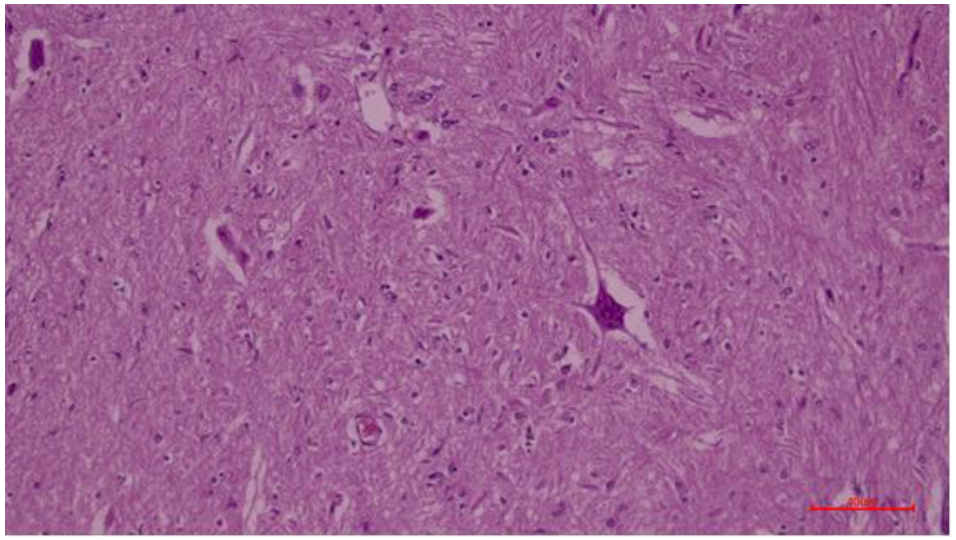

Figure 2. Medulla oblongata in male Norduz sheep (10×), hematoxylin-eosin staining.

with the Shtereom I package program by using large points to prevent unnecessary point counting [17]. Male and female comparisons were made over the calculated volume values. While comparing the volume values, the Mann–Whitney U analysis was applied and the results were compared statistically. The obtained results were presented to the literature as reference values. In studies using the Cavalieri principle, the coefficient of error (CE) must be calculated in order to decide whether the number of sections and, accordingly, the section spacing and the frequency of the dots on the dotted area test chart are sufficient. The mean standard error, or coefficient of error, is a value that confirms the resulting volume values using the Cavalieri principle. In our study, the CE value was calculated to determine the accuracy of the volume values obtained [18]. With this value, the number of sections required for the study and the adequacy of the dot frequency on the dotted area ruler were also determined. Especially in volume measurements, it is preferred that the error coefficient value be less than or equal to 5% in order to accept the results as reliable and to determine their closeness to reality [18–20].

3. Results

In this study, a total of 12 skulls were obtained from healthy Norduz sheep, 6 males and 6 females, with an average

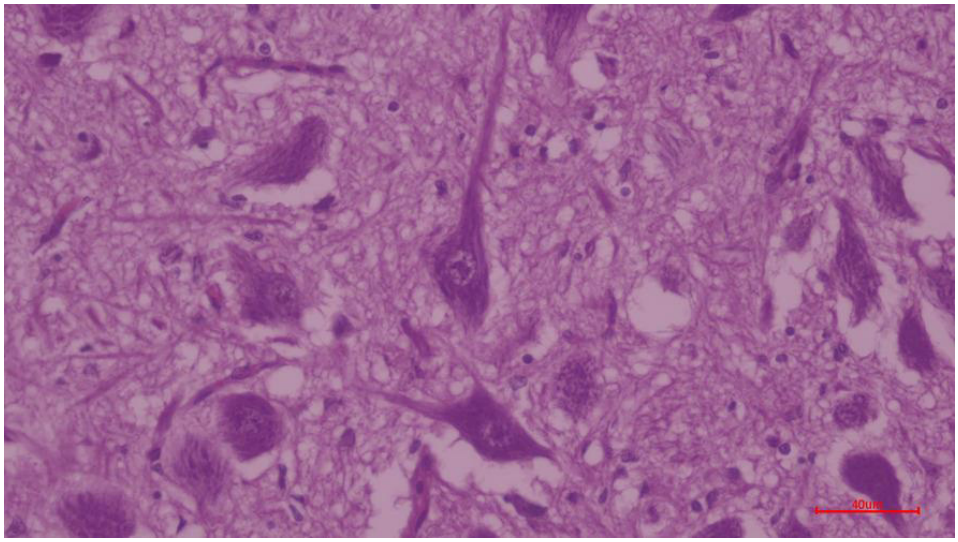

Figure 3. Medulla oblongata in female Norduz sheep (40×), hematoxylin-eosin staining.

weight of 30–40 kg and an average age of 1–3. Each of the medulla oblongata obtained from these skulls was separated from the cerebrum and fixed in formaldehyde for 1 week. After that, sections were taken from tissue samples of each animal with a microtome, and the sections were stained with hematoxylin-eosin and histological images were obtained, and volume values were calculated. In this study, the total volume, medulla and cortex volume values of the medulla oblongata of 12 Norduz sheep, 6 females and 6 males, were investigated. As a result of the evaluations, the volume values between female and male Norduz sheep were found to be different. When the average volume values were examined, it was observed that the highest average total volume value belonged to male Norduz sheep and was determined as 4.524 mm^3. When the total volumes of the medulla oblongata of female Norduz sheep were evaluated, the value of 4.123 mm^3 was calculated as the lowest average total volume value. When the medulla volume of the medulla oblongata was examined, it was determined that male Norduz sheep had the highest average volume with 2.413 mm^3, while the lowest average medulla volume was found to belong to female Norduz sheep with a value of 2.327 mm^3. When the cortex volumes of the medulla oblongata in both sexes were evaluated, it was observed that the highest average volume value was found in female Norduz sheep with 1.767 mm^3, and the lowest average cortex volume was found in male Norduz sheep with 1.520 mm^3. In addition, the total volume, medulla volume, cortex volume, the number of points in the sections (noise) and the coefficient of error (CE) values of the sections of the medulla oblongata are given below in Tables 1 and 2.

According to the data in Table 1, the highest noise value of the total volume was 4224 and was detected in M6, while the lowest noise value of the total volume was observed to belong to M5 with a figure of 3305. The highest total CE value of 0.0207 was detected in M3, while the lowest total CE value was detected in M6 with a value of 0.0172. While the mean noise value of the total medulla oblongata was determined as 3619, the mean CE value of the total medulla oblongata was determined as 0.0191. In the CE and noise values calculated in the medulla part of the medulla oblongata, the highest CE value was determined in M5 and determined as 0.0270, while the lowest CE value was observed in M2 with a figure of 0.0228. The highest noise value of the medulla part of the medulla oblongata was 2166 and observed in M2, while the lowest medulla noise value was found in M5 with a figure of 1620. The average CE value of the medulla was 0.0248, and the average noise value of the medulla was observed as 1930. When the CE and noise values of cortex are examined, the highest cortex CE value is 0.0315 in M2, and the lowest cortex CE value is 0.0304 in M1. The highest cortex noise value is 1340 and is observed in M6, and the lowest cortex noise value is 1121, which is observed in M2. The average CE value of cortex was determined as 0.0308, and the average cortex noise value was 1216.

In Table 1, the highest total noise value of the entire medulla oblongata was found in F3, as 4367, while the lowest total noise value was observed in F1 as 2452. Still, when examining the total volume value, it was stated that the highest total CE value was 0.0230 and it was determined in F6, while the lowest total CE value was calculated as 0.0173 and belonged to F3. When the average noise values

Table 1. Coefficient of error (CE) and point number (noise) values of total, medulla, and cortex parts of medulla oblongata in male and female Norduz sheep.

		Male and female coefficient (CE) and noise (N) values						
	Variables	**Number of animals**						**Mean**
		M1	**M2**	**M3**	**M4**	**M5**	**M6**	
Total	**CE**	0.0189	0.0181	0.0207	0.0191	0.0206	0.0172	0.0191
	N	3486	3775	3379	3547	3305	4224	3619
Medulla	**CE**	0.0247	0.0228	0.0260	0.0245	0.0270	0.0235	0.0248
	N	1848	2166	1861	1977	1620	2111	1930
Cortex	**CE**	0.0304	0.0315	0.0309	0.0307	0.0308	0.0305	0.0308
	N	1255	1121	1194	1164	1223	1340	1216
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	
Total	**CE**	0.0218	0.0183	0.0173	0.0214	0.0199	0.0230	0.0203
	N	2452	3544	4367	2893	3559	2977	3298
Medulla	**CE**	0.0248	0.0254	0.0219	0.0280	0.0252	0.0275	0.0254
	N	1838	1854	2387	1459	1943	1692	1862
Cortex	**CE**	0.0282	0.0250	0.0257	0.0307	0.0296	0.0328	0.0286
	N	1391	1710	1725	1226	1311	1119	1413

Table 2. Total, medulla, and cortex volume values of medulla oblongata in male and female Norduz sheep (mm^3).

	Male/female	**Total volume values**	**Medulla volume Values Values Values Değerleri**	**Cortex volume**
NUMBER OF ANIMALS	M1	4.357	2.310	1.568
	M2	4.718	2.707	1.401
	M3	4.223	2.326	1.492
	M4	4.433	2.471	1.455
	M5	4.131	2.025	1.528
	M6	5.280	2.638	1.675
	F1	3.065	2.297	1.738
	F2	4.430	2.317	2.137
	F3	5.458	2.983	2.156
	F4	3.616	1.823	1.532
	F5	4.448	2.428	1.638
	F6	3.721	2.115	1.398
	Mean	**4.123**	**2.327**	**1.767**

of the total volume were examined, this value was found to be 3298. The average CE value obtained while calculating the total volume values was 0.0203. Considering the CE and noise values of the medulla part of the medulla oblongata, the highest CE value was calculated as 0.0280 in F4. The lowest CE value was observed in F3 as 0.0219. The highest noise value of the medulla part belongs to F3, and it was determined as 2387, while the lowest noise value was determined as 1459 in F4. The average CE value of the medulla part was calculated as 0.0254 and the average noise value of the medulla part was calculated as 1862. When the CE and noise values of the cortex belonging

to the medulla oblongata were examined, the highest CE value was determined as 0.0328 in F6, while the lowest CE value was observed as 0.0250 in F2. The highest noise value of cortex is 1725 and it is found in F3, and the lowest noise value is 1119 and it is detected in F6. When the average CE values of cortex were calculated, it was found to be 0.0286, while the average noise value was determined as 1413.

When the values given in Table 2 were examined, it was determined that the highest medulla oblongata total volume value was determined as 5.280 mm^3 in M6, while the lowest total volume value was observed in M5 as 4.131 mm^3. The mean total volume of the medulla oblongata was determined as 4.524 mm^3. The highest medulla volume value was 2.707 mm^3 in M2, and the lowest medulla volume value was observed in M5 as 2.025 mm^3. The mean medulla volume value was determined as 2.413 mm^3. The highest cortex volume value is 1.675 mm^3 and it is observed in M5, the lowest cortex volume value is 1.401 mm^3 in M2. The average cortex volume value was determined as 1.520 mm^3.

According to the values obtained, the highest total volume value was found in F3 as 5.458 mm^3. The lowest total volume value belongs to F1, and it was observed as 3.065 mm^3. When the average total volume values were examined, it was determined that the highest value was 4.123 mm^3. The highest medulla volume value was found in F3 as 2.983 mm^3. It was observed that the lowest medulla volume value was found in F4 and this value was 1.823 mm^3. The mean medulla volume value is 2.327 mm^3. The highest cortex volume value of the medulla oblongata was calculated as 2.156 mm^3 in F3. The lowest cortex volume value is 1.398 mm^3. This value belongs to F6. The average cortex volume value was determined as 1.767 mm^3 (Table 2).

The ratios of the cortex volume values of the medulla oblongata to the total volume in six male Norduz sheep are given in Table 3. The highest cortex/total volume value was determined as 0.369 in M5, while the lowest cortex/total volume value was determined as 0.296 in M2.

The ratio of the cortex volume values of the medulla oblongata to the total volume value and the average cortex/total volume values were determined in 6 female Norduz sheep indicated in Table 3. The highest cortex/total volume value was observed in F1 as 0.576, and the lowest cortex/total volume value was observed in F5 as 0.368. The average cortex/total volume value is 0.435.

The highest medulla/total volume value was determined as 0.573 in M2, while the lowest medulla/total volume value was 0.490 and observed in M5. The mean medulla/total volume value was found to be 0.533 (Table 4).

It was observed that the highest medulla/total volume value was 0.749 in F1, and the lowest medulla/total volume value was 0.504 in F4. The mean medulla/total volume value was calculated as 0.572 (Table 4).

The highest medulla/cortex volume value was 1.932 and was observed in M2, and the lowest medulla/cortex volume value was 1.325 in M5. The mean medulla/cortex volume value was found to be 1.593 (Table 5).

As stated in Table 5, while the highest medulla/cortex volume value was calculated as 1.512 in F6, the lowest medulla/cortex volume value was found to be 1.084 and seen in F2. The average medulla/cortex volume value was determined as 1.328.

Table 3. Cortex/total volume values of medulla oblongata in male and female Norduz sheep.

	Male/female	Total volume values	Cortex volume DEDeğerleri	Cortex/total volume ratios
NUMBER OF ANIMALS	M1	4.357	1.568	0.359
	M2	4.718	1.401	0.296
	M3	4.223	1.492	0.353
	M4	4.433	1.455	0.328
	M5	4.131	1.528	0.369
	M6	5.280	1.675	0.317
	Mean	**4.524**	**1.520**	**0.337**
	F1	3.065	1.768	0.576
	F2	4.430	2.137	0.482
	F3	5.458	2.156	0.395
	F4	3.616	1.532	0.423
	F5	4.448	1.638	0.368
	F6	3.721	1.398	0.375
	Mean	**4.123**	**1.767**	**0.435**

Table 4. Medulla/total volume values of medulla oblongata in male and female Norduz sheep.

	Male/female	Total volume	Medulla volume Values Değerleri	Medulla/total volume ratios
NUMBER OF ANIMALS	M1	4.357	2.310	0.530
	M2	4.718	2.707	0.573
	M3	4.223	2.326	0.550
	M4	4.433	2.471	0.557
	M5	4.131	2.025	0.490
	M6	5.280	2.638	0.499
	Mean	**4.524**	**2.413**	**0.533**
	F1	3.065	2.297	0.749
	F2	4.430	2.317	0.523
	F3	5.458	2.983	0.546
	F4	3.616	1.823	0.504
	F5	4.448	2.428	0.545
	F6	3.721	2.115	0.568
	Mean	**4.123**	**2.327**	**0.572**

Table 5. Medulla/cortex volume values of medulla oblongata in male and female Norduz sheep.

	Male/female	Cortex volume	Medulla volume	Medulla/cortex volume ratios
NUMBER OF ANIMALS	M1	1.568	2.310	1.473
	M2	1.401	2.707	1.932
	M3	1.492	2.326	1.558
	M4	1.455	2.471	1.698
	M5	1.528	2.025	1.325
	M6	1.675	2.638	1.574
	Mean	**1.520**	**2.413**	**1.593**
	F1	1.738	2.297	1.321
	F2	2.137	2.317	1.084
	F3	2.156	2.983	1.383
	F4	1.532	1.823	1.189
	F5	1.638	2.428	1.482
	F6	1.398	2.115	1.512
	Mean	**1.767**	**2.327**	**1.328**

3.1. Statistical analysis

The Mann–Whitney U analysis was performed to compare sex groups in statistical evaluation. In addition, SPSS (IBM SPSS for Windows, Ver. 23) statistical package program was used for statistical calculations.

In the statistical evaluation between the sexes, no difference was found in the values obtained in the comparison between the total volume values, medulla volume values, and cortex volume values between both sexes. In addition, there was no difference between the sexes in terms of medulla/total volume ratio values. However, a value of 0.004 was obtained in terms of cortex/total volume ratio values. In this case, since $p < 0.005$, it is possible to talk about a statistically significant difference in male and female sheep in terms of this ratio value. Again, the medulla/cortex volume ratio value was calculated to be 0.041, and a significant difference was observed in terms of this value in the evaluation between sexes (Table 6).

Table 6. Statistical analysis of total volume, medulla volume, cortex volume, medulla/cortex volume, cortex/total volume, medulla/total volume, and medulla/cortex volume of medulla oblongata in male and female Norduz sheep.

	Sex	N	Mean	Standard deviation	Minimum	Maximum	p
Total volume values	**Male**	6	4.52	0.42	4.13	5.28	**NS**
	Female	6	4.12	0.84	3.07	5.46	
	Total	12	4.32	0.67	3.07	5.46	
Medulla volume values	**Male**	6	2.41	0.25	2.03	2.71	**NS**
	Female	6	2.33	0.38	1.82	2.98	
	Total	12	2.37	0.31	1.82	2.98	
Cortex volume values	**Male**	6	1.52	0.10	1.40	1.68	**NS**
	Female	6	1.77	0.32	1.40	2.16	
	Total	12	1.64	0.26	1.40	2.16	
Cortex/total volume ratios	**Male**	6	0.34	0.03	0.30	0.37	**0.004**
	Female	6	0.44	0.08	0.37	0.57	
	Total	12	0.386	0.075	0.296	0.567	
Medulla/total volume ratios	**Male**	6	0.53	0.03	0.49	0.57	**NS**
	Female	6	0.57	0.09	0.50	0.75	
	Total	12	0.55	0.07	0.49	0.75	
Medulla/cortex volume ratios	**Male**	6	1.59	0.21	1.33	1.93	**0.041**
	Female	6	1.33	0.17	1.08	1.51	
	Total	12	1.46	0.23	1.08	1.93	

NS: Not Significant, $p < 0.005$

In the statistical evaluation made in terms of the coefficient of error (CE) value between the sexes, the total volume, medulla volume, and cortex volume values are insignificant among themselves. Likewise, in terms of noise value, no statistically significant difference was found in terms of total, medulla, and cortex values in female and male sheep (Table 7).

4. Discussion

In a study, it was aimed to examine the three-dimensional reconstruction of 3 tesla magnetic resonance (MRI) images of the sheep brain, and for this purpose, cerebrum of 18 Akkaraman sheep, healthy and free of any anomaly, adult, 9 male and 9 female, were used. Cerebrums were detected in cavum cranii with 10% formaldehyde. Morphometric measurements were made in the cerebrum before imaging. According to the morphometric measurement results, it was determined that the cerebrum and left hemispherium cerebri were longer in males, and similarly, the cerebellum and medulla oblongata were longer and wider in males. In terms of directions (right/left), it was observed that the left hemispherium cerebri was longer and the right hemispherium cerebri was higher in males. In females, the left hemispherium cerebri was found to be longer [21]. When the study is examined, the fact that the Norduz sheep, which is a subvarietal of the Akkaraman breed, was chosen in terms of similarity in our study as the material used, although it adds originality to the study, the method used is different. At the same time, the fact that the medulla oblongata of male sheep is longer and wider as a result of morphometric measurements is in parallel with our study. In our study, the total volume values of the medulla oblongata were found to be higher in males than in females, similar to the study of Aydoğdu (2021). In this sense, the study presented by Aydoğdu (2021) [21] supports our study.

In a different study, it was aimed to stereologically evaluate the volume of substantia grisea and substantia alba in the brain of Akkaraman crossbred sheep, and for this purpose, brains of 8 Akkaraman crossbred sheep obtained from the slaughter were used as material. Macroscopically, serial sections were taken from the brains by means of a salami machine, corresponding to a maximum thickness of 1.6 mm. In the sections taken, substantia grisea and alba were stained with giemsa for better selection. Photographs were taken with the digital imaging system

Table 7. Statistical analysis of coefficient of error (CE) and noise (N) values obtained from medulla oblongata volume calculations in male and female Norduz sheep.

		Sex	N	Mean	Standard Deviation	Minimum	Maximum	p
Coefficient of error (CE)	**Total**	**Male**	6	0.0191	0.0014	0.0207	0.0172	**NS**
		Female	6	0.0203	0.0022	0.0230	0.0173	
		Total	12	0.0197	0.0018	0.0172	0.0230	
	Medulla	**Female**	6	0.0248	0.0016	0.0270	0.0228	**NS**
		Male	6	0.0255	0.0022	0.0280	0.0219	
		Total	12	0.0251	0.0018	0.0219	0.0280	
	Cortex	**Male**	6	0.0308	0.0004	0.0315	0.0304	**NS**
		Female	6	0.0287	0.0030	0.0328	0.0250	
		Total	12	0.0297	0.0023	0.0250	0.0328	
Noise (N)	**Total**	**Male**	6	3619.33	337.47	4224.00	3305.00	**NS**
		Female	6	3298.67	671.44	4367.00	2452.00	
		Total	12	3459.00	533.606	2452	4367	
	Medulla	**Male**	6	1930.50	199.12	2166.00	1620.00	**NS**
		Female	6	1862.17	307.94	2387.00	1459.00	
		Total		1896.33	249.799	1459	2387	
	Cortex	**Male**	6	1216.17	76.37	1340.00	1121.00	**NS**
		Female	6	1413.67	252.13	1725.00	1119.00	
		Total	12	1314.92	205.387	1119	1725	

from the obtained cross-sectional images. Using the Image program, the volumes and areas of substantia grisea and substantia alba were calculated with the Cavalieri method. As a result of this study, no difference in volume and area was found between left and right hemispheres and left and right substantia alba volume and area values in the brains of Akkaraman crossbred sheep [22]. While the Akkaraman cross was selected as the sheep breed in this study, the fact that a subvarietal of the Akkaraman breed was preferred in our study gives material similarity to both studies. However, no sex difference was observed in the study conducted by Eken (2008) [22]. In the current study, sex difference was stated and comparisons between sexes were also made. As it is a study on the nervous system, both studies overlap with each other, but both studies differ in terms of the organ chosen. Eken (2008) preferred to work at the macro level in her study [22]. However, in this study, while micro study was preferred, the physical dissector method, which is one of the foundations of stereology, was chosen at the same time. In addition, in this study, the staining of the sections was done with hematoxylin and eosin, while giemsa was used in the mentioned study. However, the use of the Cavalieri principle for both studies while obtaining the volume values brought both studies together on the basis of stereology. In addition, the Image program was chosen for volume calculations in the mentioned study. However, the fact that the Shtereom I program was used in our study distinguishes both studies from each other. In this study, while evaluating the volume of the total volume, cortex and medulla parts, the fact that substantia alba and grisea were evaluated in the mentioned study due to tissue differences also shows that there are regional evaluations in both studies. In the mentioned study, it was stated that there was no difference in volume and area between the right and left hemispheres and the right and left substantia alba in the sheep brain. In this study, however, there are slight numerical differences in the volume assessment between the sexes.

In another study by Aydoğdu (2016), it was aimed to calculate the volume values of substantia alba and grisea, ventriculus lateralis of the brain hemispheres of hair goats and Akkaraman sheep stereologically using the Cavalieri method. Six healthy male Akkaraman sheep and 6 hair goats between 1 and 2 years of age were used [23]. Transversal sections with an average thickness of 3.42 mm were taken from the hemispherium blocked on 8% agar, and Berlin Blue staining principle was applied to stain the substantia grisea parts. Images obtained from the stained sections at 600 dpi resolution were evaluated with the ImageJ program. With this program, the area and volume

measurement values of the substantia alba, grisea, and ventriculus lateralis were calculated using the Cavalieri principle. As a result of the data obtained, no asymmetrical situation was found in the right and left hemispheres of the hair goat and Akkaraman sheep. No statistically significant difference was observed between the hemispherium volume values of hair goat and Akkaraman sheep. Right ventriculus between left and right ventriculus lateralis in hair goat; a significant difference was determined in favor of left substantia alba in substantia alba. Both studies are similar in terms of animal selection and the nervous system of the material. However, the mentioned study was planned and concluded at the macro level. However, our work is at a micro level and there are differences in terms of the dyes used and the program chosen in the evaluation of volume. The part where both studies overlap is that the Cavalieri method was used when calculating the volume in the nervous system organs. In addition, no sex difference was observed in the mentioned study. In the study, it is seen that there is a focus on sex difference. As a result of the statistical analyses, although no difference was found between the volumes of the medulla oblongata of male and female sheep, numerical differences were determined.

In a study examining the morphometric changes in the spinal cord in prenatal life in sheep by stereological methods, 20 healthy sheep fetuses were divided into four groups and included in this study. Unbiased systematic random sampling method was used for stereological estimates. The Cavalieri principle was used to calculate the volume of the substantia grisea, the volume of the substantia alba, and their ratios to each other and to the volume of the central canal, and to reveal the volumes in the entire spinal cord and its various regions. As a result, a significant increase in volume ratios was observed [24]. This study is similar to the study conducted by Sadeghinezhad et al. (2018) in terms of nervous system organ selection and sheep material [24]. In addition, the use of sequential random sampling in the mentioned study differs from our study. In our study, the medulla oblongata was divided into three parts and the evaluation of each part was included in the study. It is not preferred because it is desired to study every part of the tissue with the sequential random sampling method and it is a structure that can fit into tissue cassettes. In this sense, both studies differ in method.

In a study on physical and optical dissector applications and section staining methods in neuroscience, it was seen that the use of stereological methods included unbiased approaches and provided reliable results. The quantitative morphology of the nervous system has been extensively investigated in the study. The dissector counting method introduced by Sterio in 1984 is an efficient method and a reliable solution for particle count estimation [25–27]. To obtain precise results through stereological analysis, counting items must be clearly visible on the screen. If an element in the texture cannot be seen, they cannot be analyzed even using neutral stereology. Staining and sectioning processes therefore play a critical role in stereological analysis. The aim of this study is to evaluate current neuroscience studies using optical and physical dissector counting methods. When dissector counting methods are examined, it is remarkable that despite the efficiency of the optical dissector, this method in light microscopic studies has been revealed in recent years, whereas it has been reported that the optical dissector method can be easily performed in electronic microscopic studies [27]. While the superiority of the physical dissector was stated in the aforementioned study, both its reliability and convenience were demonstrated with our study.

The region we live in has given the priority of this study to the Norduz sheep because it is a different breed. Our hope and aim is to ensure that the breed characteristic of Norduz sheep, which is a different and local breed, is included in the literature as a reference study with this study. As a result, when the existing studies were examined, no similar studies could be found on the stereological evaluation of the medulla oblongata volume in Norduz sheep. In addition, many studies have shown that stereology reflects results very close to reality and that this is an important method and supports our study. We believe that this study can be a reference source in anatomy, histology, neurobiology, neuroscience, and many other fields, and can guide future studies on the nervous system on Norduz sheep, and can also lead to different studies in the field of stereology.

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This study is summarized from the master's thesis titled "Stereological Investigation of Medulla Oblongata Volume in Norduz Sheep".

Conflict of interest
The authors of this research declare that there are no conflicts of interest.

Ethical approval
A document stating that the current study does not require ethical approval has been obtained from Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee.

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