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RESEARCH ARTICLE

Sexual dimorphism in the sheep corpus callosum using 3 tesla MRI

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Koyunlarda 3 tesla MRG kullanılarak corpus callosum'un cinsiyet açısından incelenmesi

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Öz

Amaç: Bu çalışmada formaldehit ile tespit edilmiş koyun beyninden 3T Manyetik Rezonans Görüntüleme (MRG) ile elde edilen yüksek çözünürlükteki görüntüler kullanılarak, corpus callosum'un cinsiyet açısından morfolojik ve morfometrik farklılıklarını ortaya koymak amaçlandı.

Gereç ve Yöntem: Çalışmada formaldehit ile tespit edilmiş erişkin, sağlıklı ve herhangi bir anomalisi bulunmayan 9 adet dişi ve 9 adet erkek olmak üzere toplam 18 adet Akkaraman koyun beyni kullanıldı. Koyun beynindeki morfometrik ölçümler 3T MRG'den elde edilen T2 ağırlıklı görüntülerde gerçekleştirildi. İlk önce MIMICS programında corpus callosum'un kesit yüzey alanı hesaplandı. Morfometrik ölçümlerden önce MRcronGL programında DICOM formatındaki görüntüler NIfTI formatına dönüştürüldü. Daha sonra ITK-SNAP programında standart şablon kullanılarak görüntülerin normalizasyonu yapıldı. Normalizasyonu gerçekleştirilen görüntüler ITK-SNAP programında açılarak genu corporis callosi, truncus corporis callosi ve splenium corporis callosi'de morfometrik ölçümler gerçekleştirildi.

Bulgular: Koyunlarda truncus corporis callosi'nin venticulus lateralis'in tavanını oluşturduğu alt yüzeyinin köpek ve kediye göre daha içbükey; insan, at ve tavşana göre ise daha düz olduğu tespit edildi. Corpus callosum uzunluğu, corpus callosum yüzey alanı, genu corporis callosi genişliği, truncus corporis callosi genişliği ve splenium corporis callosi genişliği'nde cinsiyet açısından fark tespit edilmedi. Corpus callosum yüzey alanı ile beyin ağırlığı ve hacmi arasındaki oranda benzer şekilde cinsiyet açısından fark gözlenmedi. Ancak yüzey alanı ile hacim arasındaki oranın insanlar ve diğer memelilerde yapılan araştırmalarda bu parametrede sıklıkla karşılaşılan farka çok yakın olduğu gözlendi.

Öneri: Bu çalışmada sağlıklı koyun beyinlerinden elde edilen bulguların sinirbilimi çalışmalarında koyunlarda oluşturulan nörodejeneratif hastalık modellerinde ve deneysel çalışmalarda kullanılabileceği düşünülmektedir.

Anahtar kelimeler: Beyin, corpus callosum, koyun, manyetik rezonans görüntüleme

Abstract

Aim: This study aims to determine the morphological and morphometric differences of the corpus callosum in terms of sex using high-resolution images obtained from formalin-fixed sheep brains by 3T Magnetic Resonance Imaging (MRI).

Materials and Methods: In the study, a total of 18 adult healthy Akkaraman sheep brains (9 females and 9 males), which had no anomaly and were fixed with formaldehyde, were used. Morphometric measurements in sheep brains were performed on T2-weighted images obtained from 3T MRI. First, the midsagittal cross-sectional area of the corpus callosum was calculated using MIMICS. Before morphometric measurements, images were converted from DICOM format to NIfTI format in the MRcronGL. Then, the normalization of the images were performed using the standard template in the ITK-SNAP. After that images were opened in the ITK-SNAP, and morphometric measurements were performed in genu corporis callosi, truncus corporis callosi, and splenium corporis callosi.

Results: In sheep, the lower surface of the truncus corporis callosi, which forms the roof of the ventriculus lateralis, was more concave than dog and cat, and flatter than human, horse and rabbit. There was no sexual dimorphism in corpus callosum length, midsagittal corpus callosum cross-sectional area, genu corporis callosi width, truncus corporis callosi width and splenium corporis callosi width. Similarly, no sexual dimorphism was observed in the ratio between midsagittal corpus callosum cross-sectional area and brain weight and volume. However, it was observed that the ratio between surface area and volume was very close to the difference frequently encountered in this parameter in studies conducted in humans and other mammals.

Conclusion: It is thought that the findings obtained from healthy sheep brains in this study can be used in neurodegenerative disease models created in sheep in neuroscience studies and experimental studies.

Keywords: Brain, corpus callosum, sheep, magnetic resonance imaging

Introduction

The corpus callosum is the largest fibre bundle linking the cerebral cortex of the right and left hemispherium cerebri. It is the largest structure formed by white matter in the brain. This link enables both hemispheres of the brain to cooperate for the whole body to act in a coordinated manner (Jason and Pajurkova 1992, Tan and Kutlu 1993). It is located horizontally between the two hemispherium cerebri on the roof of the corpus callosum ventriculus lateralis (Dursun 2008, Griffiths et al 2009).

Due to the idea that "the different mentality of men and women should have an equivalent in the human brain", studies on sexual dimorphism have often emphasized the corpus callosum (Desimone 1992, Bishop and Wahlsten 1997). Many studies have reported a difference in the corpus callosum between men and women. Many studies stated that the midsagittal surface area of the corpus callosum is larger in women than in men, especially the splenium corporis callosi is larger when the size of the brain is taken into consideration (Bishop and Wahlsten 1997, Ardekani et al 2013). Similarly, the corpus callosum volume was larger in women result of voxel-based morphometric measurements using the images obtained from Magnetic Resonance Imaging (MRI) (Shiino et al 2017). It has been stated in various studies that the surface area is larger not only in terms of sex, but also in musicians and left-handed individuals (Witelson 1985, Schlaug et al 1995).

The first study to suggest that the corpus callosum is different between men and women and the midsagittal cross-sectional surface area is larger in women was conducted by DeLacoste-Utamsing and Holloway (1982). After this research, although many studies have been conducted showing that the cross-sectional area of the corpus callosum is larger in women. However, the validity of this information has been discussed. Contrary to the existing knowledge, many studies have concluded no difference (Bishop and Wahlsten 1997, Poltana et al 2001, Ng et al 2005, Gupta et al 2011, Allouh et al 2020). In these studies, it has been stated that the belief that women think differently could not be concluded only because the splenium corporis callosi is larger (Bishop and Wahlsten 1997).

The morphology and dimensions of the corpus callosum and its relationship with the surrounding structures are frequently used not only for sexual dimorphism but also for the determination of some neurological and psychological diseases (Luders et al 2010, Woldehawariat et al 2014). Changes in the size and shape of the corpus callosum have been reported in patients with schizophrenia and bipolar disorder. There is a decrease in the surface area of the corpus callosum in patients with schizophrenia. In addition, a difference was observed between the healthy individuals and patients with schizophrenia in terms of length of the corpus callosum (Downhill Jr et al 2000, Walterfang et al 2009, Unlu et al 2014). Similarly, in diseases such as Huntington's (Di Paola et al 2012), Multiple Sclerosis (MS) (Hasan et al 2012) and Alzheimer's Disease (Di Paola et al 2010, Frederiksen et al 2011) due to atrophy formed in the brain depending on the degree of the disease, a change in the size of the corpus callosum was detected (Herron et al 2012). In a study using Diffusion Tensor Imaging (DTI), which is used to determine the direction and density of white matter fibres in the brain, a specific decrease was observed in the splenium, genu and truncus parts of the corpus callosum in individuals with autism (Alexander et al 2007). In this context, the findings obtained from the corpus callosum in healthy individuals are very important in the development of corpus callosum and assessing pathological conditions affecting the corpus callosum (Al-Hadidi et al 2021).

In recent years, the use of sheep as a model animal has increased in animal models used other than primates in neuroscience studies. The sheep brain is similar to the human brain. Therefore sheep are preferred for testing newly developed devices and perioperative technologies in neurosurgical research (Capitanio and Emborg 2008, Lee et al 2015, Pieri et al 2019). In addition, the models of neurodegenerative diseases such as Huntington (Jacobsen et al 2010) and Alzheimer's Disease (Braak et al 1994, Reid et al 2017), which cause changes in the size of the corpus callosum in humans, exist in sheep.

The morphology and morphometry of corpus callosum, numerous studies have been conducted in the human brain, especially in terms of sex, have also been examined in other mammals. In their research, Aydınlıoglu et al (2000) examined the midsagittal cross-sectional surface of the corpus callosum in dogs by dividing it into three different regions. The measurements made in these three regions revealed that the corpus callosum was larger in male dogs. In another study, no sexual dimorphism was observed in the shape and size of the corpus callosum in measurements made in rabbits, cats, dogs, cattle and horses. It was determined that the positive correlation between corpus callosum and brain weight was the highest in dogs. While a similar correlation was observed in cattle, this correlation was observed only in the posterior part of the corpus callosum in cats (Olivares et al 2000). Females had a significantly larger corpus callosum than males in measurements performed using MRI in the elephant brain (Manger et al 2010).

In neuroscience, the corpus callosum anatomy in primates has been closely studied due to the human-primate phylogenetic relationship in brain studies. In monkeys (Cebus apella), corpus callosum-brain ratio has been observed to be higher in females. Similarly, it has been reported that the corpus callosum has a larger surface area in left-handed subjects

(Phillips et al 2007, Hellner-Burris et al 2010). As a result of the examination performed in rhesus macaque (Macaca mulatta) using MRI, it was observed that especially the splenium corporis callosi was larger in females (Franklin et al 2000).

Considering the increase in neurodegenerative disease models were be formed in sheep in recent years, it is thought that the morphological and morphometric findings to be obtained from the corpus callosum in healthy sheep brains are crucial for clinical studies on these models. In this context, the motivation of the present study is the insufficient morphological and morphometric findings of the corpus callosum in terms of sex, using 3T MRI in Akkaraman sheep, a native breed.

This study aimed to determine the morphology and morphometry of the corpus callosum using 3T MRI in healthy sheep brains.

Material and Methods

The study used 18 (9 males/9 females) adult healthy Akkaraman sheep without any anomaly. The Ethics Committee approved the study procedure of Selçuk University Faculty of Veterinary Medicine (Date: 25/04/2019 and Decision no: 2019/38). Sheep heads were fixed with 10% formaldehyde before craniotomy to maintain the normal position of the brains within the cavum cranii. The skin and soft tissue were then removed from the skull, and the brains were removed from the cavum cranii. After craniotomy, the meninges were dissected respectively. After removing the pia mater encephali from the meninges, the weight of the brains was measured with a precision scale. Figure 1 shows the process (in order) performed in the brains before MRI.

Isotropic images were obtained in T2W 3D-SPACE sequence from sheep brains using a head coil in a 3 Tesla MRI (Siemens Magnetom Skyra, Erlangen, Germany) scanner. Section thickness was kept as low as possible to make precise morphometric measurements on MR images (slice thickness was 0.6 mm). T2-weighted (T2W) images were acquired following parameters: repetition time (TR) = 3200 ms; echo time (TE) = 411 ms, SNR=1, matrix=320x320. Total acquisition time was approximately 29 minutes 18 seconds per specimen.

Image analysis and morphometric measurements

The midsagittal surface area of the corpus callosum in the images was calculated in the MIMICS® 13.1 within Anatomy Department. In this step, DICOM (Digital Imaging and Communications in Medicine) format images were used.

Before the morphometric measurements were performed on the cross-sectional surface of the corpus callosum in midsagittal MR images (Figure 2), the MR images were converted to NIfTI (Neuroimaging Informatics Technology Initiative) format in the MRcronGL software. The normalization process was performed using standard templates in the image analysis of MR images obtained from the brains. The normalization of MR images obtained from sheep brain was performed using standard templates (Turone Sheep Brain Templates and Atlas) formed separately for female and male sheep by Ella and Keller (2015) in ITK-SNAP (Yushkevich et al 2006) software (Figure 3).

The normalized images were opened in the ITK-SNAP software and the following morphometric measurements were performed (Figure 4) In the same software, the 3D reconstruction of the corpus callosum was performed in the brain

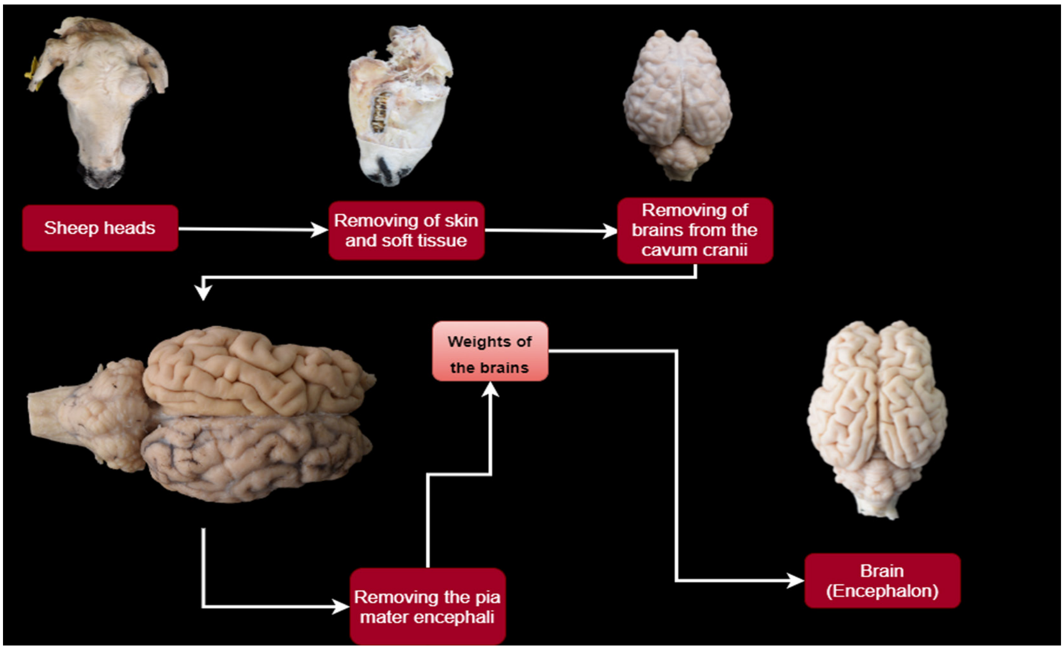

Figure 1. An overview dissection workflow of brains. Prepared diagram in web-based draw io (https://app.diagrams.net/) application

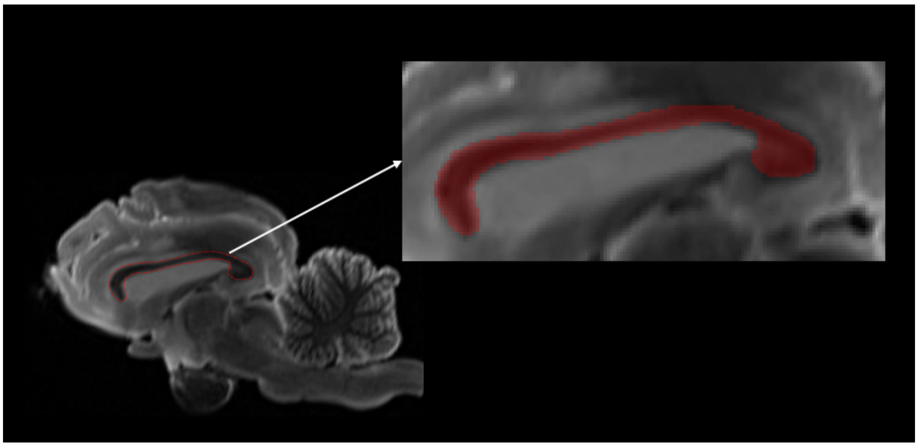

Figure 2. The midsagittal surface area of the corpus callosum

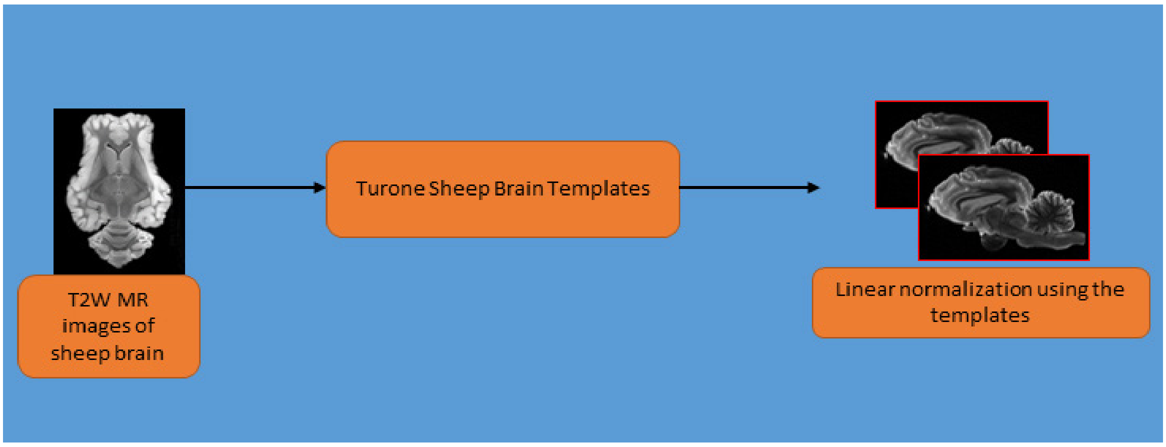

Figure 3. The normalization of T2W MR images in NIfTI format

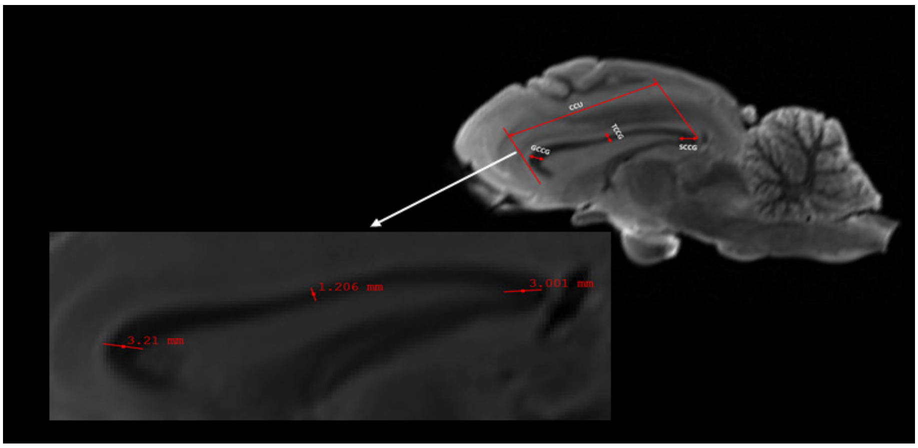

Figure 4. Morphometric measurements of the corpus callosum

of a female animal. In addition, three-dimensional reconstruction of the brains was created in the same software, and their total volume was calculated.

Morphometric measurements of the corpus callosum in MR images;

Corpus callosum length (CCL): The length of the corpus callosum in the image obtained on the median line (midsagittal corpus callosum)

Corpus callosum surface area (CCSA): The midsagittal corpus callosum cross-sectional surface area of the corpus callosum in the image obtained on the median line.

Genu corporis callosi width (GCCW): Genu corporis callosi maximum width

Truncus corporis callosi width (TCCW): Truncus corporis callosi width on the median line

Splenium corporis callosi width (SCCW): Splenium corporis callosi maximum width

SPPS 25 (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.) statistical software was used to evaluate the measurement results obtained. The homogeneity of the variances was tested using the Levene test. The normality of variables was tested using the Shapiro-Wilk test. Sexual dimorphism was performed using the Student's t-test for parametric variables and the Mann-Whitney U test for nonparametric variables. The correlation between the midsagittal surface area of the corpus callosum and the weight and volume of the brain was examined using the Pearson correlation coefficient.

Results

In the study, the corpus callosum in sheep brains was examined using MRI. It was determined that the cross-sectional surface of the corpus callosum in sheep was similar to the bovine corpus callosum in general terms. It was observed that the retroactive thinning of genu corporis callosi was more blunt than in cattle. It was determined that the splenium corpus callosi had an oval shape and the truncus corporis callosi enlarged close to the genu corporis callosi. Narrowing towards the splenium corporis callosi and then a slight enlargement was detected. It was determined that its lower surface, which forms the roof of the ventriculus lateralis, was concave, but this fold was not too much (Figure 5).

The morphometric measurements obtained on the midsagittal surface of the corpus callosum were presented in Table 1.

It was determined that the corpus callosum was long in male sheep brains, but the surface area was wide in females. Splenium corporis callosi was large in males, and genu corporis callosi was large in females. The lengths of truncus corporis callosi were very close to each other in both sexes. The morphometric measurement results obtained from the corpus callosum did not cause a statistical difference in sex ($p>0.05$).

The correlation between the midsagittal cross-sectional surface of the corpus callosum with brain weights and volumes in female and male sheep was presented in Table 2.

No correlation was determined between the cross-sectional surface of the corpus callosum with the weight and volume of the brain.

The correlation between the ratio of surface area with brain weight and brain volume was presented in Table 3.

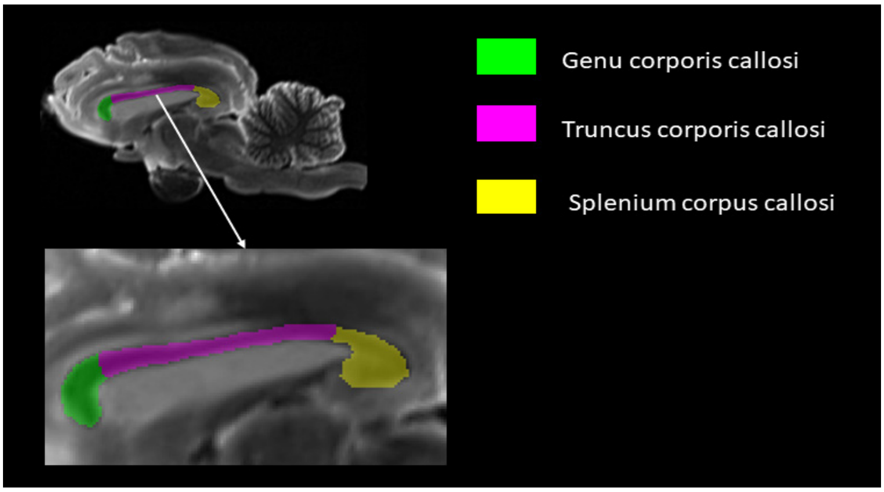

Figure 5. Subdivisions of the corpus callosum

Table 1. Morphometric measurements of the corpus callosum

Parameters	N	Male Mean±sd	Female Mean±sd	p
CCL (mm)	18	35.61 ± 1.55	34.14 ± 1.44	0.054¥
CCSA (mm^2)	18	95.78 ± 11.08	103.48 ± 10.99	0.158¥
GCCW (mm)	18	3.51 ± 0.39	3.61 ± 0.37	0.605¥
TCCW (mm)	18	1.30 ± 0.20	1.34 ± 0.11	0.565¥
SCCW (mm)	18	3.75 ± 0.50	3.44 ± 0.26	0.120¥

*p<0.05; ¥ Student's t test
CCL: Corpus callosum length; CCSA: Corpus callosum surface area CCCW: Genu corporis callosi width TCCW:Truncus corporis callosi width SCCW: Splenium corporis callosi width

Table 2. The correlation between the surface area of the corpus callosum and brain weight and volume variables by sex

	N=9		Surface Area (mm^2)
Male	Weight (g)	r	0.234
		p	0.544
	Volume (mm^3)	r	0.376
		p	0.319
Female	Weight (g)	r	-0.121
		p	0.756
	Volume (mm^3)	r	-0.102
		p	0.794

Table 3. Comparison of sex and surface area-weight (S/W) and surface area-volume (S/V) variables

	N	Male Mean±sd	Female Mean±sd	p
S/W	9	0.7325±0.08572	0.8019±0.11156	0.158¥
S/V	9	0.000798±0.000096	0.000904±0.000121	0.056¥

*p<0.05
¥ Student›s t-test

There was no significant difference between the sexes in the ratios of the cross-sectional surface area of the corpus callosum to the brain weight and volume (p>0.05). However, it was observed that the ratio between surface area and volume was very close to the difference frequently encountered in this parameter in studies conducted in humans and other mammals. Figure 6 shows 3D models of the corpus callosum and its subdivisions. The location of the corpus callosum in the brain was demonstrated in the three-dimensional model.

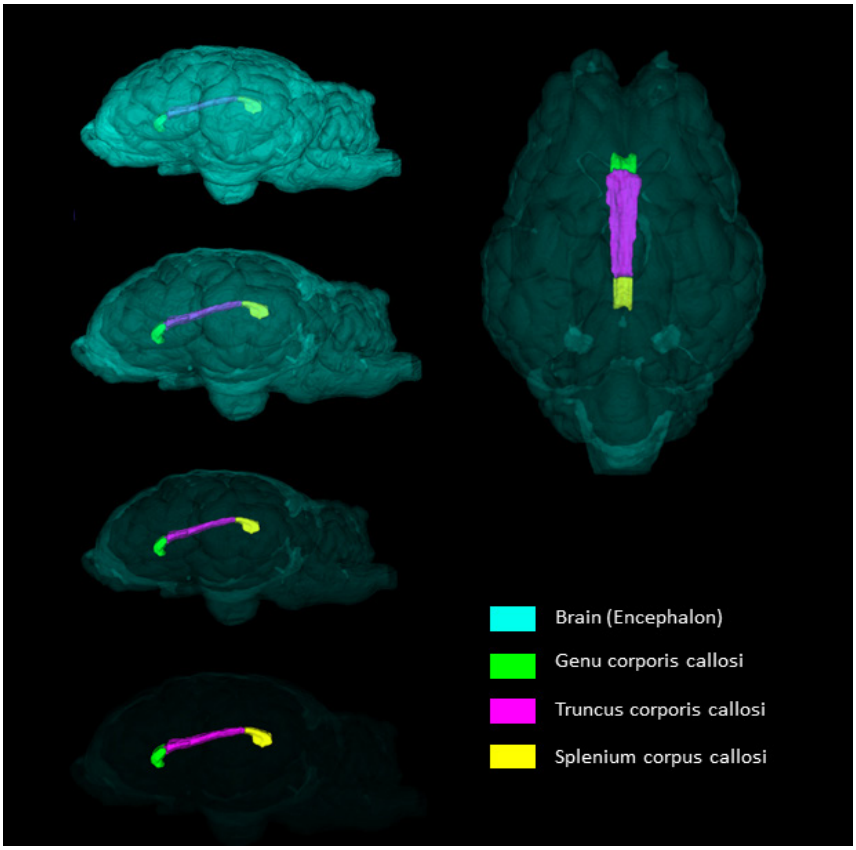

Figure 6. Three-dimensional reconstruction of the corpus callosum

Discussion

This study examined the morphology and morphometry of the corpus callosum in Akkaraman sheep, a native breed, in 3T MRI. The corpus callosum, one of the most studied anatomical structures in the brain in terms of sex in humans and primates, provides the connection between the two hemispherium cerebri. The primary purpose of the first studies on the corpus callosum was to determine its morphological counterpart in the brain of the different thinking characteristics between men and women (DeLacoste-Utamsing and Holloway 1982, Bishop and Wahlsten 1997). It is controversial that this structure is different in terms of gender in studies conducted on humans and primates. Although there are many studies reporting that the splenium corporis callosi is much larger in women, there are studies that concluded that there is no such difference (DeLacoste-Utamsing and Holloway 1982, Bishop and Wahlsten 1997, Poltana et al 2001, Ng et al 2005, Gupta et al 2011, Ardekani et al 2013, Shiino et al 2017, Allouh et al 2020). In this study, the corpus callosum was analyzed in adult sheep using morphometric measurements. Our results show that there is no difference in terms of gender. However, it was observed that the ratio between surface area and volume was very close to the difference in humans.

In recent years, the number of studies on the anatomy and function of the brain has highly increased. Most of these studies have focused on the function of the human brain. Findings obtained from experimental animals, farm animals and primates are very important for these studies. By adapting the findings obtained from these animals to humans, the application areas of experimental studies have been expanded (Capitanio and Emborg 2008, Murray et al 2019). For this purpose, the use of sheep as a model animal in neuroscience has increased in recent years (Jacobsen et al 2010, Reid et al 2017, Murray et al 2019). While degenerations were found in the brain in these models, no morphometric measurement of the corpus callosum was found. Pieri et al (2019) were present the first in vivo sheep tractography atlas. Genu corporis callosi is identified as sharply bent white matter bundles. In this study, in which anatomic images were used, it was observed that the genu corporis callosi continued by narrowing under.

In corpus callosum examined in sheep using MR images, the concave lower surface of the truncus corporis callosi forming the roof of the ventriculus lateralis was found to be flatter compared to humans, horses and rabbits. It was determined that concavity was higher compared to dogs and cats. In the study conducted by Olivares et al (2000) with horses, cattle, dogs, cats, rabbits and rats, no difference was observed in the size and shape of the corpus callosum in terms of sex. Also, in the current study, no sexual dimorphism was found in the morphometric measurements performed in the corpus callosum. While a high correlation was observed between cross-sectional surface area and brain weight in the same study, this correlation was not observed in the current study. This difference was thought to be due to the use of logarithmic data in the related study.

In another study conducted on dogs, sexual dimorphism was found on the cross-sectional surface of the corpus callosum. It was reported that the total cross-sectional surface area and the 1/5 part of posterior (close to the splenium corporis callosi) were more common in males (Aydınlıoglu et al 2000). In the study, in which dog brains fixed with formaldehyde were used, measurements were made in the cross-sectional area photographed. This difference was not observed in the present study was considered to be due to the different methods used. It was observed that the sexual dimorphism reported in the corpus callosum in cats was similar to rats but different from humans (Tan and Kutlu 1993). The fact that this difference was not observed in sheep was thought to be due to the various morphological features of the corpus callosum. In addition, the ratio of the cross-sectional surface area of the corpus callosum to the brain weight in cats and dogs was much larger compared to sheep (Manger et al 2010).

Conclusion

Consequently, it is thought that the findings obtained in the current study would be helpful for experimental studies in sheep in neuroscience. In recent years, neurodegenerative disease models have been formed in sheep.

These diseases cause changes in the morphology and morphometry of the structures in the brain. Corpus callosum is one of the structures most affected by these changes (Downhill Jr ve ark 2000, Walterfang ve ark 2009, Unlu ve ark 2014) . In the current study, it is estimated that these values obtained from healthy animals will be useful in assessing the degree of disease models formed in sheep and the effect of treatment methods on the brain.

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Conflict of Interest

The authors did not report any conflict of interest or financial support.

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Ethical Approval

The Ethics Committee approved the study procedure of Selçuk University Faculty of Veterinary Medicine (Date: 25/04/2019 and Decision no: 2019/38)

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