UNIVERSITI PUTRA MALAYSIA

MORPHOMETRIC AND HISTOLOGICAL CHARACTERISATION OF THE MENISCI OF DOMESTIC CATS (Felis catus)

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MORPHOMETRIC AND HISTOLOGICAL CHARACTERISATION OF THE MENISCI OF DOMESTIC CATS (*Felis catus)*

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CERTIFICATION

It is hereby certified that I have read this project entitled "Morphometric and Histological Characterisation of The Menisci of Domestic Cats (*Felis catus)*", by Dhiya Athirah Binti Azman and in my opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirements for the course

VPD 4999- Final Year Project,

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LIST OF ABBREVIATIONS

%	Percentage
<	Less than
>	More than
±	Plus minus
=	Equal
DBKL	Kuala Lumpur City Hall
ECM	Extracellular matrix
FPV	Faculty of Veterinary Medicine
H&E	Haematoxylin and eosin
LM	Lateral meniscus
MM	Medial meniscus
mm	Millimeter
SE	Standard error
UPM	Universiti of Putra Malaysia

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek Ilmiah Tahun Akhir.

PENCIRIAN MORFOMETRIK DAN HISTOLOGI MENISKUS PADA KUCING DOMESTIK (*Felis catus)*

Oleh

Dhiya Athirah Binti Azman

2022

Penyelia: Dr. Siti Mariam Zainal Ariffin

Meniskus adalah struktur fibro rawan yang terletak antara kondil femur dan tibia sendi stifel. Meniskus terlibat dalam distribusi dan penggalasan beban, penyerapan kejutan and penstabilan sendi. Pemahaman tentang struktur, komposisi dan ciri fungsi terhadap meniskus felin amatlah terhad. Kefahaman asas dalam anatomi normal sendi felin adalah prasyarat untuk mendalami patogenesis abnormaliti sendi. Kajian ini bertujuan untuk menentukan ciri morfometrik meniskus felin. Selain itu, kajian ini juga bertujuan untuk menentukan ciri histologi bagi kawasan dan zon meniskus felin. Sejumlah 120 meniskus telah diperolehi daripada 30 kadaver kucing yang matang rangkanya. Ukuran ekstensi

periferal, lebar, tebal, lilitan badan, lilitan badan bersama tanduk, ketinggian artikulasi dan ketinggian artikulasi unggul telah diukur. Penilaian histomorfometrik telah dijalankan bagi menentukan selulariti dan histomorfologi meniskus. Penilaian and penskoran setiap meniskus telah dijalankan mengikut kawasan (tanduk kranial, badan, tanduk kaudal) dan zon (luar, tengah, dalam). Kandungan proteoglikan telah dinilai menggunakan pewarna Safranin O. Distribusi kolagen telah dinilai menggunakan pewarna Picrosirius. Bentuk yang paling kerap dijumpai untuk meniskus medial adalah kresentik (66.67%), manakala untuk meniskus lateral adalah bentuk C (86.67%). Analisis morfometrik mendedahkan bahawa terdapat perbezaan ketara antara meniskus lateral ($P<0.05$) dan meniskus medial dalam lilitan, tebal, lebar dan ketinggian artikulasi. Ketumpatan sel tidak jauh berbeza di semua kawasan. Walau bagaimanapun, selulariti berkurangan daripada zon luar ke zon dalam meniskal. Kandungan proteoglikan tidak jauh berbeza di semua kawasan tetapi meningkat dengan ketara ($P<0.05$) di zon tengah meniskal. Kandungan kolagen jauh lebih tinggi di kawasan kranial dan zon dalam meniskal. Kajian semasa mendedahkan tentang variasi dalam morfometrik meniskus medial dan lateral dan ciri histologi kawasan dan zon meniskus felin.

Katakunci: meniskus; sendi stifel; morfometrik; histomorfologi; felin

ABSTRACT

An abstract of the project paper was presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4999 – Final Year Project.

MORPHOMETRIC AND HISTOLOGICAL CHARACTERISATION OF THE MENISCI OF DOMESTIC CATS (*Felis catus)*

By

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2022

Supervisor: Dr. Siti Mariam Zainal Ariffin

Menisci are fibrocartilaginous structures interposed between the femoral and tibial condyles of the stifle joint. The meniscus involves load-bearing and distribution, shock absorption and joint stabilisation. The basic understanding of the feline meniscus's structural, compositional and functional characteristics is limited. Understanding the menisci's normal anatomy is a prerequisite to understanding the pathogenesis of abnormality involving the stifle. The current study aimed to determine the morphometric characteristics of the feline menisci. In addition, the study also characterised the regional and zonal histological features of the feline menisci. A total of 120 menisci were obtained from 30 skeletally mature cat cadavers. The peripheral extension, width and thickness,

circumference of body and body with horns, articulating height and superior articulating length were measured. The histomorphometric assessment was conducted to determine the meniscal cellularity and histomorphology. Each meniscus was evaluated and scored in the different regions (cranial horn, body, caudal horn) and zones (outer, middle, inner). The Safranin O staining evaluated the proteoglycan content. The collagen distribution was assessed using a Picrosirius stain. The most common shape of the medial meniscus was crescentic (66.67%), and that of the lateral meniscus was C-shaped (86.67%). The morphometric analysis revealed that the lateral menisci significantly differed ($P<0.05$) in circumference, thickness, width and articulating height from the medial menisci. Cell density was not significantly different across the regions. However, the cellularity decreases from the outer zone to the deep zone of the menisci. Proteoglycan content was not found to differ in different regions but was found to be significantly increased ($P<0.05$) in the middle meniscal zone. Collagen content was considerably higher in the menisci's cranial region and deep zone. The current study revealed variations in the morphometric of medial and lateral menisci and histological features of the region and zone of feline menisci.

Keywords: menisci; stifle joint; morphometric; histological; feline

Chapter 1

INTRODUCTION

1.1 Background

The meniscus is a C-shaped fibrocartilaginous structure between the femoral condyle and the tibial plateau. The meniscus can be divided into the cranial horn, body and caudal horn (O'Connor and McConnaughey, 1978). In addition, each stifle joint possesses medial and lateral menisci. The meniscus is vital in feline life because it helps with load-bearing, shock absorption, congruence of joints, stability of joints and lubrication of joints. The multifunction of menisci is supported by the cellular activities, water and extracellular matrix (ECM), including proteoglycan and collagen fibre type I (Ariffin, 2015).

To date, data on the morphometric parameters in healthy feline menisci are not documented and the distribution of the collagen fibrils, proteoglycan and cellular density is not clearly defined. O'Connor and McConnaughey (1978) reported that the variations in the medial meniscus shape are less common than in the lateral meniscus. Prose (1984) and Ariffin (2015) reported that the feline menisci are roughly semilunar in form, with the medial meniscus larger than the lateral meniscus. However, none of these studies reported on the morphometric and quantitatively examined the dimension differences between

medial and lateral menisci. Hence, the precise morphometry of the meniscus is still unknown. Variation in the lateral and medial menisci dimensions can determine the possibility and kind of injury.

The composition and organisation of ECM in the feline menisci are not clearly defined. Although previous studies on the meniscal composition have already been reported, there remains a lack of knowledge regarding its composition and structural organisation's regional and zonal variability. O'Connor and McConnaughey (1978) stated that the collagen bundle is arranged in a herringbone pattern. Proteoglycans constitute a main component of the meniscus ECM, and these molecules have been reported to be confined in the inner border of the normal meniscus (Ariffin, 2015). A more profound understanding of the composition and organisation of normal meniscus is needed to appreciate the degenerative process that occurs in joint disease.

1.2 Justification

Characterising the morphometric and histomorphological of feline menisci will provide information on different medial and lateral menisci shapes contributing to a better delineation of meniscal anatomy. In addition, a better understanding of the meniscal structure-functional relationship will be discovered. Finally, specific findings on the anatomical variations will help facilitate surgical procedures and arthroscopy of the stifle joint in the meniscus.

1.3 Study Objectives

1. To determine the morphometric characteristics of the feline medial and lateral menisci.

2. To determine the feline menisci's regional and zonal histological characteristics.

1.4 Study Hypotheses

Objective 1:

H_0: There are no morphometric differences between feline medial and lateral menisci.

H_1: There are morphometric differences between feline medial and lateral menisci.

Objective 2:

H_0: There is no regional and zonal variation in the feline menisci compositional and structural organisation.

H_1: There is a regional and zonal variation in the feline menisci compositional and structural organisation.

Chapter 2

LITERATURE REVIEW

2.1 Feline menisci

The meniscus is a fibrocartilaginous structure interposed between the femoral condyles and the tibial plateau (Ariffin, 2015). Normal menisci have smooth, white, glistening surfaces (Sun *et al.,* 2010; Pauli *et al.,* 2011). Each stifle joint possesses medial and lateral menisci. The menisci are secured to the tibia and the femur by the cranial and caudal ligaments of the lateral and medial meniscus and by the femoral ligament of the lateral meniscus. The intermeniscal ligament connects the medial and lateral menisci. The peripheral border of each meniscus attaches to the joint capsule and the medial meniscus also has an attachment to the medial collateral ligament, so it is less mobile than the lateral meniscus (Denny and Butterworth, 2000). Much blood and nerve vessels originate from the peripheral part of the joint capsule and supply the meniscal horns. However, the meniscus's body has poor blood and nerve supply (O'Connor and McConnaughey, 1978). The meniscus plays an important role in daily feline life in load transmission, shock absorption, increases joint congruence and improves joint stability (Makris *et al.*, 2011).

2.2 Morphological studies of feline menisci

Feline menisci are normally white to blue-white, smooth, shiny, elastic and firm in appearance (Bennett, 1990; Dyce *et al.*, 2002). Each meniscus generally consists of a cranial horn, a caudal horn and a "body" (O'Connor and McConnaughey, 1978). According to Kale *et al.* (2006) (Figure 1), seven meniscus shapes exist.

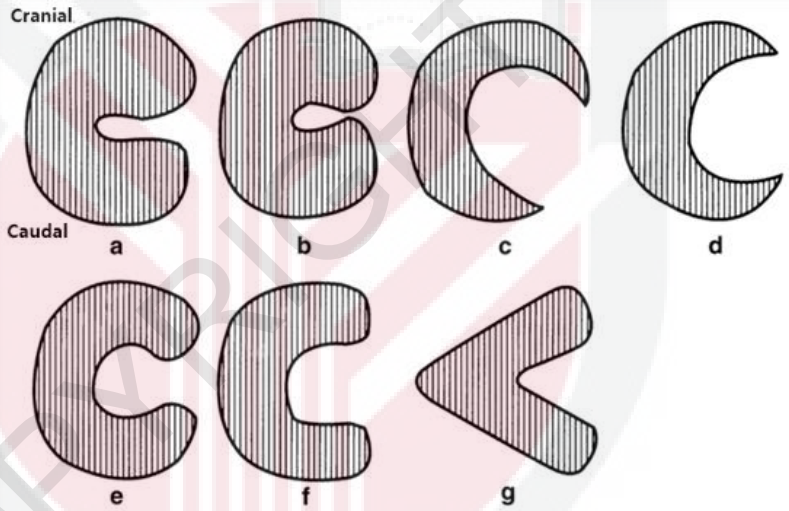

Figure 1: Diagram showing the shape of the menisci. **a** incomplete discoid, **b** complete discoid, **c** crescentic shaped, **d** sickle shaped, **e** C shaped, **f** sided U shaped, **g** sided V shaped. Image adapted from Kale *et al.* (2006).

O'Connor and McConnaughey (1978) stated that variation in shape is less in the medial meniscus than in the lateral meniscus. Prose (1984) reported that the morphology of the feline meniscus only shows a semilunar shape, with the medial meniscus being larger than the lateral meniscus. This statement is consistent with Ariffin (2015), who

stated that the medial tibial plateau is larger than the lateral; hence, the medial meniscus has a larger diameter than the lateral meniscus. However, none of the studies reported on the morphometric and quantitatively examined the differences between medial and lateral menisci. Hence, the exact size of the feline meniscus is still unknown.

2.3 Histomorphological studies of the feline meniscus

Histologically, the menisci consist primarily of cells (fibrochondrocytes or a mixture of fibroblasts and chondrocytes) and ECM. The cells synthesize and maintain the ECM, consisting predominantly of collagens, water and non-collagenous proteins (Allen *et al.*, 1995). The ECM is composed of water (70-80% of wet weight), collagen (50-60% of dry weight), proteoglycans (25-35% of dry weight) and a variety of noncollagenous proteins and glycoproteins (15-20% of dry weight) (Buckwalter *et al.*, 2005). The amount of water in the cartilage depends on the matrix's proteoglycan and collagen components.

Type II collagen makes up 90% of the total collagen in normal articular cartilage. In addition, type I, III, V, VI, IX, X and XI collagen may also be present (Mankin, 1985; Sandell, 1995). Collagen fibrils run parallel to the joint surface in the superficial layer, are arranged obliquely in the middle layer and run perpendicularly to the tidemark and subchondral bone (Zambrano *et al.*, 1982; Mankin, 1985; Hunziker *et al.*, 2007). The organization of these fibrils into a tight meshwork provides tensile strength to the articular

cartilage and helps to immobilize the proteoglycans within the tissue (Buckwalter *et al.*, 2005).

Proteoglycan is produced in the Golgi apparatus of chondrocytes, mainly found in the middle and deep layers of the articular cartilage (Renberg, 2005). Proteoglycans' distinct negative charge and hydrophilic properties help maintain the articular cartilage elasticity and stiffness under compressive loading (Mankin, 1985; Renberg, 2005). Proteoglycans are normally located within a meshwork formed by collagen fibrils and are increased in areas of articular cartilage subject to high stress (Kiviranta *et al.*, 1987). Their exact role is not certain, but they may play an important role in shock absorption, joint lubrication and stability, improving tibio-femoral congruence and assisting in weight distribution across the joint (Walker and Erkman, 1975; Bennett, 1990).

O'Connor and McConaughey (1978) stated that collagen bundles in the meniscal body are arranged in a herringbone pattern. In contrast, collagen bundles at the meniscal horn are arranged in a parallel pattern. However, this study did not mention the localization or distribution of collagen bundles across the meniscus. Herriet *et al.* (2013) stated that the meniscus has well-organized collagen bundles with fibrocytes aligned longitudinally between bundles. Ariffin (2015) stated that proteoglycan content was confined in the deep zone of the normal meniscus. No study gives complete information regarding the content of the extracellular matrix across the meniscus. Some only mention specific ECM matrix content. No study has been done on ECM's regional and zonal

distribution across the feline meniscus. Knowing the exact distribution of ECM across the meniscus could help the surgeon and veterinarian better understand the composition of the meniscus.

Chapter 3

MATERIALS AND METHODS

3.1 Cat cadavers

Thirty skeletally mature cat cadavers were obtained from the Pest Control Unit, Kuala Lumpur City Hall (DBKL). The weight (kg), age (years), breed, sex, and body condition score (BCS) of each cat were recorded. Stifle joints were examined within 24 hours *post-mortem*. In addition, each meniscus (medial and lateral menisci) was grossly examined, digitally photographed and the images will be stored.

3.2 Gross examinations

The skin around the stifle region was removed, and the fascia was bluntly dissected with scissors. All ligaments and tendons that attach around the stifle region, including the patellar tendon, lateral collateral ligament and medial collateral ligament, were resected to free the stifle joint. First, stifles were flexed, then the cranial cruciate ligament and caudal cruciate ligament were resected near their attachment to the femur to avoid cutting the menisci. The shape of the menisci was observed *in-situ*. Finally, the menisci were removed from the stifle joint and examined for the morphometric study.

3.3 Morphometric study

The dimension of each meniscus was measured according to Takroni *et al.* (2016). The circumference, width, thickness, articulating height and superior articulating length of menisci were measured using thread and RS PRO 150mm digital calliper. All morphometric measurements were measured in millimetre. Two circumference measurements were quantified using a molded plastic tape measure. The first is around the peripheral rim of the body of each meniscus from the junction of the fibre bundles of the caudal part with the body to the cranial junction with the body (grey dotted semi-circular line); the second overall circumference measurement (red dotted semi-circular line) included the body and both horns, from the tip of the caudal horn to the end of the cranial horn (Figure 2). The peripheral circumference length of both menisci was then divided into three equal regions: cranial third, middle third, and caudal thirds by using scale & color marker pens. The width (Figure 3) and the thickness (Figure 4) were taken at each point. The superior articulating length was measured from the tip of the highest point to the lowest point. For the articulating height, the measurements were taken at the middle third at three equally distanced points; a, b and c. Here, one jaw of the digital calliper was placed on the upper femoral surface, while the other was placed below the flat bottom surface. Both jaws were moved stepwise to the three locations at equally calculated distances. Data were recorded on a standardised collection sheet.

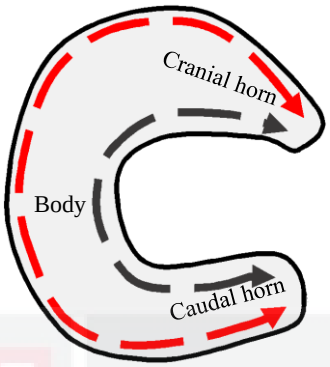

Figure 2: The measurements of two circumferences; first: along the peripheral rim circumference (grey dotted semi-circular line), and second: overall circumference (red dotted semi-circular line).

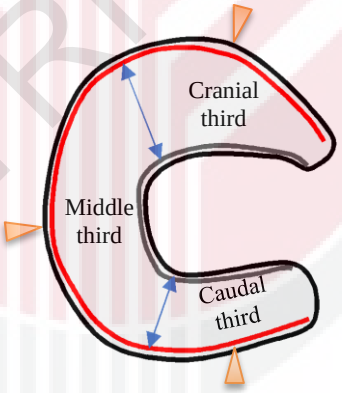

Figure 3: The meniscus was divided into three same regions, indicated by the solid blue lines. The width of the meniscus is measured from the three regions, indicated by the orange arrows.

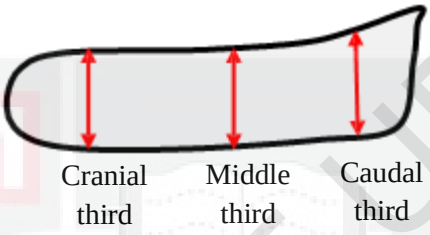

Figure 4: The solid red lines indicate the meniscus thickness measured from the three regions (cranial third, middle third and caudal third).

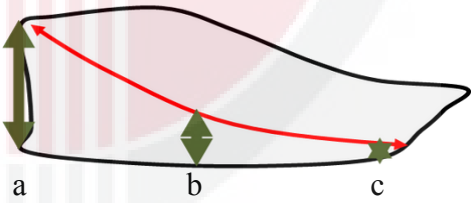

Figure 5: The superior articulating length (red line) was measured from the highest point to the lowest point and articulating height at three different points (a, b and c).

3.4 Histological assessment

3.4.1 Tissue preparation and processing

Menisci were fixed in 10% formalin for three days. Menisci were sectioned cross-radially with a scalpel for the zonal section and kept the original shape for the regional section. A portion from the cranial, body and the caudal horn was excised for the zonal section. Menisci were transferred to a ratio of 1:3 10% formalin and 10% formic acid solution for a week for decalcification purposes. The decalcified menisci when then sectioned into the cross-radial and horizontal sections. The menisci sections were then transferred into histology cassettes for the dehydration process. The dehydration process was carried out by immersing specimens in increasing ethanol concentrations until pure, water-free alcohol was reached. Specimens were embedded in Leica Surgipath Paraplast and sectioned with a Jung biocut 2035 microtome to obtain 4μm sections of the meniscus. Sections were mounted on slides while in the Roundfin water bath. Slides were left heated at 40.0 °C on HI 1220 Leica hot plate for two days to dry up.

3.4.2 Histological staining

Paraffin sections were deparaffinised by immersion in xylene and rehydrated with 100% ethanol, 95% ethanol, 80% ethanol and distilled water. The slides were stained with hematoxylin and eosin (H&E), Safranin-O and fast green, and Picrosirius red.

3.4.2.1 Haematoxylin and eosin (H&E)

All sections were routinely stained with H&E for assessment of tissue morphology. The slides were immersed in haematoxylin for three minutes and rinsed with distilled water for ten seconds. Next, the slides were immersed in tap water for five minutes to allow the stain to develop. Next, sections were dipped in acid ethanol ten times to eliminate excess haematoxylin and enhance cellular differentiation, followed by washing in tap water. Sections were counterstained in eosin for thirty seconds. Finally, sections were dehydrated with 95% ethanol and 100% ethanol before being cleared in xylene and mounted with DPX mountant.

3.4.2.2 Safranin O and fast green

Safranin O and fast green (Solarbio Life Sciences, Beijing, China) is a cationic dye that stains acidic proteoglycan in cartilaginous tissues. All sections were stained with Safranin O. Sections were deparaffinised and rehydrated. The sections were immersed in Weigert's haematoxylin working solution for five minutes and then washed in water. Following washing, sections were differentiated with acid differentiation solution for fifteen seconds and rinsed with distilled water for ten minutes. Next, the sections were stained in a fast green staining solution for five minutes. Next, sections were rinsed in weak acid for 10 seconds to remove the remaining fast green staining and then slides were air dried, followed by staining in Safranin O solution for five minutes. Finally, sections were washed in water to remove excess Safranin O. Sections were dehydrated in 95%

ethanol for three seconds, absolute ethanol (I) for three seconds and absolute alcohol (II) for one minute. The sections were cleared in xylene and mounted with DPX mountant.

3.4.2.3 Picrosirius red

Picrosirius red stain (Solarbio Life Sciences, Beijing, China) mainly consists of iron hematoxylin staining and sirius red staining solution without picric acid. It is mainly used in the study of collagen fiber. All sections were stained with Picrosirius red. Sections were deparaffinised and rehydrated. The sections were immersed in iron haematoxylin staining solution for ten minutes and then washed in distilled water to remove excess staining solution for 15 seconds. Following washing with distilled water, sections were washed with tap water for ten minutes. The sections were dripped and dyed with sirius red staining solution for thirty minutes. Sections were lightly rinsed in water to remove excess stains. Sections were dehydrated in 75% ethanol for three seconds, 95% ethanol for three seconds, absolute ethanol (I) for three seconds and absolute alcohol (II) for one minute. The sections were cleared in xylene and mounted with DPX mountant.

3.4.3 Cell density calculation

Each cell was manually calculated by clicking each one of them. Cells were calculated within five randomly chosen areas. Cells are calculated according to the regions (cranial, body, caudal) and zones (outer, middle, deep). Calculated cells were recorded in

Microsoft Excel. Formulas were key in getting the cell density per area (cell/mm^2). Cells calculated/(0.15mm x 0.15mm) = cell/mm^2.

3.4.4 Histological scoring method

The histological evaluation was evaluated in horizontal and radial-cross sections per meniscus. Each radial-cross section was divided into deep, middle and outer zones, and each zone will be histologically evaluated. Each horizontal section was divided into cranial, body and caudal regions, and each region will be histologically evaluated. The cellularity, Safranin O fast green intensity, and picrosirius red intensity across the regions and zones of menisci were assessed and scored according to Sun *et al.* (2012) (Table 1).

Table 1: Histological scoring system of feline menisci.

Features	Score	Description	Radial cross-section			Horizontal section		
			Zone			Region		
			Outer	Middle	Deep	Cranial	Body	Caudal
Cellular density (H&E) (cell/mm^2)								
Proteoglycan (Safranin O fast green)	0	None						
	1	Weak red staining						
	2	Moderate red staining						
	3	Strong red staining						
Collagen organization (Picrosirius red)	1	Weak orange-red staining						
	2	Moderate orange-red staining						
	3	Strong orange-red staining						

3.5 Software

3.5.1 Nikon NIS-Elements imaging software

All Safranin O and picrosirius red-stained slides were viewed under a light microscope. Histological images were captured using Nikon NIS-Elements imaging software. Images were saved in JPG file format. Selected images were chosen and saved as a tagged image file format for use in the thesis.

3.5.2 Motic software

All H&E stained histological slides were viewed under 20x magnification of a light microscope. Motic software is connected to the light microscope. Using Motic software, histological images were captured then grid were added to ease calculating cells. The size of the grid is 150 µm X 150 µm. Images were saved in JPG file format. Selected images were chosen and saved as a tagged image file format for use in the thesis.

3.5.3 ImageJ version 1.53v

All H&E stained histological were then imported to ImageJ software for cell density calculation. Each cell was manually calculated by clicking each one of them. Cells were calculated within five randomly chosen areas.

3.5.4 Microsoft Excel

All data were tabulated and saved in Microsoft Excel.

3.7 Statistical analysis

Statistical analysis was conducted using Graphpad prism version 9. Data were expressed as mean and standard deviation. Two-tailed, paired T-tests were used to determine the morphometric differences between medial and lateral menisci. Two-tailed, unpaired T-tests were used to determine the morphometric differences between left and right medial menisci and left and right lateral menisci. One-way ANOVA was used to determine cellular density differences across meniscal regions and zones. The Kruskal-Wallis test was used to determine the localisation and distribution of proteoglycan and collagen across meniscal regions and zones. All morphometric data were expressed as mean and standard error (SE). $P<0.05$ is considered statistically significant.

Chapter 4

RESULTS

4.1 Morphological study of the feline menisci

A total of 120 menisci from 30 cats were evaluated. All menisci appeared morphologically normal, smooth and glistening upon gross examination. The numbers of medial and lateral menisci of the left and right stifle joints with different morphology are summarized in Tables 2 and 3, respectively. Each cat cadaver had the same medial and lateral menisci shape despite different joints. In the present study, three morphological types of the medial menisci were determined in which 66.67% of the left and right medial menisci were crescent, 16.67% of the left and right medial menisci were C-shaped, and 16.67% of the left and right medial menisci were discoid.

Regarding the shape of the lateral menisci, two morphological types of the menisci were determined, in which 86.67% of the left and right lateral menisci were C-shaped, and 13.33% of the left and right lateral menisci were crescent. Generally, the most common morphology for the medial meniscus is crescent and C-shaped for the lateral meniscus (Figure 6). No discoid was observed in the lateral meniscus of both sides, left and right stifle joints.

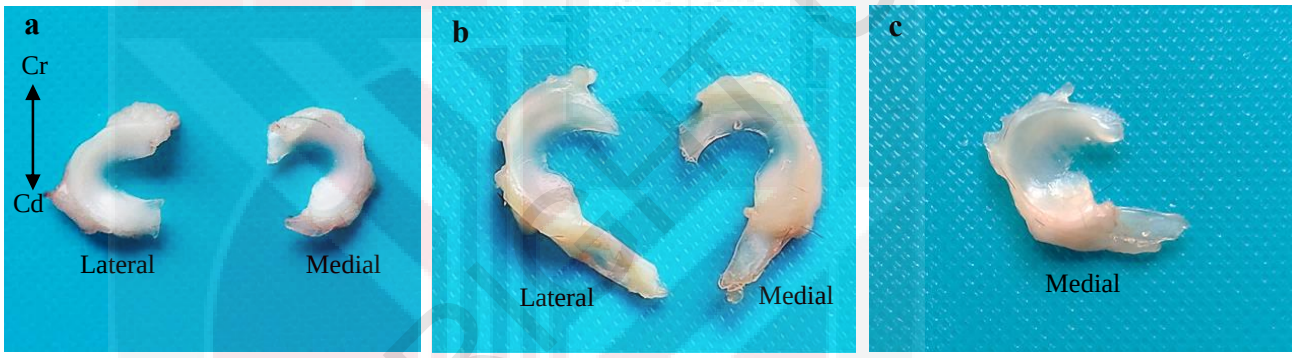

Figure 6: Gross morphology of menisci shows (**a**) C-shaped lateral and medial menisci; (**b**) crescent shape lateral and medial menisci while (**c**) discoid shape of the medial meniscus.

Table 2: The numbers of medial and lateral menisci of the left stifle joints with different morphology. The total number of cats is 30.

		Crescent	Sickled	C-Shaped	Sided U	Sided V	Discoid
Medial meniscus	Number of menisci	20	0	5	0	0	5
	Percentage	66.67%	0%	16.67%	0%	0%	16.67%
Lateral meniscus	Number of menisci	4	0	26	0	0	0
	Percentage	13.33%	0%	86.67%	0%	0%	0%

Table 3: The numbers of medial and lateral menisci of the right stifle joints with different morphology. The total number of cats is 30.

		Crescent	Sickled	C-Shaped	Sided U	Sided V	Discoid
Medial meniscus	Number of menisci	20	0	5	0	0	5
	Percentage	66.67%	0%	16.67%	0%	0%	16.67%
Lateral meniscus	Number of menisci	4	0	26	0	0	0
	Percentage	13.33%	0%	86.67%	0%	0%	0%

4.2 Morphometric study of the feline menisci

Measurement and statistical significance of the results of the dimensions of the medial and lateral menisci in the left and right stifle joints are summarized in Table 4. Dimensions of the lateral menisci were generally larger than medial menisci in all measurements, except the thickness and width of cranial horn measurements for both left and right joints. The left lateral menisci had the longest overall circumference measurement, 23.55 ± 0.39mm, followed by the right lateral menisci, 23.10 ± 0.35mm.

The overall circumference of the lateral menisci (23.55 ± 0.39 mm) was significantly different ($P<0.01$) from the medial menisci (21.95 ± 0.37 mm). In addition, the peripheral rim circumference of the lateral menisci (13.31 ± 0.44 mm) was significantly different ($P<0.05$) from the medial menisci (11.90 ± 0.36 mm).

There was a significant difference in thickness measurement of the middle third and caudal third of both menisci. The thickness of the middle third of the lateral menisci (1.69 ± 0.06 mm) was significantly different ($P<0.0001$) from the medial menisci (1.37 ± 0.06 mm). Likewise, the thickness of the caudal third of the lateral menisci (1.92 ± 0.08 mm) was significantly different ($P<0.05$) from the medial menisci (1.52 ± 0.09 mm). However, the thickness of the cranial third of the medial menisci (2.26 ± 0.09 mm) was not significantly different ($P= 0.0514$) from that of the lateral menisci (2.09 ± 0.07 mm).

The width of the lateral menisci was not significantly different (P>0.05) from that of the medial menisci for all three regions. The articulating height was a parallel continuation to the peripheral height taken at the periphery, decreasing gradually toward the inner edge. Statistically significant differences were found in articulating height measurement in all zone of both menisci. The articulating height of the periphery of the lateral menisci (1.67 ± 0.06 mm) was significantly higher (P<0.0001) than the medial menisci (1.35 ± 0.06 mm). The articulating height of the middle zone of the lateral menisci (0.68 ± 0.03 mm) was significantly different (P<0.001) from the medial menisci (0.52 ± 0.02 mm). The articulating height of the inner edge of the lateral menisci (0.30 ± 0.02 mm) was significantly higher (P<0.05) than that of the medial menisci (0.25 ± 0.01 mm). The sloped superior articular length in the lateral menisci (3.81 ± 0.11 mm) was not significantly different (P=0.2120) from that of the medial menisci (3.67 ± 0.13 mm).

The overall circumference of the lateral menisci (23.10 ± 0.35 mm) was not significantly different (P=0.0999) from the medial menisci (22.27 ± 0.51 mm). Likewise, the peripheral rim circumference of the lateral menisci (12.68 ± 0.41 mm) was not significantly different (P=0.5249) from the medial menisci (12.29 ± 0.50 mm).

There was a difference in thickness measurement of the middle third and caudal third of both menisci. The middle third of the lateral menisci (1.74 ± 0.07 mm) was significantly thicker (P<0.0001) than the medial menisci (1.34 ± 0.05 mm). In addition, the caudal third of the lateral menisci (1.94 ± 0.08 mm) was significantly thicker (P<0.01)

than the medial menisci (1.47 ± 0.07 mm). However, the thickness of the cranial third of the medial menisci (2.17 ± 0.09 mm) was not significantly different (P=0.7252) from that of the lateral menisci (2.16 ± 0.56 mm).

The middle third of the lateral menisci (3.87 ± 0.10 mm) was significantly wider (P<0.01) than that of the medial menisci (3.45 ± 0.12 mm). The articulating height was a parallel continuation to the peripheral height taken at the periphery, decreasing gradually toward the inner edge. There was a significant difference in the articulating height measurement of both menisci's periphery and middle zone. The articulating height of the periphery of the lateral menisci (1.71 ± 0.07 mm) was significantly higher (P<0.0001) than the medial menisci (1.34 ± 0.05 mm). In addition, the articulating height of the middle zone of the lateral menisci (0.63 ± 0.03 mm) was significantly higher (P< 0.01) than the medial menisci (0.55 ± 0.03 mm). However, the articulating height of the inner edge of the lateral menisci (0.28 ± 0.02 mm) was not significantly different (P=0.5138) from that of the medial menisci (0.26 ± 0.03 mm). The sloped superior articular length in the lateral menisci (3.87 ± 0.10 mm) was significantly different (P<0.01) from that of the medial menisci (3.45 ± 0.12 mm). A comparison of the dimensions between the different joints of the same side of the meniscus is represented in Table 5.

Table 4: Dimensions, in millimetres, of the medial and lateral menisci in the left and right stifle joints.

	Mean ± SEM Medial meniscus	Mean ± SEM Lateral meniscus	P-value
Left joint			
Circumference			
Overall	21.95 ± 0.37	23.55 ± 0.39	0.0013**
Peripheral rim	11.90 ± 0.36	13.31 ± 0.44	0.0118*
Thickness			
Cranial third	2.26 ± 0.09	2.09 ± 0.07	0.0514
Middle third	1.37 ± 0.06	1.69 ± 0.06	<0.0001****
Caudal third	1.52 ± 0.09	1.92 ± 0.08	0.023*
Width			
Cranial third	3.66 ± 0.12	3.52 ± 0.08	0.2051
Middle third	3.67 ± 0.13	3.81 ± 0.11	0.2120
Caudal third	3.08 ± 0.11	3.15 ± 0.09	0.4337
Articulating height			
Periphery	1.35 ± 0.06	1.67 ± 0.06	<0.0001****
Middle	0.52 ± 0.02	0.68 ± 0.03	0.0002***
Inner edge	0.25 ± 0.01	0.30 ± 0.02	0.0333*
Superior articulating length	3.67 ± 0.13	3.81 ± 0.11	0.2120
Right joint			
Circumference			
Overall	22.27 ± 0.51	23.10 ± 0.35	0.0999
Peripheral rim	12.29 ± 0.50	12.68 ± 0.41	0.5249
Thickness			
Cranial third	2.17 ± 0.09	2.16 ± 0.56	0.7252
Middle third	1.34 ± 0.05	1.74 ± 0.07	<0.0001****
Caudal third	1.47 ± 0.07	1.94 ± 0.08	0.0004***
Width			
Cranial third	3.58 ± 0.09	3.58 ± 0.08	0.9775
Middle third	3.45 ± 0.12	3.87 ± 0.10	0.0023**
Caudal third	3.13 ± 0.11	3.14 ± 0.10	0.9574
Articulating height			
Periphery	1.34 ± 0.05	1.71 ± 0.07	<0.0001****
Middle	0.55 ± 0.03	0.63 ± 0.03	0.0079**
Inner edge	0.26 ± 0.03	0.28 ± 0.02	0.5138
Superior articulating length	3.45 ± 0.12	3.87 ± 0.10	0.0023**

Left medial meniscus (n = 30); left lateral meniscus (n = 30); right medial meniscus (n = 30); right lateral meniscus (n = 30). Statistically significant differences with asterisks, where (*) indicates significance levels with P-values <0.05, (**) indicates significance levels with P-values <0.01, (***) indicates significance levels with P-values <0.001 and (****) indicates significance levels with p-values <0.0001.

Table 5: Comparison of the dimensions (mm) between the different joints of the same side of the meniscus.

Parameters	Medial meniscus			Lateral meniscus		
	Left joint	Right joint	*p*-value	Left joint	Right joint	*p*-value
Circumference						
Overall	21.95 ± 0.37	22.27 ± 0.51	0.6078	23.55 ± 0.39	23.10 ± 0.35	0.3950
Peripheral rim	11.90 ± 0.36	12.29 ± 0.50	0.5357	13.31 ± 0.44	12.68 ± 0.41	0.3045
Thickness						
Cranial third	2.26 ± 0.09	2.17 ± 0.09	0.5932	2.09 ± 0.07	2.16 ± 0.56	0.4123
Middle third	1.37 ± 0.06	1.34 ± 0.05	0.7493	1.69 ± 0.06	1.74 ± 0.07	0.5953
Caudal third	1.52 ± 0.09	1.47 ± 0.07	0.6738	1.92 ± 0.08	1.94 ± 0.08	0.8732
Width						
Cranial third	3.66 ± 0.12	3.58 ± 0.09	0.6074	3.52 ± 0.08	3.58 ± 0.08	0.5931
Middle third	3.67 ± 0.13	3.45 ± 0.12	0.2186	3.81 ± 0.11	3.87 ± 0.10	0.6563
Caudal third	3.08 ± 0.11	3.13 ± 0.11	0.7119	3.15 ± 0.09	3.14 ± 0.10	0.9036
Articulating height						
Periphery	1.35 ± 0.06	1.34 ± 0.05	0.9219	1.67 ± 0.06	1.71 ± 0.07	0.6594
Middle	0.52 ± 0.02	0.55 ± 0.03	0.5667	0.68 ± 0.03	0.63 ± 0.03	0.2497
Inner edge	0.25 ± 0.01	0.26 ± 0.03	0.8308	0.30 ± 0.02	0.28 ± 0.02	0.4179
Superior articulating length	3.67 ± 0.13	3.45 ± 0.12	0.2186	3.81 ± 0.11	3.87 ± 0.10	0.6563

The values are expressed as the *p*-value. Left medial meniscus (n = 30); left lateral meniscus (n = 30); right medial meniscus (n = 30); right lateral meniscus (n = 30).

4.3 Histomorphological characterisation of the meniscus

There was no difference in the cell density across the regions of the menisci (Figure 7). However, the cell density decreases from the outer to deep zones (Figures 8). Proteoglycan content was not found to differ across the regions (Figure 9). However, proteoglycan content was significantly increased in the middle zone (Figures 10 and 13). In addition, collagen content was considerably higher in the third cranial region (Figures 11 and 14) and deep zone (Figures 12 and 14).

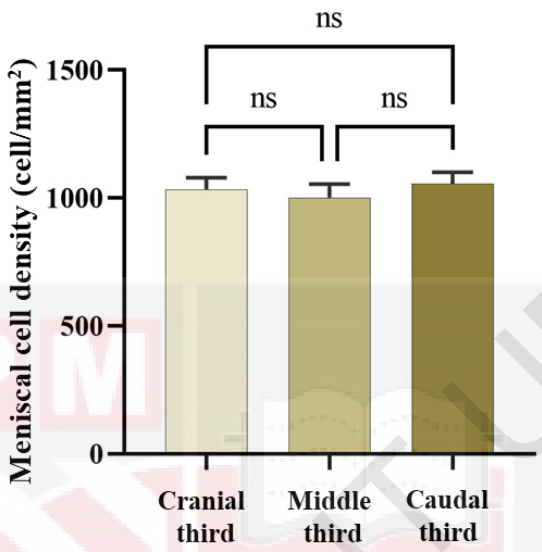

Figure 7: Showing cellular density (cell/mm^2) in the different regions of the menisci. Data are presented as the mean ± SEM. (n=30). There is no significant difference in cellular density of different regions of menisci. (One-way ANOVA, ns: not significant represents P>0.05).

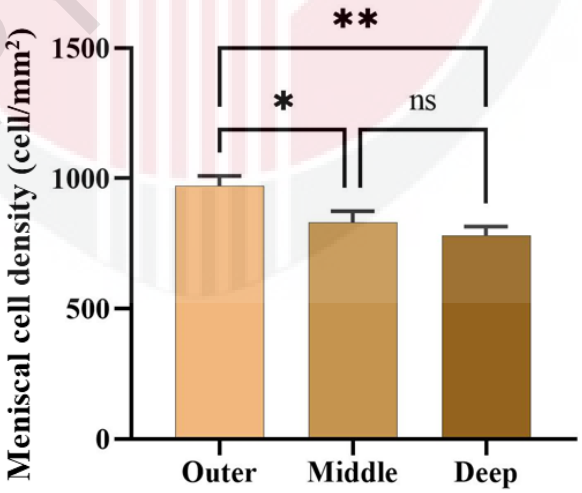

Figure 8: Showing cellular density (cell/mm^2) in the different zones of the menisci. Cellularity decreases from outer zone to deep zone. Data are presented as the mean ± SEM. (n=30). (One-way ANOVA, * represents P<0.05, ** represents P<0.001, ns: not significant represents P>0.05).

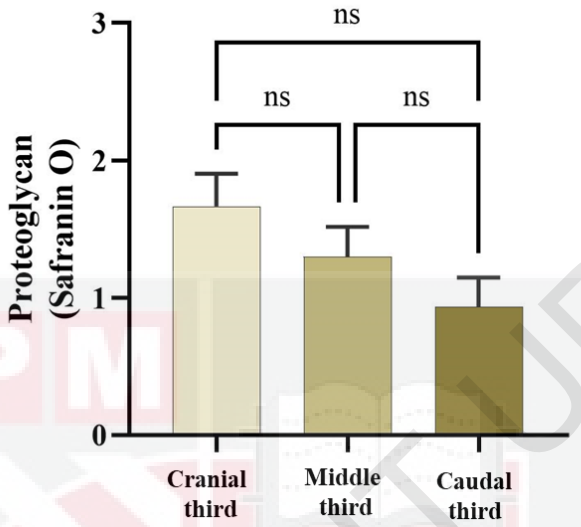

Figure 9: Histological scores of proteoglycan in the different regions of the menisci (n=30). There is no significant difference in proteoglycan content of different regions of menisci. Data are presented as the mean ± SEM. (One-way ANOVA, ns: not significant represents P>0.05).

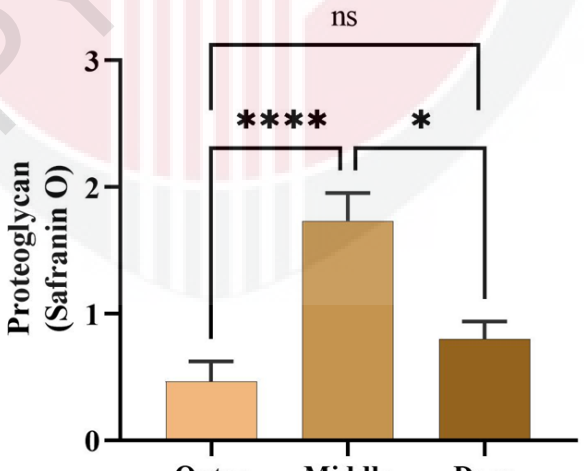

Figure 10: Histological scores of proteoglycan in the different zones of the menisci (n=30). The proteoglycan content evaluated by the Safranin O score is significantly higher in middle zone than in the outer and deep zones of the menisci. Data are presented as the mean ± SEM. (One-way ANOVA, **** represents P<0.0001, * represents P<0.05, ns: not significant represents P>0.05).

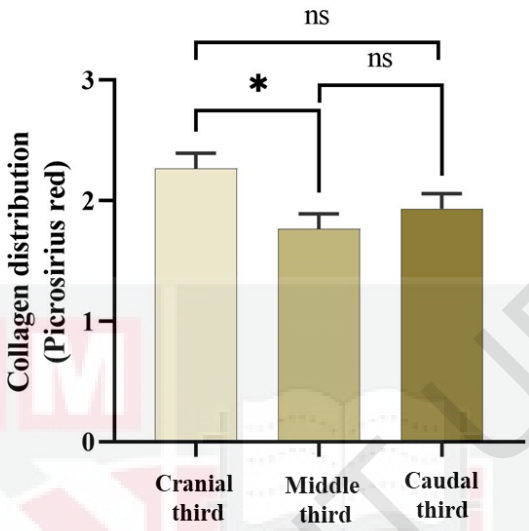

Figure 12: Histological scores of collagens in the different regions of the menisci (n=30). The collagen content was determined using Picrosirius red staining. The collagen content is significantly higher in cranial region than in the caudal and body of the menisci. Data are presented as the mean ± SEM. (One-way ANOVA, * represents P<0.05, ns: not significant represents P>0.05).

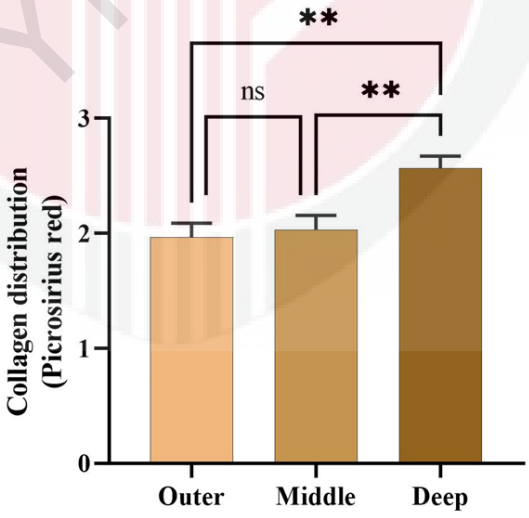

Figure 11: Histological scores of collagens in the different zones of the menisci (n=30). The collagen content was determined using Picrosirius red staining. The collagen content is significantly higher in deep zone than in the middle and outer zones of the menisci. Data are presented as the mean ± SEM. (One-way ANOVA, ** represents P<0.001, ns: not significant represents P>0.05).

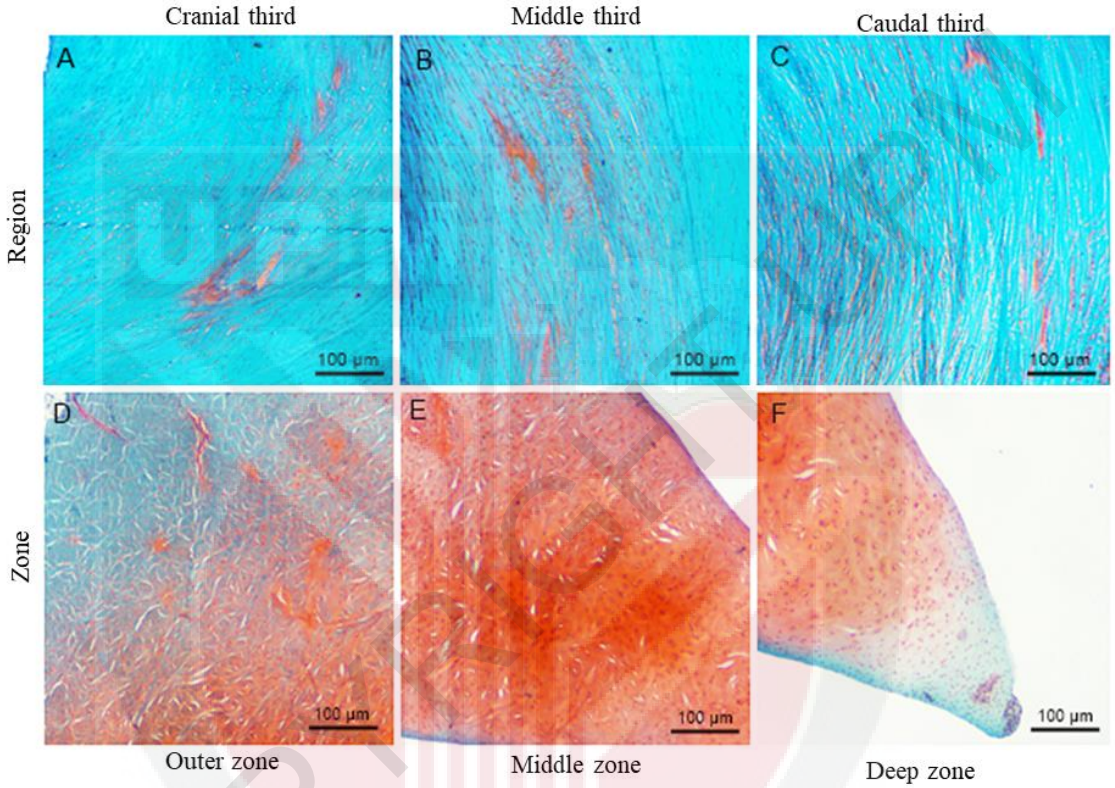

Figure 13: Safranin O staining for proteoglycan showed that feline menisci had proteoglycan content. The proteoglycan stained more intensely in the middle zone of the menisci. A-F: 40X magnification.

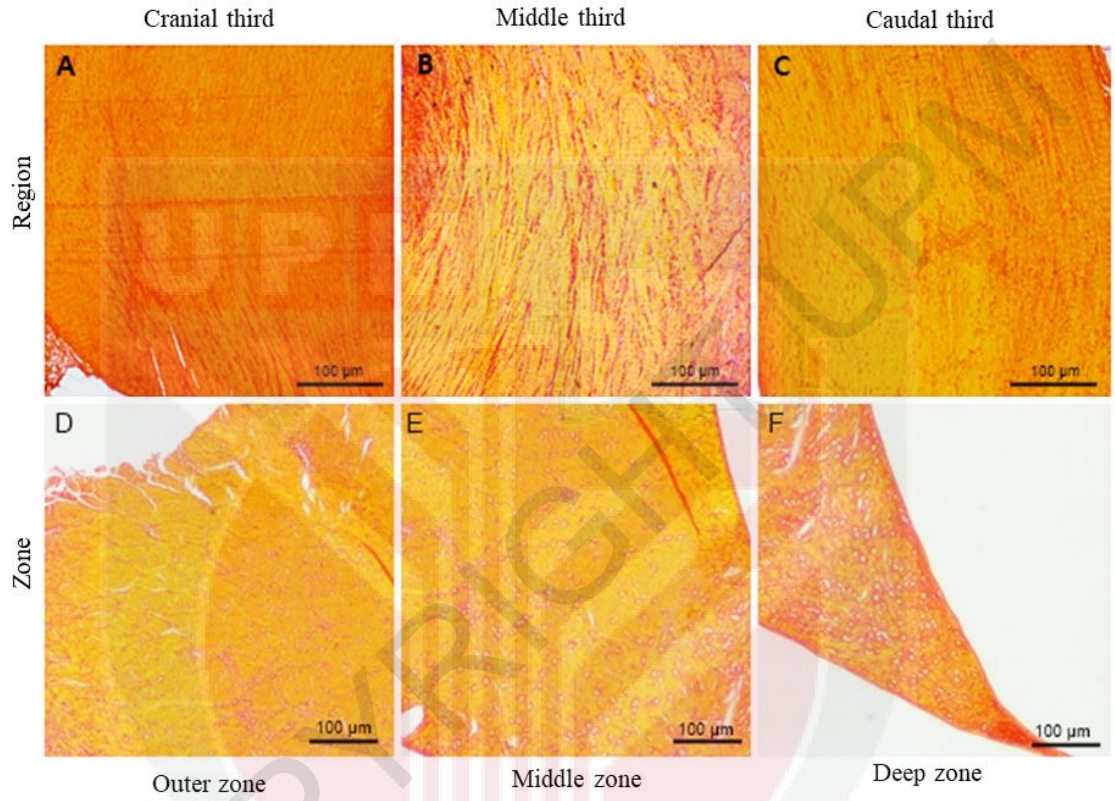

Figure 14: Picrosirius red staining for collagen fibers of menisci. The collagen stained more intensely in the menisci's cranial third region and deep zone. A-F: 40X magnification.

Chapter 5

DISCUSSION

5.1 Morphological study of the meniscus.

Due to recent advancements in radiographic and surgical procedures like arthroscopy, computed tomography, and magnetic resonance imaging, the data of morphology, morphometric and histological variations of knee joint meniscus have become significant. Therefore, it is crucial to investigate these variations to establish morphological and histomorphological features for clinical diagnosis and surgical treatments (Kale *et al.*, 2006).

There is a great deal of data regarding meniscus in humans; however, only a few studies have been done on the meniscus of animals, especially feline species. It has been said that in meniscal allograft transplantation, it is the obligation of the tissue bank delivering the graft to provide a meniscal allograft that matches the recipient's knee's meniscus in terms of size and shape (Haut *et al.*, 2000). Meniscal shape anomalies are categorised as hypoplasia or hyperplasia in human (Gupta *et al.*, 2022). Numerous research has focused on meniscal hyperplasia (discoid menisci) in human since discoid menisci are often observed in human. The discoid shape is a typical morphology of a developing embryo and is occasionally retained because the central region of the discoid

cannot be absorbed during the foetal stage (Wang and Gao, 2017). The highest prevalence rate of a discoid medial meniscus reported is 0.3% in human (Chen *et al.*, 2012). In the present study, 16.67% of the medial meniscus of both left and right stifle joints were discoid in shape. The study also found that the medial meniscus has more morphology variation than the lateral meniscus. This finding is contradicted by the study done by Oconnor and Mcconaughey in 1978, who stated that the medial meniscus has less variation in shape.

5.2 Morphometric study of the meniscus.

The was a significant difference between the medial and lateral sides of the same side. The morphometric analysis revealed that the lateral menisci significantly differed from the medial menisci in both parameters for circumference. The current morphometric study found that the circumference of the lateral menisci was generally larger than the medial menisci. However, these findings do not support the previous study by Prose (1984) and Ariffin (2015), which reported that the feline medial meniscus is larger than the lateral meniscus. Moreover, the morphometric results match those observed in other species, such as pigs, sheep and human (Takroni *et al.*, 2016; Goyal *et al.*, 2020).

There were no significant differences in the medial and lateral menisci width. However, the results revealed that the lateral menisci significantly differed from the

medial menisci in thickness at the middle third and caudal third. Lateral menisci are larger than medial menisci. When comparing the thickness of the middle third of both menisci, it was observed that the middle third of the medial meniscus is thinner than the lateral meniscus. This finding may explain the higher incidence of injuries in the medial meniscus of cats (Beale, 2009). A study by Braz and Silva (2010) stated that the middle third of the medial meniscus is decreased in thickness and mobile, which causes the middle third to be prone to stress and injury. This may be related to the menisci having two fixed points, the horns, while the remainder point (middle third) is mobile, making it more prone to stress. This alone would justify this anatomical thicker feature.

Although there were no significant differences in the articulating height, the results were consistent with Takroni *et al.* (2016) stating that articulating height decreases gradually towards the inner edge in pigs, sheep and human. A morphometrics study is simply a quantitative way of addressing the shape and variation of any anatomical structure. Data reported herein should help understand the feline menisci's anatomical measurements.

5.3 Histomorphological characterisation of the meniscus

In the present study, the cell density was not significantly different across the regions. However, the cell density decreases from the outer zone to the deep zone. This result agrees with Cengiz *et al.* (2015), who stated that the cell in the outer zone, which is

well vascularised, is denser than the avascular middle and deep zones. Moreover, high cell density may promote an intrinsic repair process (Chevrier *et al.*, 2019; Yan *et al.*, 2021). This means the outer zone heals faster than the middle and deep zone if the injury happens.

Proteoglycan content was not found to differ across the regions but was found to be significantly increased in the middle zone. This finding can be supported by a study by Nerurkar *et al.* (2011). They stated that proteoglycans provide the middle zone with a high capacity to resist large compressive loads and maintain stiffness. Collagen content was considerably higher in the third cranial region and deep zone. Starke *et al.* (2009) reported that tibiofemoral compressive loads cause considerable tensile forces at the cranial horn in porcine. Collagen fibers have great tensile strength and contribute to the load transmission in the stifle joint. Thus, high collagen distribution found in the third cranial region in felines may justify the similar function of collagen in providing compressive properties to the menisci.

The limitation of this study was the small sample size. A larger sample size is better for observing more morphological and morphometric variations. Another limitation was not measuring the circumference of menisci *in-situ* in the study. After the meniscal samples were completely separated from the tibial plateau, a change of the arc or the curve of the menisci may contribute to inaccurate lengths, especially at the end of the meniscus, which has insertions to the ligaments. *In-situ* measurements were difficult to access as the cat's menisci were too small.

Chapter 6

CONCLUSION AND RECOMMENDATIONS

As for the conclusion, there were variations in morphometry between the medial and lateral menisci. In addition, there were regional and zonal variations in the meniscus compositional and structural organisation. As for the recommendation, further comparative morphometric studies could be done with other quadruped stifle animal species as feline species. Next, to do further study to establish the collagen-proteoglycan-water relationship. Lastly, immunohistochemical assessment is needed to localize different types of collagens as this study only localizes the overall collagen distribution.

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