UNIVERSITI PUTRA MALAYSIA

HISTOLOGICAL AND HISTOMORPHOMETRICAL ASSESSMENTS OF FELINE OSTEOARTHRITIS (OA) MENISCI

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HISTOLOGICAL AND HISTOMORPHOMETRICAL ASSESSMENTS OF FELINE OSTEOARTHRITIS (OA) MENISCI

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CERTIFICATION

It is hereby certified that I have read this project entitled "Histological and Histomorphometrical Assessments of Feline Osteoarthritis (OA) Menisci" by Nur Salina Binti Sukry, and in my opinion, it is satisfactory in terms of scope, quality and presentation as partial fulfilment 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
ECM	Extracellular matrix
FPV	Faculty of Veterinary Medicine
H&E	Haematoxylin and eosin
MM	Meniscal mineralisation
mm	Millimeter
OA-MM	Osteoarthritis with meniscal mineralisation
OA	Osteoarthritis
P	Significant difference
r_s	Correlation coefficient
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.

PENILAIAN HISTOLOGI DAN HISTOMORFOMETRI BAGI MENISKUS OSTEOARTRITIS (OA) KUCING.

Oleh

Nur Salina Binti Sukry

2023

Penyelia: Dr Siti Mariam Binti Zainal Ariffin

Osteoartritis kucing (OA) adalah proses degeneratif kronik terutamanya yang menjejaskan sendi sinovia kucing. Meniskus adalah salah satu komponen sendi sinovia yang terletak di antara kondil femur dan dataran tibia. Ia adalah struktur rawan gentian yang terdapat secara berpasangan di sisi dan medial, dan bertanggungjawab untuk pengagihan beban, penyerapan kejutan, penstabilan dan pelinciran sendi. Pemahaman tentang anatomi meniskus adalah prasyarat kerana ia mempunyai peranan penting dalam permulaan dan perkembangan OA menyekat. Kajian semasa bertujuan untuk menilai perubahan histologi dan histomorfometrik dalam meniskus OA kucing. Selain itu, kajian ini memberi tumpuan untuk menentukan hubungan antara mineralisasi meniskus dan

degenerasi dalam OA kucing. Sebanyak tiga puluh lapan tisu meniskus karkas kucing yang terbenam parafin secara arkib, dengan (n=15), tanpa OA (n=8) secara semula jadi dan OA dengan mineralisasi meniskus (MM) (n=15) telah digunakan. Tisu terbenam parafin yang diarkibkan dipotong pada 5.0µm menggunakan mikrotom dan diletakkan pada slaid kaca. Keratan rentas membujur telah diwarnakan menggunakan hematoksilin & eosin (H&E) dan Safranin O untuk penilaian meniskus. Penilaian histologi dinilai secara mikroskopik dan diskor menggunakan sistem penggredan histologi berdasarkan parameter seperti integriti permukaan, organisasi kolagen dan keamatan safranin O. Selain itu, penilaian histomorfometrik dilakukan menggunakan mikroskop cahaya pada pembesaran X4 dan X10 yang disambungkan ke komputer yang dilengkapi dengan perisian Motic Image Devices. Parameter yang dinilai ialah ketumpatan selular (sel/mm^2) dan saiz MM (mm^2). Secara keseluruhannya, analisis histologi mendedahkan bahawa skor global dalam menisci normal adalah berbeza dengan ketara ($P<0.05$) dengan OA meniskus. Walau bagaimanapun dari segi ketumpatan selular, tidak terdapat perbezaan yang ketara antara meniskus normal dan OA, tetapi keputusan bertentangan ditemui di antara OA (tanpa MM) dan OA (dengan MM). Terdapat juga korelasi sederhana yang signifikan (r_S=0.6712) antara kawasan MM dan skor global OA (dengan MM). Kajian semasa mendedahkan perubahan luar biasa OA kucing dalam parameter histologikal dan histomorfometrikal.

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

ABSTRACT

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

HISTOLOGICAL AND HISTOMORPHOMETRICAL ASSESSMENTS OF FELINE OSTEOARTHRITIS (OA.) MENISCI

By

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Feline osteoarthritis (OA) is a chronic degenerative process affecting feline synovial joints. Menisci are one of the components of synovial joints that are located between femoral condyles and the tibial plateau. It is a fibrocartilaginous structure found in pairs laterally and medially, responsible for load distribution, shock absorption, stabilisation and joint lubrication. Understanding meniscal anatomy is a prerequisite, as it has a significant role in the initiation and progression of stifle OA. The current study aimed to evaluate the histological and histomorphometrical changes in feline OA menisci. Besides,

this study focuses on determining the association between meniscal mineralisation and degeneration in feline OA. A total of thirty-eight archived paraffin-embedded skeletally mature cat cadavers' meniscal tissues, with (n=15), without naturally occurring OA (n=8) and OA with meniscal mineralisation (MM) (n=15), were used. The archived paraffin-embedded tissues were sectioned at 5.0µm using a microtome and mounted onto glass slides. The longitudinal cross sections were stained using haematoxylin and eosin (H&E) and Safranin O for meniscal evaluation. The histologic assessment was evaluated microscopically and scored using a histological grading system based on parameters such as surface integrity, collagen organisation and safranin O intensity. In addition, histomorphometric evaluations were performed using a light microscope at X4 and X10 magnifications connected to a computer equipped with Motic Image Devices software. The parameters assessed were cellular density (cell/mm^2) and the size of MM (mm^2). Histological analysis revealed that the global score of OA menisci was significantly higher ($P<0.05$) than the normal menisci. There was no significant difference in cell density between normal and OA menisci, but opposite results were found between OA (without MM) and OA (with MM). There was also a significant moderate correlation (r_S=0.6712) between the MM area and OA (with MM) global score. The current study revealed remarkable changes in feline OA in histological and histomorphometrical parameters.

Keywords: meniscus; stifle joint; morphometric; meniscal mineralisation; feline

CHAPTER 1

INTRODUCTION

1.1 Background

Osteoarthritis (OA) is a common disease affecting older cats, but the clinical signs are always unrecognised because most cats will not exhibit lameness (Kimura et al., 2020). Osteoarthritis is characterised by progressive cartilage degradation that will influence changes in other intra-articular structures such as synovium, subchondral bone, and menisci (Ariffin, 2015).

The stifle meniscus plays an important role in load transmission, shock absorption, stabilisation, joint lubrication, and proprioception (Dyce et al., 2017). Meniscal injury, mineralisation, or degeneration are thought to contribute to the development or progression of stifle OA (Voss et al., 2017). Most OA meniscus microstructural changes are known from humans (Yadaz et al., 2022) and murine model studies (Ramos-Mucci et al., 2020). Osteoarthritic menisci may show changes in cellular, structural and extracellular matrix (ECM) properties (Ariffin, 2015). However, a quantitative histomorphometric analysis of this joint component has not been investigated, attracting much attention in the field.

Voss et al. (2017) previously reported that meniscal degeneration is severe in the meniscus with mineralisations, but it did not seem to be consistently associated with the size of the mineralisation. Nevertheless, meniscal mineralisation may affect the quality and strength of the meniscus. However, no literature has been found that examines the relationship between meniscal mineralisation and the histological changes in the meniscus caused by degeneration.

1.2 Justification

Determining histological and histomorphometrical changes of the meniscus in feline OA will provide a better understanding of the significance of meniscus changes seen in stifle OA, as comprehensive evaluation at the microscopic level is needed. In addition, mineralisation of the menisci may reflect degenerative changes as part of the OA process affecting the stifle joint. Besides, very limited pathological literature on this disease has been published. To establish their interconnection, a complete understanding of feline OA requires a combination of histological and histomorphometrical assessments with its pathological characteristics. This is important in understanding the pathophysiology of feline OA and paving the way for developing disease-modifying drugs for OA therapy. Moreover, the connection between OA and meniscus degeneration is intricate, making it challenging to determine which comes first. Meniscus degeneration is a multifaceted process linked to ageing, and the discovery of a degenerative meniscal tear frequently indicates early stifle OA (Farrah et al., 2021).

1.3 Objectives of the Study

1. To evaluate the histological and histomorphometrical changes in the OA menisci
2. To determine the association between meniscus mineralisation and degeneration

1.4 Research Hypothesis

Objective 1:

H_0: OA exhibits tissue integrity, well-organised collagen fiber, safranin O intensity, and cellular density similar to normal menisci.

H_1: OA menisci exhibits poor tissue integrity, disorganisation of collagen fibres, high safranin O intensity and low cellular density as compared to normal menisci

Objective 2:

H_0: There is no association between meniscal mineralisation and its degenerative changes

H_1: There is an association between meniscal mineralisation and its degenerative changes

CHAPTER 2

LITERATURE REVIEW

2.1 Stifle Osteoarthritis (OA)

Feline Osteoarthritis (OA) is a chronic degenerative joint disease affecting synovial joints. Feline OA is a common but under-recognised chronic disease that negatively impacts the life quality of senior cats. Clinically, it is described as a slowly progressing joint condition marked by a gradual onset of joint pain, stiffness and restricted movement. Pathologically, it is defined as a non-inflammatory disorder of synovial joints characterised by the breakdown of joint cartilage and new bone formation at the joint surfaces and margins (Ariffin, 2015). The stifle joint is frequently affected by OA, and the prevalence rates ranged from 60 to 86.2% (Kimura et al., 2020; Freire et al., 2010). Ariffin (2015) also reported that the stifle joint is found to be the second highest for radiographic prevalence, followed by hip, tarsal, shoulder and carpal joints, which are 23.9%, 21.1%, 17.7%, 6.7% and 6.4% respectively. In stifle OA, morphological changes occur in all joint compartments, including the menisci.

2.2 General Gross Anatomy and Histology of Meniscus

The meniscus is a crescent wedge-shaped pad of fibrocartilage structure interposed between the femoral condyle and tibial plateau. Typically, a normal meniscus

exhibits a smooth, translucent, white and glistening surface (Sun et al., 2010; Pauli et al., 2011; Ariffin, 2015). Besides, this structure also possesses a concave shape proximally to accommodate the convexity of the femoral condyles and flattened distally for articulation with the tibial plateau (Allen et al., 1995; Dyce et al., 2017; Ariffin, 2015). Each meniscus consists of different regions: a cranial horn, a body and a caudal horn (O'Connor and McConnaughey, 1978; Ariffin, 2015). Moreover, the menisci are attached to the tibia and femur by five meniscus ligaments and are connected by a sole intermeniscal ligament.

The five ligaments in this meniscus include cranial tibial ligaments of the lateral and medial meniscus, caudal tibial ligaments of the lateral and medial meniscus, and femoral ligament of the lateral meniscus (Briggs, 2004). The menisci play an important role in load transmission, shock absorption, joint lubrication and proprioception (Dyce et al., 2017).

Histologically, the main components of the meniscus are cells (fibrochondrocytes or a mixture of fibroblasts and chondrocytes) and extracellular matrix (ECM). These cells produce and sustain ECM, primarily composed of collagens (type 1), water and non-collagenous proteins such as proteoglycan. However, the amount of proteoglycans present within the ECM in a normal meniscus is significantly lower than in hyaline articular cartilage (Graverand et al., 2001). According to Fox et al. (2011), Chen et al. (2017) and Murphy et al. (2019), proteoglycans are responsible in maintaining hydration and granting tissue a substantial ability to withstand compressive forces.

2.3 Degenerative Changes in OA menisci

Grossly, discolouration of the meniscus from white to dark yellow, light brown or reddish colour can be observed (Pauli et al., 2011). Meanwhile, histologically, a few microscopic changes can be seen in OA menisci, including the tissue surface, collagen fiber organisation, and safranin O intensity. Fibrillation, undulation and disruption of tissue surface may be observed in OA menisci. Ariffin (2015) stated that the meniscus of feline OA showed fibrillation and increased proteoglycan compared to the normal menisci. Besides, meniscus degeneration occurs within the fibrocartilage.

Another study by Voss et al. (2016) reported that meniscal degeneration showed surface roughening with clefts or tears and loss of collagen fibre organisation and cells. Similar microscopic changes were found in studies done in human and animal models of OA. Generally, OA menisci showed uneven tissue surfaces and unorganised collagen fibres. Increases in proteoglycan were seen in OA menisci as depicted by high Safranin O intensity; however, there were not much differences in cell density (Pauli et al., 2011; Long et al., 2019; Ramos-Mucci et al., 2020 and Yadav et al., 2022).

In addition, Deng et al. (2019) found that rabbits with induced OA showed similar degenerative changes, such as rough tissue surface with indentations, irregular thickness and disorganisation of collagen fibres, and decreased cartilage cell numbers. However, according to Katsuragawa et al. (2010), cell density within the menisci varied in different

regions in the rabbit model of OA, showing both increases and decreases. Additionally, clusters of cells were commonly observed in the degenerated areas.

2.4 Meniscal mineralisation and its significance

Meniscal mineralisation can be defined as calcium crystal deposition within the meniscus. The cranial horn of domestic cats' medial meniscus of the stifle joint commonly exhibits meniscal mineralisation (Voss et al., 2016). Based on Sun et al. (2010), meniscal mineralisation may impact elasticity, induce stiffness and thus modify its biomechanical properties, resulting in a gradual decline in meniscus functionality. This could influence its shock-absorbing ability, potentially elevating mechanical loading on the medial femoral condyle and medial part of the tibial plateau, resulting in OA.

Three hypotheses exist on the types of meniscal mineralisation within the feline medial meniscus. First is an intrameniscal ossification involving trabecular bone with marrow spaces entirely embedded within the meniscus. This formation could be due to chondro-osseous transdifferentiation from fibrocartilage tissue and mineral deposition. Subsequently, this process leads to trabecular bone and bone marrow formation, most likely attributed to mechanical stress resulting from gait abnormalities or abnormal hind limb conformation, which may be induced by the pain associated with OA. For the second explanation, meniscal mineralisation is an intrameniscal ossification consisting of trabecular bone with marrow spaces and is characterised by a distinct articular surface

covered by a cartilage cap. This represents a meniscal sesamoid bone, known as a lunula, and they are believed to be either a normal anatomical feature or a vestigial anomaly without clinical importance. Lastly, this process is also called intrameniscal mineralisation with areas of chondro-osseous metaplasia (Freire et al., 2010; Ariffin, 2014; Voss et al., 2016;).

Yet, mineralisation in the meniscus might not solely represent degenerative calcification, and it could also signify the existence of a sesamoid bone, which is considered a normal characteristic of the feline meniscus. Apart from that, meniscal degeneration was generally more severe in stifles with mineralisations. However, the relationship between meniscal mineralisation and degeneration remains unclear (Voss et al., 2016).

CHAPTER 3

MATERIALS AND METHODS

3.1 Archived samples

Thirty-eight skeletally mature cat cadavers were included in this study, and these animals were part of a previous study conducted by Ariffin (2015). Archived paraffin-embedded meniscal samples, which had 15 OA menisci, 15 OA-MM menisci (with meniscal mineralisation), and 8 healthy menisci, were used. OA meniscal samples obtained from naturally occurring OA were five neutered males, six females and four spayed females, with a mean age of 7.3 years (range: 2-20 years). Healthy meniscal samples were from four neutered males, two females, and two spayed females, with a mean age of 2.7 years (1-5 years).

3.2 Tissue sectioning and staining

Specimens were sectioned longitudinally with a Jung Multicut RM2045 microtome to obtain 5µm sections of the meniscus. Each section was then mounted on glass slides in the Roundfin water bath at 42ºC. Then, slides were placed on an HI 1220 Leica hot plate at 40ºC for 3 days to remove moisture. After drying up, the slides were stained with haematoxylin and eosin (H&E) and Safranin O.

3.2.1 Haematoxylin and eosin (H&E)

All sections were routinely stained with H&E for histological and histomorphometrical assessment. The slides were immersed in haematoxylin for five minutes and rinsed with distilled water for ten seconds. After that, the slides were dipped in acid ethanol for ten seconds to eliminate excess haematoxylin and enhance cellular differentiation, followed by rinsing with tap water. Next, each section of slides was counterstained with eosin for 30 seconds and subsequently dehydrated with 70%, 80%,95% and 100% ethanol before being cleared in xylene and mounted with DPX mountant.

3.2.2 Safranin O and fast green

Safranin O is a basic dye that stains cartilage (proteoglycans, chondrocytes and type II collagen), mucin and mast cell granules in tissue sections in shades of red. All sections were stained with Safranin O. Deparaffinization and rehydration were done, and sections were immersed in Weigert's haematoxylin solution for five minutes and then rinsed with distilled water for 10 minutes. Next, the sections were stained with the fast green solution for five minutes and rinsed with weak acid for 12 seconds to remove the remaining fast green staining. The slides were then air-dried and stained with Safranin O staining solution for five minutes. Lastly, the slides were dehydrated at 90% and 100% for a minute before being cleared in xylene and mounted with DPX mountant.

3.3 Histological & histomorphometrical assessments

3.3.1 Histological scoring method

The histological evaluation was performed in longitudinal sections per meniscus. The scoring system comprised three important microscopic features: tissue surface, collagen fiber organisation and Safranin O intensity. The total histological score was determined by summing up the total individual scores. Meanwhile, a global score was determined based on an overall assessment of the severity of the histological changes. The scoring system (Table 3.1) was developed based on the combination of histological scoring systems adapted from Pauli et al. (2011), Ariffin (2015) and Voss et al. (2016).

3.3.2 Cell Density Calculation

Five areas of meniscal regions (cranial horn, body, caudal horn) were captured per meniscus under X100 magnification using Motic Image Device software. Cell nuclei were counted by using a multipoint tool in ImageJ software. Firstly, the cell counts per grid in one of the meniscus regions were determined by calculating the number of cell nuclei per grid ($1.5mm^2$) (Weber et al., 2015) as shown in Figure 3.1. This step was repeated for another four selected grids. Then, the average number of cell nuclei per area was determined by calculating the total number of cell counts per grid in five selected grids and dividing by five. Finally, the same formula was used for another four meniscal regions and the estimated cellular density of a meniscus was determined by summing up the

average cell counts per area divided by five meniscal regions (Figure 3.2). Calculated cell density was recorded and tabulated.

Table 3.1: Histological scoring of feline menisci

Histological features		**Score**	**Description**
Menisci	Tissue surface	0	Smooth
		1	Slight fibrillation or slightly undulating
		2	Moderate fibrillation or markedly undulating
		3	Severe fibrillation or disruption
	Collagen fiber organisation	0	Collagen fibres organised, homogenous eosinophilic staining of extracellular matrix
		1	Collagen fibres organised, diffuse foci of hyaline mucinous degeneration
		2	Collagen fibres unorganised, confluent foci or bands of hyaline or mucinous degeneration, fraying
		3	Collagen fibres unorganised, fibrocartilaginous separation (oedema, cystic formation), severe fraying and tears
	Safranin-O intensity (Proteoglycan)	0	No stain
		1	Slight staining
		2	Moderate staining
		3	Strong staining
	Global score	0	No abnormality (Total histologic score 0)
		1	Mild (Total histologic score 1-3)
		2	Moderate (Total histologic score 4-6)
		3	Severe (Total histologic score 7-9)

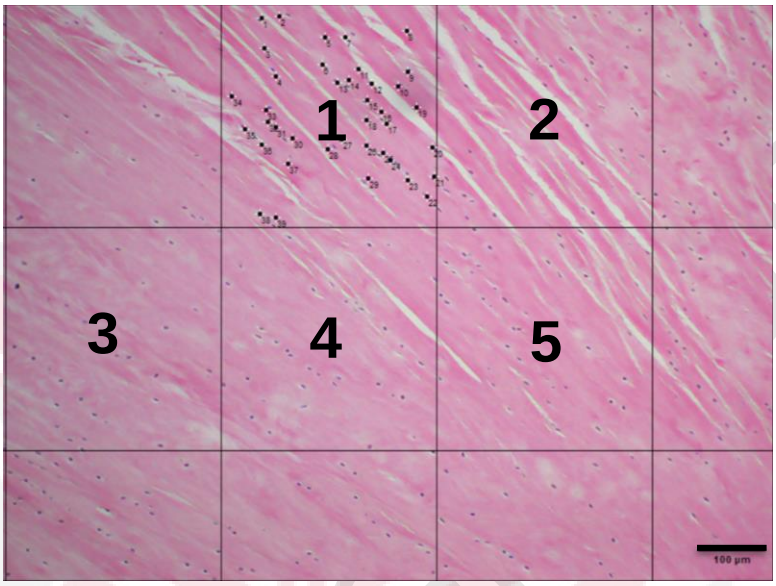


Figure 3.1: This figure shows an image of a meniscal region. The multipoint tool in Image J software calculates the number of cell nuclei per grid. Each box labelled with a number (1, 2, 3,4 and 5) represents the number of the selected grids.

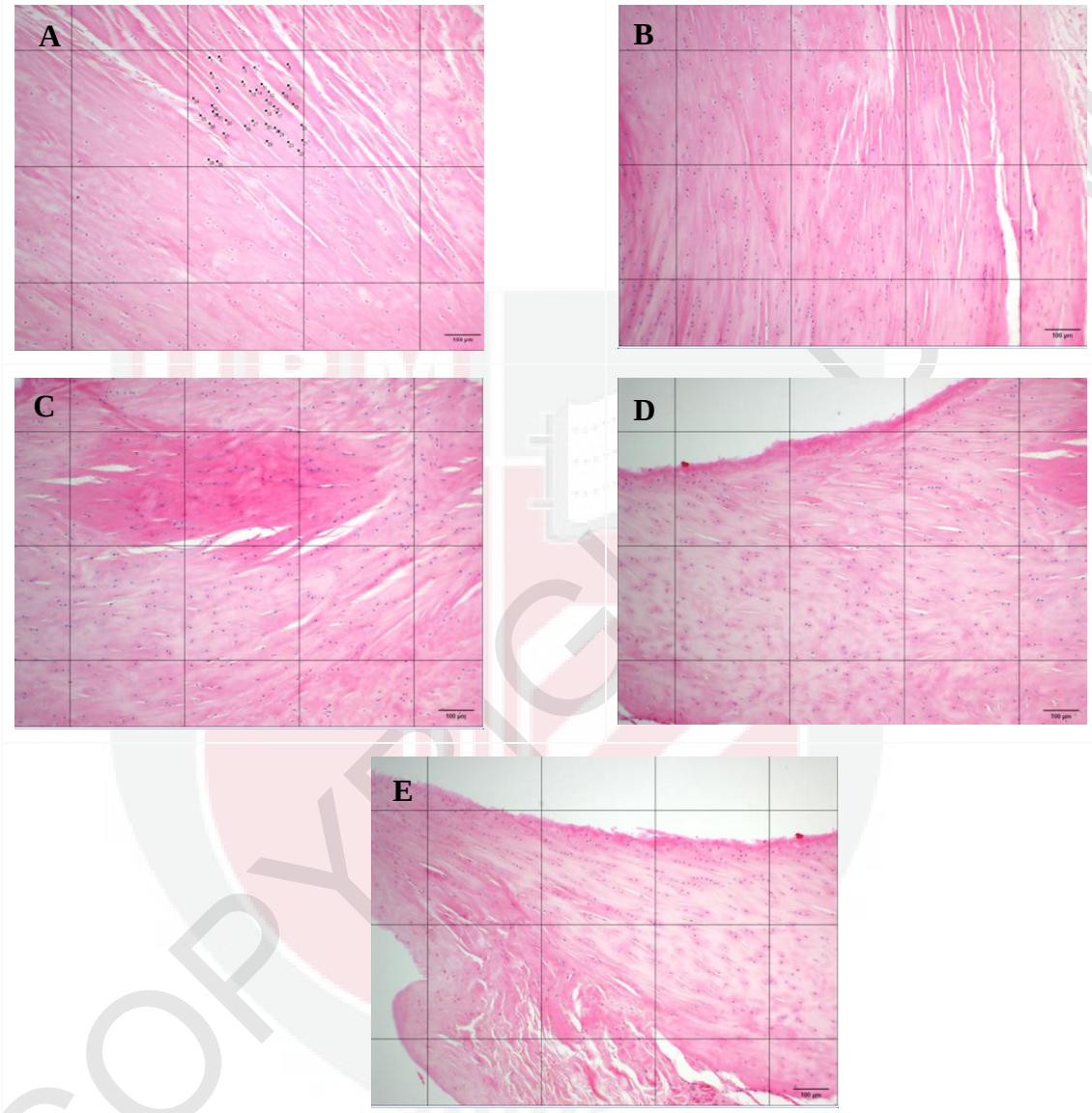
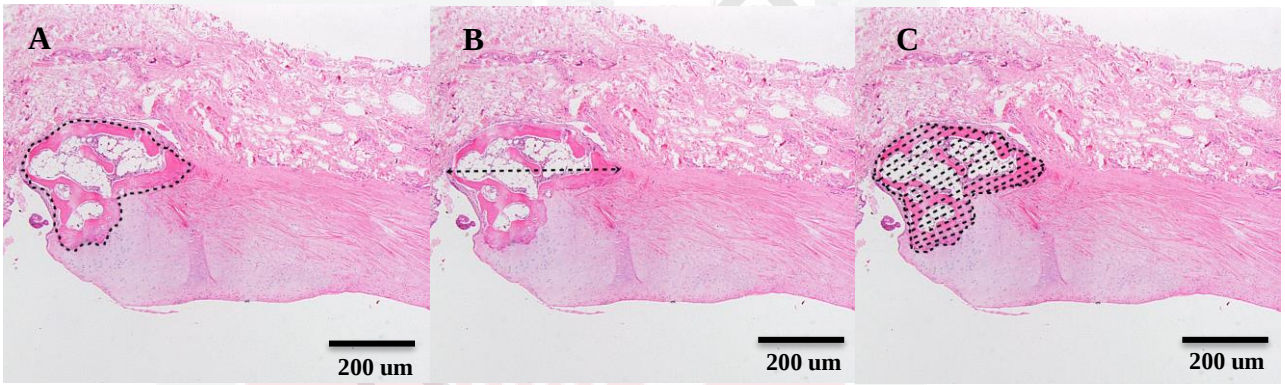


Figure 3.2: This figure shows five different segments (A, B, C, D and E) of a meniscus. The estimated cellular density of a meniscus is determined by summing up the average cell counts per area divided by five meniscal regions.

3.3.3 Measurement of meniscal mineralisation size

The meniscal mineralisation size includes perimeter (mm), length (mm) and area (mm^2) (Figure 3.3). This Measurement was performed using a dragging tool in ImageJ software and all parameters were automatically calculated. Calculated meniscal mineralisation sizes were then recorded and tabulated.



Perimeter (mm) Length (mm) Area (mm^2)

Figure 3.3: Measurement of meniscal mineralisation size, where (A) perimeter (mm); (B) length (mm); (C) area (mm^2).

3.4 Image viewing and capturing

3.4.1 Motic Image Device Software

All H&E and Safranin O-stained slides were viewed under X40 and X100 magnification of a light microscope. This software is connected to a light microscope. Using Motic Image Device software, histological images were captured and saved in JPG and tagged image file format (TIF). TIF images were included in the thesis.

3.4.2 Image J Java 8

All H&E and Safranin O stained histologically captured images were imported to Image J software for cell density calculation and meniscal mineralisation measurements. Each cell nuclei were counted using a multipoint tool in ImageJ software for cell density calculation. A dragging tool measured meniscal mineralisation (perimeter, length, and area).

3.4.3 Microsoft Excel

All data were then recorded and tabulated in Microsoft Excel.

3.5 Statistical Analysis

Statistical analysis was performed by using GraphPad Prism version 10. Data were expressed as mean and standard deviation. D' Agostino-Pearson omnibus normality test was performed to determine the normal distribution of the recorded data. Two-tailed, one-way ANOVA was used to evaluate the comparison of the histological scores of the normal, OA menisci and OA-MM menisci. In addition, two-tailed, one-way ANOVA was conducted to assess the cellular density differences among normal, OA and OA-MM menisci. Besides, Spearman's correlation test was done to determine the correlation between meniscal mineralisation and meniscal degeneration. Correlation coefficient (r_s) results were interpreted according to a modified categorisation of that used by Dancey and Reidy (2004) (Table 3.2). All histomorphometrical data were expressed as mean and standard deviation (SD). $P<0.05$ was considered as statistically significant.

Spearman's correlation coefficient (r_s)	**Descriptions**
0.0	No correlation
0.10 - 0.20	Slight correlation
0.21 - 0.40	Fair correlation
0.41 - 0.70	Moderate correlation
0.71 - 0.80	Good correlation
0.81 - 0.99	Almost perfect
1.0	Perfect correlation

Table 3.2: Interpretation of Spearman's correlation coefficient.

CHAPTER 4

RESULTS

4.1 Histological evaluation of feline OA menisci

A total of 38 histological slides from 38 meniscal archived samples were evaluated. Meniscal samples were categorised into normal, osteoarthritic (OA) and osteoarthritic with meniscal mineralisation (OA-MM). In the present study, OA and OA-MM showed higher histological total scores than normal meniscal tissue (Figure 4.1). However, the difference between the histological total score of OA and OA-MM was not statistically significant ($P>0.05$). Meanwhile, the histological total scores were significantly lower in normal menisci compared to OA and OA-MM menisci ($P<0.01$) (Figure 4.1).

Figure 4.2 shows a representative micrograph of the tissue surface and collagen fiber organisation of normal and OA menisci. A normal meniscus showed a smooth tissue surface and well-organised collagen fibres. Conversely, OA menisci exhibit varying degrees of fibrillation, disruption on their tissue surfaces, and disorganisation of the collagen fibres. Besides, various red shades of safranin O intensity can also be observed in OA menisci, as shown in Figure 4.3.

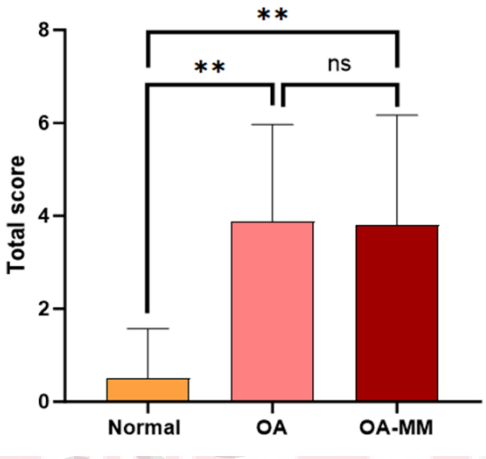


Figure 4.1: Showing comparison of the histological total scores of the OA menisci, OA-MM menisci and normal menisci. The histological scores were significantly higher in OA and OA-MM menisci than in normal menisci. (One-way ANOVA, **represents P<0.01; ns: not significant P>0.05).

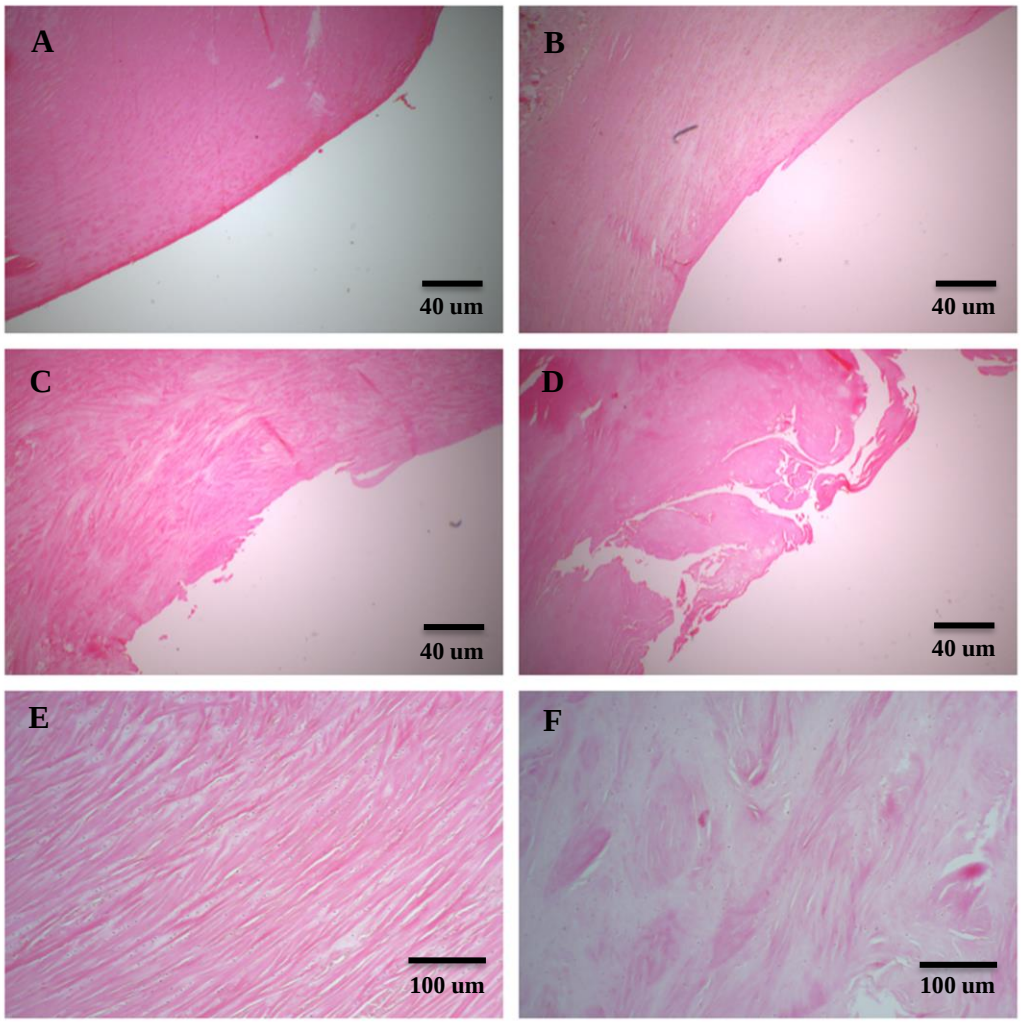


Figure 4.2: Representative micrographs of the OA and normal menisci. A-D; Tissue surface of menisci (X40): A – Normal; B – Slight fibrillation; C – Moderate fibrillation and undulating; D – Severe fibrillation with disruption. E-F; Collagen fiber organization (X100): E – Well organized collagen bundles; F – Disorganisation of the collagen bundles.

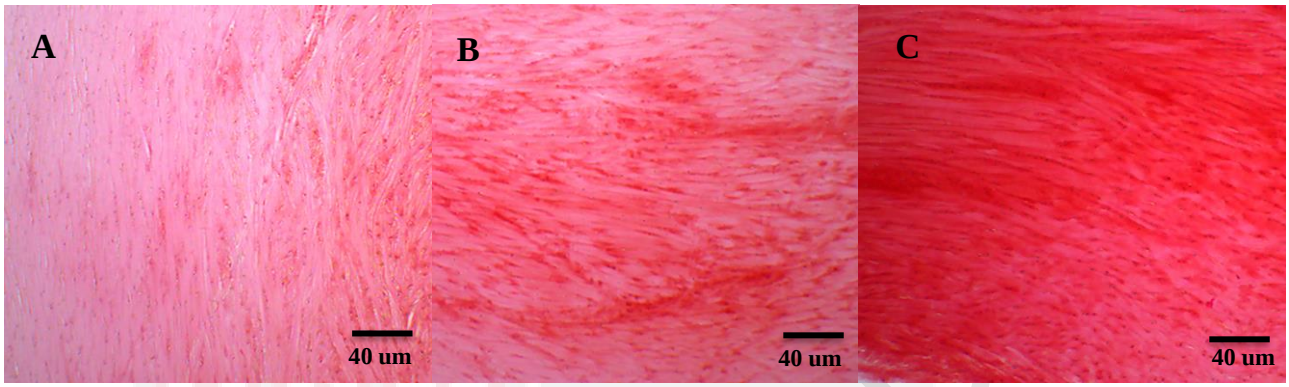


Figure 4.3: Safranin O intensity in OA menisci (X40) magnification. A – Slight staining; B – Moderate staining; C – Strong staining.

4.2 Histomorphometrical evaluation of feline OA menisci

4.2.1 Cellular density (cells/mm^2)

Cellular density across meniscus regions was compared among normal, OA and OA-MM menisci using one-way ANOVA, whereby the result was considered statistically significant only if $P<0.05$. As shown in Figure 4.4, OA meniscus tissue had the greatest cellular density (25.5±6.8 cells/mm^2), followed by normal (23.6±6.6 cells/mm^2) and OA-MM (18.9±3.8 cells/mm^2) meniscus. Aside from that, the results also showed no significant differences between normal meniscus and OA and OA-MM meniscus tissues in terms of cellular density ($P>0.05$). In contrast, the cellular density of OA-MM menisci was significantly lower than the OA menisci ($P<0.001$).

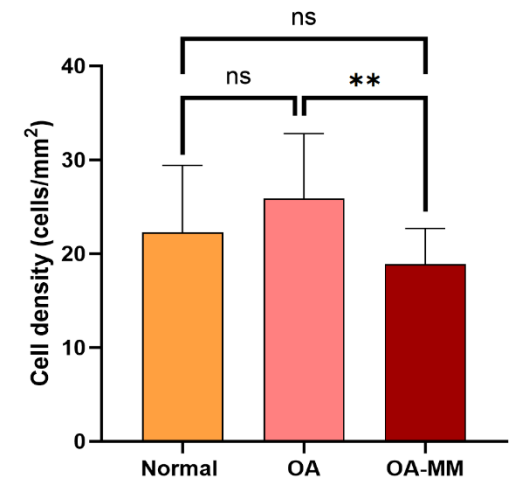


Figure 4.4: Cell density evaluations in OA, OA-MM and normal menisci. Data are compared using one-way ANOVA (**: P<0.001 was considered significant; ns: not significant).

4.2.2 Meniscal mineralisation size

Sizes of meniscus mineralisation were measured, including the perimeter (mm), length (mm), and area (mm^2). The mean±SD of the MM perimeter, length and area were 2.79±1.86 mm, 0.99±0.64 mm and 0.31±0.22 mm^2, respectively (Table 4.1). The Spearman's correlation test was performed to determine the correlation between meniscal mineralisation size and degeneration. There was a significant moderate positive relationship between meniscal degenerative score and MM perimeter (r_s =0.61, P<0.05), length (r_s =0.68, P<0.05) and area (r_s =0.67, P<0.05) (Figure 4.5).

	Meniscal mineralisation		
	Perimeter (mm)	Length (mm)	Area (mm^2)
Mean ± SD	2.79 ±1.86	0.99 ± 0.64	0.31 ± 0.22
Range	1.26 - 8.96	0.43 - 2.71	0.08 - 0.84

Table 4.1: The meniscal mineralisation size was measured according to the perimeter (mm), length (mm) and area (mm^2).

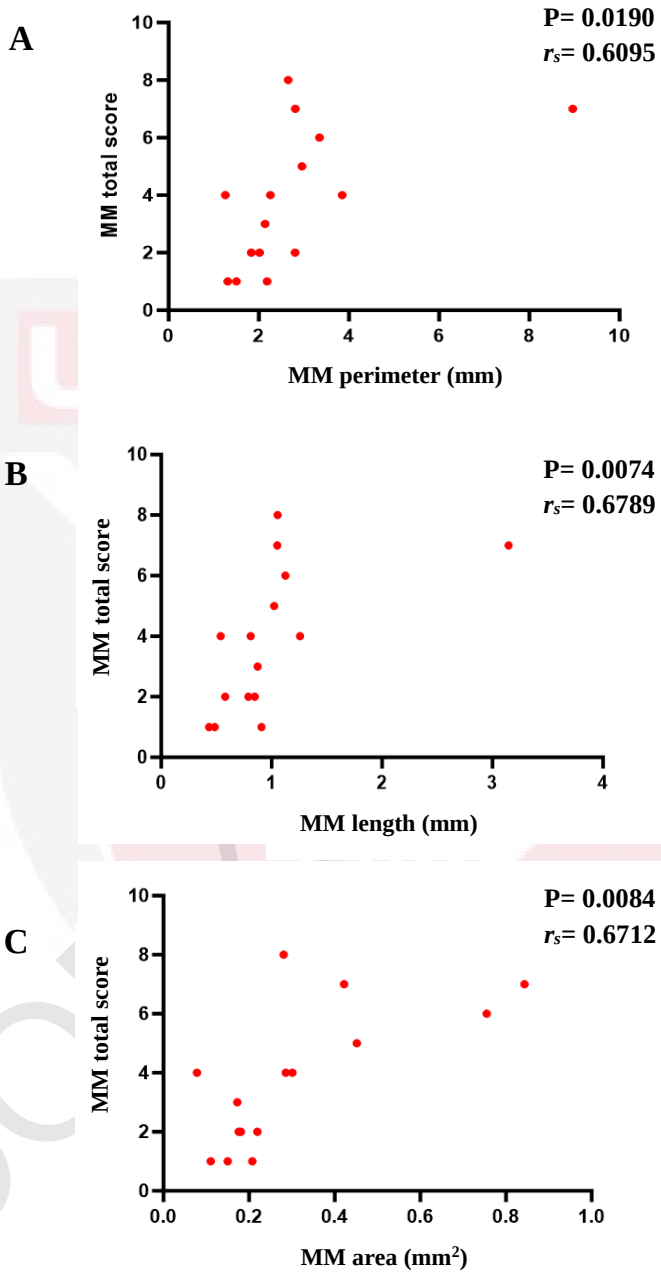


Figure 4.5: Correlation analysis of total meniscal degenerative scores and meniscal mineralisation sizes. (a, b & c) A significant moderate positive relationship between meniscal degenerative total scores and meniscal mineralisation sizes (perimeter, length and area).

CHAPTER 5

DISCUSSION

5.1 Histological evaluation of feline OA menisci

The results of the present study demonstrated the presence of degenerative changes in OA and OA-MM menisci. The total degenerative score of OA and OA-MM menisci was significantly higher than that of normal menisci. Osteoarthritis is a common condition that affects the stifle joint, with meniscal damage being one of its major complications. The menisci become fragmented or degenerated, further exacerbating the degenerative changes in the stifle joint. This finding is consistent with a study conducted in human and animal models of O.A. Krupkova et al. (2018) mentioned that menisci taken from arthritic joints possess higher degenerative scores. Besides, there is also a significant increase in the degenerative score of the medial menisci of the OA porcine stifle joint compared to the control group (Kreinest et al., 2016). Aside from that, menisci that were taken from ageing and arthritic human joints possess higher degenerative scores (Pauli et al., 2011).

The current study examined the microscopic features of normal and osteoarthritic meniscal tissue, as depicted in Figure 4.2, to highlight the distinct characteristics differentiating degenerated osteoarthritic meniscus from healthy meniscus. One common finding was the thinning and fraying of the meniscus. The meniscus is composed of fibrocartilage, which undergoes degradation with the progression of OA. As a result, the

meniscus becomes weaker and more susceptible to tears or ruptures (Sun et al. 2012). These defects in the meniscus can lead to increased joint pain and instability, further exacerbating the severity of OA.

Osteoarthritis menisci exhibited a significant influence on the staining intensity of Safranin O, with Safranin O staining appearing more intense in OA menisci. The increased staining intensity can be attributed to increased proteoglycan content within the OA menisci. Proteoglycans, which are complex chains of protein and sugar molecules, play a crucial role in the structure and integrity of the cartilage matrix (Pordzik et al., 2020). They bind to water within the matrix, providing lubrication and cushioning during joint movement. In OA menisci, increased proteoglycan content suggests that there may be an attempt to compensate for the loss of cartilage by increasing the production of matrix components (Monnibi et al., 2020).

5.2 Histomorphometrical evaluation of feline OA menisci

5.2.1 Cellular density

The histomorphometrical analysis conducted in this study revealed a significant difference in cellular density between OA and OA-MM menisci. However, no significant differences were observed between normal and OA menisci. However, despite structural changes in OA menisci, previous studies found that cell density did not differ significantly from normal menisci. These findings are in agreement with human studies reported by

Katsuragawa et al. (2010) and Battistelli et al. (2019), where no reduction in cell density was observed among normal, osteoarthritic, or injured menisci. On the other hand, Lopez-Franco et al. (2016) reported a significant decrease in meniscal cell count in OA-affected menisci compared to those younger individuals without O.A. Pauli et al. (2011) also mentioned a decline in cellularity in OA menisci, which was not observed in non-OA menisci from elderly individuals.

The cellular density was notably reduced in OA with meniscal mineralisation. This phenomenon can be explained by the stiffening effect within the meniscus tissue when mineralisation occurs. When calcium deposits accumulate within the meniscus, they lead to a hardening of the tissue (Sun et al., 2012). As a result, the viable cells (fibrochondrocytes) within the affected region are significantly reduced. The reduced number of viable cells in calcified areas of the meniscus can be attributed to several factors. Firstly, the stiffness and increased rigidity caused by mineralisation hinder the normal cellular activities and migration patterns of the fibrochondrocytes. The altered biomechanical environment (e.g. mineralisation) may interfere with cellular metabolism, reducing cell number (Bajpai et al., 2020). Additionally, calcified deposits can alter the cellular signalling mechanisms that regulate cell proliferation and differentiation. The presence of mineralisation can lead to the inhibition of growth factors and cytokines that promote cellular growth (Proudfoot, 2019), further contributing to the reduction in cellular density.

On the other hand, it is important to note that no published study currently specifically examines the quantification of cellular density in feline osteoarthritic menisci with meniscus mineralisation. Further research is necessary to understand the cellular changes in this feline model of OA comprehensively. By quantifying the cellular density in feline osteoarthritic menisci with meniscus mineralisation, scientists can further explore the mechanisms behind this phenomenon and contribute to developing targeted therapeutic interventions.

5.2.2 Meniscal mineralisation size

Spearman's correlation coefficient test was performed to determine the correlation between meniscal mineralisation size and degenerative changes. The results revealed a moderate positive correlation between the total histological scores and meniscal mineralisation size (perimeter, length and area). This finding indicates that high histological scores were correlated with meniscal mineralisation size. Meniscal mineralisation is the process by which fibrocartilage tissue undergoes chondro-osseous transdifferentiation, resulting in mineral deposition (Freire et al., 2010). This process leads to trabecular bone and bone marrow formation within the menisci. Meniscal mineralisation has been linked to mechanical stress caused by gait abnormalities or abnormal hind limb conformation, which could be caused by the pain of OA (Ariffin, 2015). The significance of meniscal mineralisation lies in its potential impact on the structure and function of the meniscus. The presence of mineralised tissue can disrupt the biomechanical properties of the meniscus, impairing its role as a shock absorber and

stabiliser in the stifle joint. When the size of the mineralisation becomes excessively large, it can have detrimental effects on the meniscus's function and structural integrity. Results from this study indicate that the size of the mineralisation in the meniscus is directly associated with the severity of the meniscal degeneration. This suggests that the size of the mineralisation can be used as a reliable indicator of the advanced stage of degeneration.

CHAPTER 6

CONCLUSION AND RECOMMENDATION

In conclusion, the osteoarthritic (OA) menisci exhibited poor tissue integrity, disorganised collagen fiber organisation, and high safranin O intensity compared to normal menisci. Furthermore, only OA-MM demonstrated low cell density. Additionally, meniscal degeneration was found to be associated with meniscal mineralisation size. In terms of recommendations, further investigation can be conducted to validate the histological grading score. This score should include interobserver and intraobserver studies to ensure reliability and validity. Additionally, future studies can be conducted to compare the expression of matrix genes between OA and control menisci. By examining the differences in gene expression, valuable insights can be gained into the molecular mechanisms underlying meniscal degeneration in OA.

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