COMPREHENSIVE CHARACTERIZATION OF CANINE MENISCAL PATHOLOGY

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Master of Science

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by
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The undersigned, appointed by the Dean of the Graduate School, have examined the thesis entitled

COMPREHENSIVE CHARACTERIZATION
OF CANINE MENISCAL PATHOLOGY

Presented by Jill Kristine Luther,

A candidate for the degree of Master of Science,

And hereby certify that in their opinion it is worthy of acceptance.

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DEDICATION

Some Very Special People

I want to thank you, Lord, for some special people that I love,
Special people who love me just because I'm me,
People who believe that I'm important, as I am,
People who can stand me even when I'm sour and disgusting.
People who listen when I spit out my feelings,
People who wait when I cannot find the words,
People who shake me when my spirit falls asleep.

For all those very special people
I want to shout
And shout and shout with thanks.

Those are the people, one today, one tomorrow,
Who look for that part of me that's me,
Who groan with me until that part of me is free,
Who will love whatever is left of me when the day is over.

For all those very special people
I want to sing
and sing and sing with love.

For ones like that, Lord, mean more to me
than anything on earth, and sometimes even more than you.
For only through someone like that do I believe that
you are really true.
For when someone like that
accepts me in my sorry little mess,
Then so do you, my Lord,
Then so do you...

Norman Habel

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With sincere gratitude, Jill

~We make a living by what we get, we make a life by what we give~ Winston Churchill

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COMPREHENSIVE CHARACTERIZATION OF CANINE MENISCAL PATHOLOGY

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ABSTRACT

Meniscal injury is one of the most common causes of pain and dysfunction in the human knee and canine stifle joint. In the canine patient, meniscal injury is usually secondary to cranial cruciate ligament rupture, and the resulting instability in the joint. Despite the prevalence of meniscal disease, the literature contains relatively few reports addressing mechanisms of disease for cranial cruciate ligament-associated meniscal injury. Diagnosis of meniscal tears can be challenging based on clinical signs and history alone, and diagnostic tests to confirm tearing can be expensive, invasive, and unavailable in some areas. Additionally, controversy remains regarding standard of care for treatment of the canine meniscus. Therefore, our overall line of research was to comprehensively characterize canine meniscal pathology with focus on three areas: 1) comparison of clinical and bench top measures of meniscal pathology in early meniscal disease, 2) comparison of diagnostic modalities for pathology of the caudal portion of the medial meniscus, and 3) investigation of the effects of a commonly utilized treatment of the medial meniscus.

CHAPTER 1

CANINE MENISCUS

1. Meniscal anatomy, ultrastructure and function

The menisci are paired fibrocartilages interposed between the tibial plateau and femoral condyles. They are C-shaped when viewed dorsally and wedged-shaped when viewed in cross section. The abaxial rims of the menisci are deep, convex, and attached to the joint capsule, while the axial edges are thin, concave, and free (Figure 1).[1] The lateral meniscus is slightly thicker and forms a greater arc than the medial meniscus. The menisci can be divided into three main anatomic regions: a body and cranial and caudal horns. The menisci are attached to bone by six ligaments: the craniolateral, craniomedial, caudolateral, and caudomedial meniscotibial ligaments, the attachment of the meniscal horns to the tibia; the intermeniscal ligament, traversing between the menisci cranially; and the meniscofemoral ligament of the lateral meniscus, the only attachment of the menisci to the femur.[2] The medial meniscus has an additional attachment to the collateral ligament, which the lateral meniscus is lacking.[3] The peripheral one third of the meniscus is vascular, supplied by the lateral and medial distal genicular arteries.[3,4] The innervation to the menisci is supplied by nerves from the perimeniscal tissue; the nerves penetrate approximately the peripheral one third of the menisci. There is a greater concentration of nerve fibers and sensory receptors in the meniscal horns than in the body.[5,6]

The canine meniscus is composed of 63-65% water.[7,8] The remaining dry matter is largely made up of collagen (60-80%), predominantly collagen type I, which provides the structural scaffolding, and to a much lesser extent (less than 2% each of total collagen content), collagen types II, III, V, and VI.[9-11] Collagen fiber bundles are arranged in a distinct pattern that is integral to meniscal function. The surface fibers are arranged radially, while the deeper fibers are arranged circumferentially and are continuous with the cranial and caudal meniscotibial ligaments. There are also radial fibers scattered throughout the deep zone weaving throughout

the circumferential fibers.[12,13] This fiber arrangement allows for the weight-bearing axial (compression) forces to be transferred into radial forces and dispersed along the circumferential fibers, which is known as the "hoop stress" mechanism. The remaining extracellular matrix is comprised of proteoglycans and glycoproteins, although only about 10% of that of hyaline cartilage. Proteoglycans consist of a protein core with multiple glycosaminoglycan units covalently attached. These large, hydrophilic molecules interact with the collagen fibers to form a strong meshwork that is resistant to tension, compression and shear stresses.[13] The canine meniscal glycosaminoglycan concentration has been elucidated and varies from that of the human meniscus. The canine meniscus consists of 60% chondroitin 6-sulphate, 25% chondroitin 4-sulfate, 10% chondroitin, and 5% dermatan sulfate.[7] Hyaluronic acid makes up about 6-7% of the total uronic acid of the canine meniscus.[7] Minute amounts of glycoproteins are also found in the extracellular matrix including elastin (< 1%), potentially to aid in maintaining the shape of the meniscus, and adhesion molecules such as thrombospondin and fibronectin.[10] Type VI collagen also falls under the description of a glycoprotein, due to its dumbbell structure and the lack of crosslinks seen in the fibrillar collagens, although the specific function of the molecule has not been well-described.[10,14] Fibrochondrocytes make up the cellular component of the meniscus; they secrete the fibrocartilagenous matrix, reside within lacunae, and are round to oval in shape depending on the location within the tissue.[15,16]

Many studies evaluating the biochemical and ultrastructural meniscal composition have revealed regional differences within the meniscus.[7,8,17-24] Regional differences in gene expression of the meniscus have also been discovered.[25,26] A pervasive finding is that the inner, or axial, portion of the meniscus has a composition closer to that of hyaline cartilage, as indicated by increased glycosaminoglycan content (aggrecan, specifically) and increased collagen type II content. Additionally, the peripheral, or abaxial portion of the meniscus has shown, in comparison to the inner portion, increased lipid content and decreased water content;[22] increased overall collagen content and decreased GAG content;[23] increased mRNA expression for collagen

I, matrix metalloproteinase (MMP)-2, and MMP-3;[25] and increased collagen synthesis in both normal and degenerative medial menisci.[21] A study investigating the meniscotibial insertional regions of the menisci showed both types I and II collagen were localized in these regions, which is presumably in response to tensile forces as well as compressive and shear forces.[18] In this same study type X collagen was only localized to the fibrocartilagenous zone of the meniscotibial ligament insertion site, perhaps indicating its role in maintaining calcification. Two separate studies have shown regional variations within the central portion (in reference to cranial, central, and caudal regions) of the canine medial meniscus. Adams et al showed a decrease in uronic acid while Stephan et al showed decreased water and GAG content within the central medial meniscus versus the same region in the lateral meniscus.[7,8]

There have been regional differences demonstrated in cellular morphology as well. Ghadially has characterized both human and rabbit meniscal cellular composition.[15,27] Both species demonstrated differences in cell morphology based on a superficial zone (closest to the articular surfaces) and a deep zone. The cells of the superficial zone were more oval or fusiform with short cellular projections and those of the deep zone were rounded or polygonal with elaborate cellular processes. Additionally, the author described elongated or spindle-shaped fibroblasts near the periphery where the menisci joined with the joint capsule as well as cells throughout all zones that appeared to be intermediate between fibroblasts and chondrocytes. More recently, further work on the lapine meniscus utilizing immunohistochemical staining discovered four distinct cell types: two in the abaxial region, one in the axial region, and one along the margins of the superficial tissue.[16] This study supports the idea that the differences in cellular phenotype are necessary to produce and maintain the unique structure and morphology of the tissue to allow the menisci to perform their required functions.

The functions of the meniscus include load bearing and distribution, increased joint congruency, joint stability, shock absorption, joint lubrication, and proprioception.[13,28,29] Approximately 65% of the weight bearing of the tibial plateau is transmitted through the

menisci.[30] This is achieved largely due to the collagen fibers – their complex arrangement and tensile stiffness and strength. The menisci serve to increase congruency through the incongruent femorotibial joint, thereby providing a protective effect to the articular cartilage.[31] Additionally, the increased congruency of the joint equates to increased joint stability; the menisci have been proven to be secondary stabilizers of the stifle joint.[32,33] Meniscal tissue has been shown to have low compressive stiffness and permeability when compared to hyaline cartilage, allowing the meniscus to dissipate and absorb energy, functioning as an efficient shock absorber.[13] The menisci also serve in providing joint lubrication and synovial fluid distribution. Meniscal fibrocartilage has been described as biphasic, having a fluid phase and a solid phase.[34] With compressive forces, the fluid phase, consisting mostly of water, is forced to flow through the solid matrix of collagen, proteoglycans, and proteins, and exuded out into the joint. Once the compression subsides, the fluid is imbibed back into the meniscal tissue.[13] This cycle of fluid flow into and out of the meniscal interstitium provides cell nourishment and joint lubrication to reduce friction. Finally, the menisci have a role in proprioception. Histology of the human meniscus identified axons in the perimeniscal tissue that penetrated into the peripheral 2/3 of the meniscus and were most concentrated at the horns.[35] These findings indicated that the meniscus is capable of afferent input to the central nervous system, which has importance for the biomechanical function of the joint.

2. Meniscal pathology and healing potential

Meniscal injury is one of the most common causes of pain and dysfunction of the stifle joint in the canine.[36,37] Both medial and lateral meniscal tears have been reported; however, the medial meniscus is much more commonly affected with clinically relevant pathology.[1,37-41] While human beings commonly experience primary meniscal injuries, dogs and people frequently incur meniscal tears secondary to rupture of the cranial cruciate ligament (CCL) or anterior cruciate ligament (ACL), respectively. Approximately 40-80% of ACL tears in people and 20-77% of CCL tears in dogs will have associated meniscal lesions at the time of index surgery.[1,36,37,42,43]

Canine meniscal lesions have been divided into five major categories of tear configuration: vertical longitudinal, radial, horizontal, flap and complex.[44,45] The most common type of meniscal injury seen in the canine is a bucket handle tear, or a variation of the longitudinal tear in which the torn portion is displaced axially (Figure 2).[1,37,46]

The etiology of canine meniscal disease is largely due to the altered biomechanics of the CCL deficient stifle joint. Research on both the human knee and the canine stifle have illustrated the secondary stabilizing effect of the medial meniscus which, in the absence of the CCL, becomes a primary stabilizer of the joint.[32,33] The caudal aspect of the medial meniscus acts as a wedge between the femur and the cranially advancing tibia during weight bearing, thus increasing the load transmission and shear forces on this region of the meniscus and predisposing it to injury.[33,47,48] The firm attachments of the medial meniscus to the tibia via the caudal meniscotibial ligament and the medial collateral ligament cause a tethering effect, whereas the lateral meniscus has more mobility. Other work has suggested that it is likely the excess internal rotation seen with CCL ruptures that causes the crushing effect and eventual tearing of the medial meniscus, rather than the cranial caudal instability.[46] The contributions of biologic factors must also be considered in meniscal disease. Meniscal cells have been shown to be exquisitely sensitive to factors in the microenvironment such as inflammatory cytokines, and are also strongly influenced by the mechanical environment.[49-52] Changes in the intraarticular environment cause the meniscus to respond by changes in its own metabolic activity, alterations in the biochemical composition, and production of detrimental cytokines, which could predispose the tissue to degeneration or tearing.

Few studies have shown significant risk factors for development of meniscal pathology in the CCL deficient canine stifle. A 2002 study by Necas et al demonstrated increased risk for meniscal damage in CCL deficient dogs weighing 25-45 kilograms (kg) compared with dogs weighing less than 24kg; however, this study did not take into account body condition score.[53] Obesity is a known risk factor in human beings for meniscal damage.[54] It is hypothesized that an

increased body mass index in people increases the strain and torque in the knee joint and blood supply is limited to these menisci through vascular compression or association with cardiovascular risk factors. No similar association with body condition scoring in dogs has been investigated. A study by Jandi et al found associations between the extent and duration of CCL rupture and meniscal injury in the clinical canine patient: chronic CCL ruptures and complete tears of the CCL were more likely to have meniscal damage than more acute ruptures or partial CCL tears.[55] Additionally, in a separate study, Jandi et al found that remaining stifle instability following stifle stabilization surgery increased the risk of meniscal injury.[56]

Meniscal tissue has some healing capacity, but this is limited to the vascular abaxial portion of the meniscus. Lesions of the vascular region may heal within about 10 weeks experimentally; however, it is unclear when functional healing takes place.[57,58] A remodeling process can occur in the avascular portion of the meniscal tissue to partially restore the missing portion with fibrous tissue.[59-62] This remodeled tissue has been shown to be functionally inferior.[63,64] Both the vascular portion of the meniscus and the surrounding synovial vasculature have been shown to contribute to the reparative processes.[60] However, the most common location for meniscal tears to occur is in the avascular portion in both the canine and human patient, making successful restoration of function in the torn meniscus less likely.[37,46,65]

3. Diagnosis and treatment of canine meniscal pathology

Meniscal pathology in the canine patient occurs secondary to a CCL tear prior to surgical treatment or as a sequela to pathologic progression and/or altered kinematics following stifle surgery. Stifle exploration via arthrotomy or arthroscopy at the time of initial surgical treatment of CCL deficiency is considered the current standard-of-care diagnostic method for meniscal pathology, as this allows for both visualization and palpation of the menisci as well as concurrent treatment. However, the diagnosis of subsequent meniscal tears often presents a challenge. History and physical examination findings that may raise the index of suspicion for meniscal injury include acute onset of a severe lameness on the previously operated limb, a meniscal click

heard and palpated on flexion and extension of the stifle, and joint effusion.[66] However, in many cases a diagnosis of meniscal injury is not clear cut and advanced imaging is necessary. The reported diagnostic techniques for the canine meniscus include weight-bearing radiographs, ultrasonography, computed tomography (CT), magnetic resonance imaging (MRI), and arthroscopy.

Weight-bearing radiography of the knee joint is commonly used in human medicine to follow the progression of osteoarthritis, and an association has been found between the joint space width and the presence of meniscal injury.[67] Weight-bearing radiography is uncommonly used in veterinary medicine due to the challenge of maintaining proper positioning in the standing, non-sedated animal.[68] Necas et al investigated a non-weight bearing stressed tibia craniocaudal projection for detecting medial joint collapse in the sedated dog. The study reported a sensitivity of 52% and specificity of 71% for the diagnosis of medial meniscal damage based on medial joint collapse.[53] A recent study sought to determine the usefulness of joint space measurements in the non-weight bearing non stressed radiographic views of the canine cadaveric limb and found that these views were limited in value based on the variation in joint space width between specimens.[69]

The diagnostic value of ultrasonography for meniscal pathology has been studied for both clinical and research purposes in several species including human beings, dogs, horses, and rabbits.[36,70-75] Although most recent reports in the literature cite sensitivities and specificities greater than 80% for ultrasound (Table 1), there is still some controversy over the accuracy of this technique.[76] The advantages to ultrasound include the non-invasiveness, low cost, ability to scan multiple joints, ability to repeat the scan to monitor progression of disease, and ability to perform a dynamic investigation.[77] In veterinary medicine, an additional advantage can include the portability of the ultrasound machine and the ability to perform the examination with only light sedation or manual restraint. The technique does require technical skill for obtaining and interpreting the images, which may play a role in the discrepancies in the accuracy seen in the

medical literature and the lack of widespread use of ultrasound in clinical veterinary practice to date. An additional limitation is that individual ultrasound images represent a portion of the complete cross-sectional anatomy.[78] Also, the ultrasound beam cannot penetrate mineral-containing structures very well, and thus, does not give information regarding any pathology of the bone.[78] Finally, the joint space is likely too narrow to comprehensively image the meniscus of small dogs.[78]

Computed tomographic arthrography is a technique that has been reported to be less operator dependent than ultrasoundwith respect to obtaining and interpreting meniscal imaging.[79] Accuracy for diagnosis of meniscal injury is generally over 75%, as reported from both cadaveric and clinical studies (Table 2) and the technique has been used clinically in human and canine patients.[79-81] Ionic contrast material is injected intra-articularly and the knee joint is put through full range of motion to distribute the contrast material throughout the joint.[79,82] This distribution is often checked via radiography or fluoroscopy in the human patient. Three dimensional CT has also been reported and allows for evaluation of the ligamentous structures of the knee joint in people.[83] Three dimensional CT arthrography has a reported sensitivity and specificity of over 98% for medial meniscal pathology in one study.[84] The advantages of CT (arthrography or 3D) over ultrasound include high spatial resolution, high contrast resolution, diagnostic ability for the tear configuration, and multiplanar capability of study.[82] Disadvantages to CT arthrography include the invasiveness of the contrast injection, artifacts created by metallic implants, patient exposure to ionizing radiation, need for general anesthesia, and cost compared to ultrasound. Additionally, the availability of the equipment can hinder its use. The plane of image appears to be important, as dorsal plane images have shown greater accuracy than images in the transverse plane in the canine.[79,85]

Magnetic resonance imaging has proven to be a highly accurate diagnostic tool for human meniscal pathology with recently reported accuracy of over 85%. Two systematic reviews of the accuracy of MRI for diagnosis of meniscal pathology in the human literature have reported

sensitivities of 91.4%[86] and 93.3%[87] and specificities of 88.1%[86] and 88.4%.[87] MRI is considered the gold standard for noninvasive imaging of the human knee joint, but the technique is less prevalent in veterinary medicine due to cost and availability. While the CCL transected canine model has been used in many studies evaluating various aspects of osteoarthritis research, there are few studies using MRI to study these joints in vivo.[88-91] Additionally, there are only three studies in dogs reporting the accuracy of MRI for diagnosis of meniscal pathology with sensitivities ranging from 64-100% (Table 3).[92-94] A recent comparison of the use of sonography, CT, and MRI of the normal canine stifle concluded that MRI allows for the best imaging of the stifle intra-articular structures due to the high resolution of the imaging modality.[95] While MRI has proven superior for soft tissue structure imaging to other modalities, the technique is cost prohibitive, technically demanding, requires general anesthesia, and uncommonly used in the clinical canine patient to date. Similar to CT, metallic implants can distort the images, although non-ferrous implants have been shown to have very little effect.[92] MRI is less sensitive to calcium than CT, and is therefore, typically not the imaging modality of choice for boney imaging.[96] Additionally, the size of the joint and patient play a role in the ability to obtain diagnostic images; small dogs may not be ideal candidates for stifle MRI.[89] MR arthrography is used in humans for diagnosis of post-operative meniscal tears and the technique has been described in dogs, but is considered more invasive due to the intra-articular contrast injection.[97]

Arthroscopy has been the standard of care for assessment of the menisci for at least three decades and is the reference standard for most imaging studies in medicine and veterinary medicine.[36,89,93,98-101] At the time of stifle stabilization surgery in the canine patient, arthroscopy has been shown to be superior to arthrotomy due to the detrimental effects of arthrotomy on the overall health and function of the joint, as well as the improved diagnostic accuracy of arthroscopy when compared to arthrotomy.[102,103] Another advantage of arthroscopy over arthrotomy for diagnosis is the magnification that allows for increased visualization of small defects in the articular cartilage and menisci.[37] An advantage of arthroscopy over advanced

imaging techniques that cannot be overstated is the ability to both diagnose and treat meniscal pathology using the same modality, saving the client money, and the patient time under general anesthesia.

The sensitivity and specificity for diagnosis of meniscal pathology were reported in a canine *ex vivo* study for arthrotomy and arthroscopy.[103] The sensitivity and specificity were 80% and 95%, respectively, for arthroscopy and 37% and 84% for a craniomedial arthrotomy. A clinical report of arthroscopy and arthrotomy for stifle evaluation prior to stifle stabilization showed a 3.8% subsequent tear rate for patients undergoing arthroscopy and 5.1-6.8% subsequent tear rate for patients undergoing arthrotomy.[104] It was speculated that perhaps these subsequent tears were actually tears missed at the time of initial stifle arthrotomy. The less invasive nature of arthroscopy versus arthrotomy has allowed for second look arthroscopic procedures in the canine patient, which has helped to advance the understanding of CCL disease, meniscal disease, and management of these conditions.[66,105] Contrary to these reports, there is at least one persistent report in the literature of improved outcomes with arthrotomy over arthroscopy based on decreased lameness scores six months post-operatively.[106] The author of that study states a belief that the canine stifle joint may be too small for adequate evaluation of the entire meniscus. Disadvantages to arthroscopy are the need for advanced, expensive equipment and the learning curve associated with stifle arthroscopy.[45,106] Recent reports of novel joint distracters have allowed for improvements in visualization and treatment of the meniscus.[107,108]

Treatment of the torn meniscus by veterinary surgeons is quite variable. While surgeons remain who do not advocate assessment of intra-articular pathology, standard of care has moved toward joint assessment via arthrotomy or arthroscopy.[104,106,109-111] Current therapeutic and prophylactic strategies for meniscal injury include addressing CCL deficiency, meniscectomy, meniscal repair, and meniscal release (Figure 3).

To date there is no gold standard for treatment of canine CCL disease, but many stifle stabilization procedures are currently in use to address the biomechanical alterations associated

with CCL deficiency.[112-114] No procedure reported has eliminated the problems of progression of osteoarthritis or post-operative meniscal tearing with reported post-operative meniscal tear rates of 2.8% to 17.5%.[115,116] Some very early anectodal reports for the tibial plateau leveling osteotomy and tibial tuberosity advancement procedures described up to 100% medial meniscal tear rates when meniscal release was not performed.[117]

Based on the lack of vascularity, and therefore healing potential, in the axial 2/3 of the meniscus, tears in this region are often treated with meniscectomy.[118] While total meniscectomy was promoted in the past, the current paradigm is preservation of as much grossly normal meniscus as possible.[119] Partial meniscectomy with preservation of an intact peripheral rim has been shown to have less detrimental effects on the articular cartilage than total meniscectomy.[120,121] Techniques for meniscectomy have been described via both arthrotomy and arthroscopy and care must be taken during the procedure not to damage intra-articular structures.[119]

Meniscal repair is commonly performed on human beings, but few reports exist for repair on veterinary patients, other than in experimental models.[122] This is likely related to the configuration and location of the meniscal tears, differences in pathogenesis of meniscal disease, and economics of treatment, all which dictate standards of care. Recent work by Thieman et al has focused on the contact mechanics of different configurations of canine meniscal tears and different suture repair techniques.[44,123] Findings included evidence that longitudinal tears could be repaired rather than meniscectomized in order to restore contact mechanics of the stifle joint. However, previous *in vivo* work on the canine model suggests that healing may not be complete even in the vascular portion of the meniscus with primary repair of longitudinal tears.[124] Experimental work utilizing bioabsorbable conduits for avascular region tears in the canine meniscus has shown promise for promotion of healing, but has not been employed for use in the canine patient.[125]

Medial meniscal release (MR) is reported to be performed in conjunction with stabilization of CCL deficient stifles in an attempt to prevent subsequent meniscal injury due to remaining instability in cranial translation and internal rotation.[104,111,113,126,127] MR can be performed at the caudal horn-caudal meniscotibial ligament junction (caudal) or at mid-body (central), and can be done via full arthrotomy, mini-arthrotomy, or arthroscopy.[111,127] Meniscal release, whether caudal or central, completely disrupts the circumferential fibers and destroys the hoop stress mechanism critical to tissue and joint function.[13,28,32,47,63,128] Biologically, the meniscus will not undergo functional healing after meniscal release.[60,63,64,128-130] Biomechanically, MR causes deleterious alterations in joint load transmission[47] and joint stability[33] equivalent to meniscectomy. Clinical evidence suggests that MR may not be advantageous for preventing subsequent meniscal injury as intended.[104,131] In addition, an *ex vivo* study evaluating three open and arthroscopic approaches for performing meniscal release concluded that all three approaches evaluated were lacking in completeness and accuracy of release, and all caused injury to associated ligaments and/or articular cartilage.[111] Force plate analysis ≥ 4 months post Tibial Plateau Leveling Osteotomy (TPLO) showed no significant differences in limb function between meniscectomy and MR.[132] Similarly, no detectable differences in subjective clinical outcomes were seen when comparing dogs with MR, without MR, or undergoing partial meniscectomy in conjunction with TPLO treatment for CCL deficiency.[104]

4. Study purposes

Meniscal disease is an important orthopedic problem in both human and veterinary medicine. In 2005 1.32 billion dollars were spent on canine CCL disease in the United States alone.[133] Given the incidence of up to 77% of patients having associated meniscal injury, there is quite a financial impetus for improvement of knowledge of the pathophysiology of meniscal disease and options for prevention, diagnosis, and treatment of meniscal injuries. Human meniscal disease is also a very common orthopaedic problem, and much of the research that has

been done for it using animal models of cruciate ligament and meniscal disease may be extrapolated to our clinical veterinary patients. Likewise, comparative research on the canine as a model of disease may further the understanding of meniscal disease in the human and in other species such as the horse. Therefore, the overall objective for our of research was to comprehensively characterize canine meniscal pathology with focus on three areas: 1) comparison of clinical and bench top measures of meniscal pathology in early meniscal disease, 2) comparison of diagnostic modalities for pathology of the medial meniscus, and 3) investigation of the effects of a commonly utilized treatment of the medial meniscus.

CHAPTER 2

STUDY 1: CHARACTERIZATION OF CLINICAL, IMAGING, HISTOLOGIC, BIOCHEMICAL, AND MOLECULAR CHANGES IN THE MENISCUS OF THE CRANIAL CRUCIATE LIGAMENT DEFICIENT CANINE STIFLE

1. Experimental purpose and hypothesis

Despite the prevalence of meniscal disease, the literature contains relatively few reports addressing mechanisms of disease for cranial cruciate ligament-associated meniscal injury. While reports do exists on the biomechanical, biochemical, structural, molecular, and histologic properties of the normal and pathologic meniscus, there is not one report that comprehensively characterizes all, or even most, of these entities, as well as correlating them to practical, clinical measures of disease. Therefore, the purpose of this study was to comprehensively characterize early meniscal pathology in the cranial cruciate ligament deficient canine stifle eight weeks post cranial cruciate ligament transection. Secondarily, we wanted to compare various clinical and basic science measures of disease. We hypothesized that there would be significant differences in all outcome variables among CrCL deficient dogs and the positive and negative controls.

2. Materials and Methods

a. Animals

Four adult mongrel dogs (mean weight 19.6 kg) were used in this study with approval from the Animal Care and Use Committee of the University of Missouri. Pre-operatively, the dogs were judged healthy and free of orthopaedic disease based on CBC, serum chemistry panel, heartworm test, radiographic examination of the hip and stifle joints, complete physical and orthopaedic examination, and clinical lameness evaluation. The dogs were housed individually in runs approved by the Association for Assessment and Accreditation of Laboratory Animal Care and fed a commercially available maintenance diet for the duration of the study.

b. Surgery

On the day of surgery the dogs were pre-medicated with xylazine [0.5 mg/kg intramuscularly (IM)], morphine (0.5 mg/kg IM) and atropine (0.04 mg/kg IM), anesthetized with thiopental [10-20 mg/kg intravenously (IV)], and maintained with isoflurane in oxygen. Cefazolin (22 mg/kg IV) was administered at induction and every 90 minutes until extubation. Using aseptic technique, a lateral parapatellar arthrotomy was performed on one randomly chosen stifle per dog. The stifles were inspected for evidence of pathology and the cranial cruciate ligament was transected (CrCLX group) using a number 11 blade in two dogs, the caudal cruciate ligament (CdCLX group) was transected in one dog, and in one dog a sham operation was performed in which the ligaments were palpated with a blunt probe rather than transected. The contralateral unoperated limbs served as internal controls. The dogs were recovered and administered post-operative analgesia with morphine [0.5mg/kg subcutaneously (SQ)] as needed for 48 hours. The dogs were allowed unrestricted use of the limbs and were made to walk on the affected limb each day.

c. MRI

Magnetic resonance imaging was performed on both stifles of one CrCLX dog and the sham-operated dog five weeks post-operatively. Scans were acquired with a 1.5T magnet (Symphony, Siemons). The dogs were pre-medicated with xylazine (0.5 mg/kg IM), butorphanol (0.5 mg/kg IM), and glycopyrrolate (0.005-0.01 mg/kg IM) and anesthesia was induced and maintained with pentobarbital (5-15 mg/kg IV). The following imaging protocols were performed: T2W 3D gradient recalled echo (GRE), 3D spoiled gradient with fat suppression (SPGR), T1W spin echo sequences, and delayed gadolinium-enhanced magnetic resonance imaging of cartilage (dGEMRIC). Sagittal and dorsal images were acquired and slice thickness was 4mm with 1.2mm spacing.

d. Lameness Evaluation

Eight weeks post-operatively clinical lameness scores were assigned to each dog by two veterinary orthopaedic surgeons blinded to the surgical treatment of the dogs. The scores were assigned based on visual examination of gait using a validated scoring system.[134] (Appendix 1) The dogs were then humanely euthanatized for further outcome measures.

e. Ultrasonographic evaluation

Sonographic examination (Logiq 500 MD, General Electric, Fairfield, CT; with a 12-14 MHz high-resolution probe) of both stifles of each dog was performed by one veterinary radiologist unaware of the surgical procedure undergone by each dog. The dogs were positioned in lateral recumbency with the limb of interest uppermost for ultrasound examination which included assessment for meniscal displacement, echogenicity, and shape as well as presence of joint effusion, synovial thickening, and osteophytosis.[36] Subjective evaluations were recorded; no statistical analysis was performed.

f. Gross and histologic evaluation

Both stifles of each dog were disarticulated, examined, and photographed. The menisci were harvested and subjectively scored for gross pathology (Appendix 2).
Each meniscus was divided into three anatomic regions: cranial, middle, and caudal. These regions were then sectioned into three portions for histologic, biochemical and molecular analyses. For the purpose of statistical analysis, the three anatomic regions were evaluated together.

For histologic assessment, 5-micron sections were cut from each sample and stained with hematoxylin and eosin (H&E) and toluidine blue. Each section was subjectively assessed by one investigator blinded to the sample group using a scoring system specifically for meniscal tissue based on three important categories: tissue architecture and tissue loss, cell and matrix (proteoglycan and collagen) content and morphology, and proliferative response (Appendix 3).

g. Biochemical analysis

i. The wet weight of the meniscal tissue was obtained immediately following tissue harvest and sectioning. The samples were then lyophilized and the dry weight was obtained. Water content of the menisci was calculated as follows: Water content (%) = [(Wet weight – Dry weight)/Wet weight] x 100. The lyophilized tissues were digested overnight at 65°C in 1 ml of papain digestion solution (0.3 mg/ml papain (14 U/mg), 20 m*M* sodium phosphate buffer containing, 1 m*M* EDTA, 2m*M* DTT). Digested tissues were stored at -20°C until subsequent biochemical assays were performed.

ii. Total sulfated GAG content was quantified using the dimethyl-methylene blue (DMMB) assay.[135] Total GAG content was determined by addition of 245µl of DMMB solution to a 5-µl aliquot of the digest solution and absorbance was measured at 530nm using a Synergy HT (Bio-TEK). Known concentrations of bovine tracheal chondroitin sulfate Ag were used to construct the standard curve. Results are reported as µg GAG/mg tissue dry weight.

iii. Total collagen content was determined using a colorimetric assay to measure the HP content.[136] The assay was modified to a 96-well format. A 50µl sample from the papain digested tissues was mixed with an equal volume of 4N sodium hydroxide in a 1.2ml deep-well 96-well polypropylene plate. The plate was covered with a silicon sealing mat, a polypropylene cover was placed on top of the mat, and the plates were stacked. The plates were sealed by compression with a C-Clamp, and autoclaved at 120°C for 20 min to hydrolyze the sample. Chloramine T reagent (450µl) was mixed with each sample, and incubated for 25 min at 25°C. Ehrlich aldehyde reagent (450µl) was mixed with each sample and incubated for 20 min at 65°C to develop the chromophore. Known concentrations of HP (Sigma, St. Louis, MO) were used to construct a standard curve. A portion (100µl) of each sample was transferred to a new 0.2ml 96-well plate, and absorbance read at 550nm using a Synergy HT (Bio-TEK). Values obtained were standardized to the dry weight of the meniscal explant and reported as µg HP/mg tissue dry weight.

iv. Total prostaglandin E2 ($PGE2_)$ content of the menisci was quantified using the ACE™ enzyme immunoassay system (Caymen Chemical, Ann Arbor, MI). The samples were run in duplicate in a 96 well plate and compared to a known concentration standard curve. The samples and standards were incubated with 50µl of goat polyclonal anti-mouse IgG antibody and 50µl of an acetylcholinesterase (AChE) -linked tracer. The plate was covered with a plastic film and incubated for 18 hours at 4ºC. Just before developing the plate, Ellman's Reagent was reconstituted with UltraPure water. The plate was rinsed with Wash Buffer and Ellman's Reagent was added to the wells. The plate was covered and allowed to develop in darkness while on an orbital shaker for 60-90 minutes. The absorbance was measured at a wavelength of 410nm. The results are reported as pg PGE2/ml of sample used for purification.

h. Gene expression analysis

Total RNA was extracted from snap frozen meniscal samples using the TRISpin method as described previously.[137] Snap frozen tissues were powdered, then homogenized in Trizol (Invitrogen, Carlsbad, CA) in an o-ring screw cap microcentrifuge tube using 1.0 mm diameter Zirconia Beads and a mini-bead beater (BioSpec Products, Bartlesville, OK), at 5,000 rpm for 2 minutes. Chloroform was added to the homogenates, the phases separated by centrifugation, and the upper aqueous phase was transferred to a new tube. Ethanol was added to a final concentration of 35%, and passed through a Minelute RNeasy minicolumn (Qiagen, Valencia, CA). The column was washed according to the manufacturer's protocol, and the RNA eluted off of the column with 14µl of RNase free water. RNA concentration was determined by UV spectrophotometry.

Total RNA (500ng) was converted to cDNA using random hexamer primers and the StrataScript™ RT enzyme (StrataGene, La Jolla, CA) according to the manufactures guidelines in a 20µl reaction. cDNA samples were diluted to 200µl with RNase free water to insure accurate pipetting, and stored at -20°C until used for Real-Time PCR analysis.

Real-Time PCR was performed with the Rotor-Gene RG-3000 (Corbett Research, Sydney, Australia) using the Quantitect SYBR® green PCR kit following the manufacturers guidelines. The PCR profile for all tests consisted of an initial incubation at 94°C for 15 minutes, followed by 55 cycles of 5 seconds at 94°C, 10 seconds at 57°C and 20 seconds at 72°C. After the PCR profile a melt curve analysis was done to ensure specific amplification for each sample. SYBR green fluorescence was monitored during each extension step of the PCR profile, take off values (Cts) and amplification efficiencies were determined using the comparative quantification setting in the Rotor-Gene software. Canine specific PCR primers were designed for each gene analyzed using canine sequence information found in Genbank. For genes without canine sequence available, degenerate primers were designed using sequence information from multiple species to amplify gene specific transcripts from canine tissue sources. The transcripts were sequenced, compared to Genbank using BLAST to ensure specificity, and canine specific PCR primers were designed using this sequence information. Each sample was tested in duplicate for structural matrix macromolecules (collagen types I, II, III and VI, aggrecan and decorin), matrix metalloproteinases (MMP-1, -2, -3 and -9), aggrecanases (ADAMTS 4, 5), tissue inhibitors of matrix metalloproteinases (TIMP-1, TIMP-2, and TIMP-3), inflammatory indicators [interleukin 1 beta (IL-1β), nitric oxide synthase (iNOS), cyclooxygenase 1 and 2 (COX-1 and COX-2)], and the house keeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

i. Statistical Analysis

All statistical analyses were performed using a computer software program (Sigma Stat®, San Rafael, CA). Data were pooled for the anatomic meniscal regions and comparisons were made between and within each operative, sham, and control group for the medial and lateral menisci. A one way ANOVA on ranks was used for statistical analysis and statistical significance was set at $p < 0.05$.

3. Results

a. MRI

There was no evidence of meniscal pathology on MRI images; however, articular cartilage proteoglycan content in dGEMRIC images of the medial femoral and tibial condyles was lower in the CrCLX stifles compared to all other groups (Figure 4).

b. Lameness evaluation

At eight weeks post operatively, the two CrCLX dogs were lame (scores 2+/-0.5 and 1.5 +/-0.5), while the CdCLX and sham-operated dogs were judged to be sound (0+/-0).

c. Ultrasonographic evaluation

Ultrasonographic evaluation revealed pathologic changes in the CrCLX stifles, with minimal to no changes seen in the CdCLX, sham or control stifles. The sham dog showed slight soft tissue thickening adjacent to the joint. The CdCLX stifle had mild joint effusion and minimal extracapsular soft tissue thickening. Changes in the two CrCLX stifles included increased joint fluid at the mid-body and caudal horn of the medial menisci, mild medial meniscal enlargement and displacement, and extracapsular soft tissue thickening (Figure 5).

d. Gross and histologic evaluation

No tears or other gross pathology were noted for either meniscus in any stifle. Histologically, CrCLX menisci had higher proliferation scores compared to CdCLX, sham and control groups, which approached statistical significance ($p=0.06$).

e. Biochemical analysis

i. The mean total water content for all meniscal sections was 62.47%. The means for medial and lateral menisci were 64.34% and 60.60%, respectively. The water contents for the treatment groups are listed in Table 4. There was a statistically significant increase of water content in the medial menisci of the CrCLX group when compared to the control group ($p=0.011$).

ii. The total sulfated glycosaminoglycan content of the menisci was not significantly different between groups or lateral and medial menisci. The mean values are listed in Table 5.

iii. The total collagen content of the menisci was not significantly different between groups or lateral and medial menisci. The mean values are listed in Table 6.

iv. The PGE2 content of medial menisci of all operated groups was significantly higher than that of the control group ($p=0.011$). Mean values are listed in Table 7.

f. Gene expression analysis

Analysis of gene expression in the menisci revealed several significant differences among groups when compared to controls including the following (Figures 6-11): **Medial** – upregulation of MMP-1 in CrCLX menisci, upregulation of COX-2 in CrCLX menisci, downregulation of TIMP-2 and ADAMTS-5 in the CrCLX menisci, and downregulation of MMP-3 and TIMP-3 in both CrCLX and CdCLX menisci. **Lateral** – upregulation of MMP-13 in the CrCLX menisci, upregulation of COL-6 in the CdCLX menisci, upregulation of MMP-1 and COL-3 in both CrCLX and CdCLX menisci, and downregulation of MMP-3 and TIMP-3 in the CdCLX menisci.

Changes were only considered significant in the CrCLX or CdCLX groups if they were not also significant in the sham operated group.

4. Discussion

Early meniscal pathology was detected in both medial and lateral menisci in this cranial cruciate ligament transection model based on sonographic, biochemical and molecular outcome measures. There were structural, inflammatory, and degradative changes in meniscal tissue that were neither detected grossly nor on MRI. These are novel and important findings with respect to diagnostics and prevention and treatment strategies in meniscal disease with immediate clinical ramifications.

While the CrCLX model of stifle osteoarthritis is commonly used in the research setting, there are few reports describing the associated effects on menisci. To the author's knowledge, there are no studies comprehensively assessing early meniscal pathology using both basic science and clinical measures of disease. Limitations of this study include the short time course of the study, the lack of multiple time points for the detection of temporal changes, and the very small

number of subjects utilized. Despite this, the outcomes are supported by previous work and do elucidate new data regarding pathophysiology of early meniscal disease.

Caudal cruciate ligament deficiency is rarely seen in the canine stifle, but can be a component of stifle derangement due to a traumatic event such as a vehicular accident. Multiple studies evaluating the effects of experimental transection of the CdCL reported minimal clinical or histologic effects.[46,138] In the work by Harari et al, there was detectable lameness in some of the dogs one month post transection, but not at any other time point. Radiographic effusion was detected as late as six months post transection but low volumes of synovial fluid were obtained via arthrocentesis. Throughout the study, abnormal caudal drawer motion was detectable via palpation. At six months post transection there was no articular cartilage or meniscal pathology detected via gross or histologic examination. Pournaras et al hypothesized that the lack of osteoarthritis changes seen following CdCLX can likely be attributed to the lack of rotary instability.[46] Therefore, this model was chosen as a positive control, as there are biomechanical alterations that do not apparently translate into clinically significant or histologically detectable degenerative changes as long as six months post operatively. A cadaveric human study on the effect of posterior cruciate ligament transection (PCLX) on meniscal strain has been performed.[139] This study demonstrated increases in meniscal strain following PCLX and the authors hypothesized that this strain could contribute to the osteoarthritis typically seen in people with PCL ruptures. While obvious differences exist in the biomechanics of the canine stifle and the human knee joint, perhaps increased strain on the menisci of the CdCLX canine stifle could be partially responsible for the complex molecular changes noted in the current study. The severed ends of the caudal cruciate ligament may also serve as a nidus of inflammatory and degradative mediators that could affect intra-articular structures, including the menisci.[46]

There was no detectable meniscal pathology noted on magnetic resonance images at five weeks post transection. However, there was pathology of the medial menisci of CrCLX dogs at eight weeks post transection noted on ultrasound. Direct comparisons of the imaging techniques

cannot be made due to the difference in timing of the evaluations. Ultrasound has been shown to be sensitive and specific for meniscal pathology in the CrCLX animal model and clinical canine patient.[36,71,78] Magnetic resonance imaging is the current imaging standard for meniscal evaluation in humans and is growing in use in the research and clinical settings for animals. Intravenous administration of gadolinium has been shown to be beneficial for highlighting articular cartilage pathology as it was in the current study, but has not proven to highlight meniscal pathology beyond what has been shown on non-contrast sequences.[88,97] This difference in imaging capabilities may be due to the differences between hyaline cartilage and fibrocartilage GAG content, as the negatively charged contrast agent is theorized to distribute in inverse relation to the negatively charged GAG molecules.[140] Intra-articular injection of gadolinium has been described for magnetic resonance arthrography the human and canine patients and, although more invasive than conventional techniques, may increase accuracy for diagnosis of meniscal lesions.[97,141]

Lameness was detected in the two CrCLX dogs, but not in the sham or CdCLX dogs. It is likely that this lameness was attributable in part to the biomechanical alterations of the cranial cruciate ligament deficient stifle. Additionally, cartilage pathology was noted on the dGEMRIC magnetic resonance images as well as joint effusion on the stifle ultrasound of CrCLX dogs are indicative of secondary effects and plausible explanations for stifle pain and dysfunction.

Higher histologic proliferation scores were seen for the menisci of CrCLX dogs when compared to CdCLX, sham and control dogs. The medial and lateral meniscal samples were pooled for comparison between dogs. No differences were seen between groups for collagen or GAG content and morphology or for tissue architecture disruption, which fits well with the lack of changes noted on MRI. An early increase in cellular proliferation within the medial meniscus following CrCLX has previously been shown in the lapine model.[142] At two weeks post transection in the study by Hellio Le Graverand et al there was evidence of cellular proliferation as detected via immunohistochemical staining with Ki-67, a protein expressed by cycling cells,

whereas Ki-67 was not detected in normal medial menisci. They showed positive staining in menisci, and therefore cellular proliferation, with no other morphologic changes, consistent with our findings. It was hypothesized in that study that proliferation is just one of several steps in the formation of cellular clusters within the degenerative lapine meniscus.

There was a significant increase of water content of the medial menisci of the CrCLX dogs. This increase in water content post CrCLX has been demonstrated previously,[143,144] even as early as one week post transection in the canine model.[145] The increase in tissue hydration is characteristic of tissue degeneration and has been seen concurrently with changes in collagen and/or glycosaminoglycan (GAG) concentration in other studies.[143,145] The breakdown of the collagen fiber matrix allows the imbibition of water and loss of GAG.[146] It is interesting that the current study showed changes in water content before appreciable changes in either collagen or GAG content on the protein level and may indicate that water content has the earliest detectable change on the tissue level when compared with either GAG or collagen. While the water content of the sham subject's medial meniscus was also increased, even more so than the CrCLX study group, there was likely too much variation in the samples tested or too few samples to show significance. Although not statistically significant, the water content of medial menisci of all groups, including controls, was greater than that for the lateral menisci. In two studies looking at water content of the normal canine meniscus there was no difference noted between the medial and lateral menisci.[7,8] Interestingly, one of the studies found regional variation in that the central portion of the medial meniscus had *decreased* water content when compared to the central portion of the lateral meniscus.[8] The GAG concentration was also decreased in this central region of medial menisci. These authors hypothesized that the relative decrease in water content of the medial central region may be directly related to the decrease in GAG content and may contribute to the frequency of mechanical failure of this region.

There is relatively little comprehensive data on gene expression of the normal meniscus. An expression profile of both human hyaline and fibrocartilage has been performed using cDNA

microarray technology containing 23,040 genes.[147] This study revealed that the patterns of gene expression for fibrocartilage and articular cartilage were very similar, but Col-I expression was abundant in fibrocartilage and Col-II in hyaline cartilage, consistent with other studies.[26,147,148] Aggrecan has also been noted to be expressed in greater concentrations in the articular cartilage when compared to fibrocartilage.[26] Additionally, regional variations have been identified in constitutive mRNA levels for meniscal fibrocartilage. The axial region of the meniscus expresses higher mRNA levels for aggrecan, Col-II, and NOS-2 (nitric oxide synthase 2) than the abaxial region, while the abaxial region expresses higher levels of Col-I and MMP-2 and -3.[25,26] One study comparing the normal medial and lateral meniscus of rabbits showed that mRNA levels were generally higher for the medial meniscus and that there were increased levels of certain molecules unique to the medal or lateral meniscus.[149]

Early changes of the menisci following cranial cruciate ligament transection have previously been described in the lapine model. [144,150] In the work of Hellio Le Graverand et al describing the gross, histologic, immunohistochemical, and molecular changes in rabbit menisci three and eight weeks post CrCLX, the menisci had structural and cellular alterations that corresponded with complex mRNA changes. Although this study describes pathology as "early" these rabbits had severe gross tearing of the medial menisci. The meniscal damage in the current study may represent an even earlier point in the pathophysiology of meniscal disease secondary to CrCLX, given the lack of gross pathology.

MMP-1 was upregulated in both medial and lateral menisci of CrCLX and also in the lateral meniscus of the CdCLX dog. MMP-13 was upregulated in the lateral menisci of the CrCLX dogs. A similar early upregulation of MMP-1 has been described in lapine CrCLX medial and lateral menisci,[150,151] as well as porcine meniscal cells cultured under static or dynamic compression conditions.[152] MMP-13 has also been noted to have dramatic early upregulation at two weeks post CrCL transection with subsequent stable meniscal tissue levels.[151] MMP-1 and MMP-13 are also known as collagenase 1 and 3, respectively, and are associated

with the initial degradation of collagen fibrils in articular cartilage. The upregulation seen here may indicate an imbalance between synthesis and degradation of meniscal collagen.

MMP-3, or stromelysin, expression is generally upregulated very early after cartilage injury, and downregulated in late-stage osteoarthritic cartilage.[153,154] Its functions include the processing of other proteases and growth factors.[153] The downregulation of MMP-3 in medial and lateral menisci of CrCLX and CdCLX of this current study is not surprising based on this known pattern in articular cartilage. Bluteau et al found peak upregulation of MMP-3 in medial meniscal tissue two weeks post CrCLX in the rabbit model, and decreased MMP-3 concentrations at subsequent timepoints.[151]

ADAMTS-5 was downregulated in the medial meniscus of CrCLX stifles, but there were no other differences for ADAMTS-4 or -5. The work by Bluteau et al. found similar results, with medial menisci showing stable ADAMTS-4 levels and decreased ADAMTS-5 levels at 4 weeks post CrCLX transection in rabbits.[151] In that study it was suggested that aggrecanase activity may be regulated by posttranscriptional mechanisms based on the relative stability of aggrecanase mRNA levels despite the increased release of aggrecanase-generated aggrecan catabolites. An *in vitro* study of immature bovine meniscal explants revealed that aggrecanase-mediated aggrecanolysis may play a physiologic role in the development of the bovine meniscus, and that IL-1 induced loss of glycosaminoglycans was primarily due to MMPs or aggrecanases other than ADAMTS-4 and -5.[155] Because large proteoglycans play such a minor role in the total makeup of meniscal fibrocartilage compared to collagens, it is intuitive that collagenases might play a greater role in the degradation of meniscal tissue when compared to aggrecanases.

The inhibitors of MMPs, the TIMPs, showed a pattern of downregulation. TIMP-2 was decreased in the medial meniscus of CrCLX stifles, while TIMP-3 was decreased in the medial meniscus of CrCLX and CdCLX stifles as well as the lateral meniscus of CdCLX stifles. There were no significant differences for TIMP-1. It has been previously shown that gene expression for TIMP-1 in both rabbit and human menisci was relatively stable despite degeneration of the

tissue, thereby supporting the theory that degeneration is in part due to an imbalance of MMPs and their inhibitors.[151,156] This theory of imbalance is further supported by our results in which these MMP inhibitors were either stable or downregulated.

Cox-2 was upregulated in the medial menisci of the CrCLX group. Hellio Le Graverand and co-workers found dramatic upregulation of inflammatory mediators Cox-2 and iNOS at 3 weeks post CrCLX in the medial meniscus of the rabbit.[150] There were no significant differences in any other markers of inflammation in the current study, which may indicate that Cox-2 is either primed and ready for an earlier response or that the molecule responds more vigorously as an inducible enzyme. It has been previously shown in normal rabbit menisci that Cox-2, among other molecules, is expressed in higher levels in the medial than lateral meniscus.[149] Results from the PGE-2 assay in this current study indicated an increase in all operated groups when compared to controls, likely an indication of inflammation related to the surgical insults.

Col-III and Col-VI were both upregulated in the lateral menisci of CrCLX and CdCLX dogs and Col-III was also upregulated in the lateral meniscus of the CdCLX dog. Collagen upregulation in the menisci of the CrCLX canine model has been previously shown in the work of Wildey et al.[148] Concentrations of both Col-I, the major structural collagen, and Col-VI, the major repair collagen type, were upregulated in the CrCLX menisci compared to controls at three and 12 weeks post-transection. Both medial and lateral menisci showed upregulation, but the medial was greater than the lateral. It was concluded from that study that this upregulation of collagen is likely a protective anabolic response of the meniscal tissue. The Wildey study included a harsher biomechanical environment than the current study (outdoor pen exercise) and showed gross meniscal damage. In another study using the lapine CrCLX model, the gene expression of medial meniscal cartilage matrix molecules (Col II, Col X, aggrecan) varied little during the progression of osteoarthritis at two, four and nine weeks post CrCL transection, causing the authors to draw the conclusion that results indicated poor repair capacity of the meniscal tissue.[151] The molecules tested were chosen to compare hyaline and fibrocartilage and

were more of a reflection of the normal constituents of articular cartilage than meniscus. Therefore, the conclusion must be interpreted in light of the lack of collagens more specific to meniscal tissue. Very interestingly, another lapine CrCLX study at three weeks post transection found that the lateral meniscus had more of a fibroblastic phenotype with upregulation of Col-I and Col-III and downregulation of fibromodulin while the medial meniscus had more of a fibrochondrocytic phenotype with upregulation of Col-I, Col-II, Col-III, and aggrecan.[150] Those mRNA results translated to the protein level in which both menisci had increased immunohistochemical staining for Col-I and –III and the medial meniscus alone had increased staining for Col-II.[144] The lack of gross degeneration and lack of upregulation of matrix molecules in the medial meniscus in our study could indicate less degeneration of the meniscus, and therefore, less impetus for tissue repair and subsequent upregulation of the structural matrix molecules when compared to previous studies. It is unclear why there was upregulation of collagen seen in the lateral meniscus alone but certainly could be a part of the complex biomechanical changes that occur in the individual stifle compartments when the stabilizing effects of the cruciate ligaments are altered. Biologically, the medial and lateral menisci have innately unique compositions,[7,149] which may be a result of different mechanical stresses, and also allow for the tissues to respond differently to changes in mechanical stresses.

The molecular changes seen in this study are complex, but they are consistent with other studies, supporting our methods and findings. The upregulation of inflammatory and degradative molecules and downregulation of antidegradative molecules suggests that the meniscal tissue may play an initiating and/or perpetuating role in osteoarthritis of the stifle joint.

Outcomes of this study appear to suggest very early changes in the menisci participating in the pathophysiology of the degenerative process of the joint; however, additional time points should be included in further studies in order to establish a temporal understanding of the degenerating canine meniscus. Additional studies should also include larger numbers of subjects.

This will allow further investigation of the separate regions of the menisci, both the axial and abaxial zones, as well as the cranial, middle and caudal regions.

CHAPTER 3

STUDY 2: COMPARISON OF DIAGNOSTICS FOR THE PATHOLOGIC CANINE MENISCUS

1. Experimental purpose and hypothesis

Currently in veterinary medicine, visualization of the meniscus via arthrotomy or arthroscopy is considered the gold standard for clinical diagnosis of meniscal pathology. However, both are invasive and costly techniques requiring general anesthesia and causing morbidity. While exploration of the stifle at the time of stifle stabilization surgery is imperative for diagnosis, treatment, and prognostication, it would be ideal to have a minimally invasive technique for diagnosis of tears prior to surgery. This would allow for more precise surgical planning and accurate communication with the client. Additionally, meniscal tears that occur post-stifle stabilization surgery present an even larger challenge, as only 27.6% of these tears will have physical examination findings that allow for a probable diagnosis to be made prior to surgical exploration.[66]

Ultrasonography has been reported as a sensitive and specific method for accurate noninvasive diagnosis for medial meniscal tears, and magnetic resonance imaging (MRI) is being utilized increasingly in both research and clinical settings to aid in diagnosis of joint pathology. Arthroscopy is becoming more popular for stifle exploration as a less invasive surgical option, especially for diagnosis and treatment of subsequent tears. While there are reports on both imaging modalities and arthroscopy for the canine model of osteoarthritis and the canine patient, there are no reports directly comparing the techniques using thorough gross examination as the reference standard and comparing all techniques to histology.

The purpose of this study was to compare ultrasonographic, arthroscopic, and MRI of the canine meniscus using comprehensive gross examination as the gold standard. Additionally, we compared the imaging techniques to histopathology of the medial menisci. We hypothesized that each of these diagnostic modalities would have a strong ($r>0.7$) positive correlation to the gold standard measure of presence and severity of meniscal pathology in dogs.

2. Materials and Methods

a. Animals

All procedures were in accordance with the University Animal Care and Use Committee. Twenty-one purpose-bred hound dogs (mean age 19.3 months; mean weight 19.9 kg) were used for a blinded prospective study evaluating a proprietary compound for treatment of osteoarthritis. All dogs included were judged healthy and free of orthopaedic disease based on complete physical and orthopaedic examinations, complete blood count, serum biochemistry profile, heartworm test, and evaluation of orthogonal radiographic views of the hips and stifles. The dogs were housed individually in Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)–approved runs and fed a commercially available maintenance diet for the duration of the study.

b. Surgery and post-operative procedures

On the day of surgery all dogs were premedicated (glycopyrrolate @ 0.005 mg/kg (IM), xylazine @ 0.5 mg/kg (IM), morphine @ 0.5 mg/kg (IM)) and anesthetized (thiopental @ 10-20 mg/kg (IV), isoflurane inhaled @ 1-4% in O_2). The right stifle was prepared for aseptic arthroscopic surgery. Cefazolin (0.5 gram, IV) was administered at induction and extubation. Arthroscopy was performed with a 2.7mm 30° foreoblique arthroscope (Smith and Nephew, Andover, MA) with digital image capture using craniomedial and craniolateral portals to examine all major intra-articular structures in all joint compartments. Craniolateral arthroscope and craniomedial instrument portals were established by making a 5 mm stab incision in the respective location to allow cannula placement. Complete arthroscopic exploration was

performed prior to CrCL transection with a #11 scalpel blade. Intra-articular structures were examined once more prior to incision closure. Dogs were given morphine (0.5 mg/kg SQ) as needed for pain post-operatively for 48 hours. The dogs were leash-walked ten minutes per day for eight weeks at a pace that encouraged the use of the operated hindlimb. The dogs received either the experimental compound or placebo twice daily *per os*.

Eight weeks post-operatively, MRI and second-look arthroscopy were performed on the operated stifle, and ultrasound examinations were performed on both stifles prior to humane euthanasia. Disarticulation of the stifle joints was performed for gross examination and removal of the menisci for histologic processing.

c. MRI

Magnetic resonance imaging was performed on the right stifle of all 21 dogs with a 1.5T magnet (GE Horizon LX) and custom-designed coil (Pfizer Canine Knee Coil). The stifle was positioned in extension with the patient in lateral recumbency. Sequences included: 3D spoiled gradient with fat suppression (1.0mm slice thickness, 20º flip angle, TR=42/TE=7, matrix 512x256, field of view = 8), T2W 3D gradient recalled echo (1.0mm slice thickness, 30º flip angle, TR=30/TE=14, matrix 512x256, field of view = 8), and Fast Fat Suppressed Dual Echo Spin Echo (1.5mm slice thickness, TR=2500/TE = 15/60, matrix 256x256, field of view = 8). Sagittal images were obtained.

d. Ultrasonography

Ultrasonographic examination was performed using a 12-14 MHz high-resolution linear matrix array probe and a 9-10 MHz intraoperative/small parts linear probe (Logiq 9, General Electric, Fairfield, CT). The hair was clipped for increased resolution on the medial and lateral aspects of the stifle joint. The dogs were placed in lateral recumbency and the stifle joint partially flexed. Acoustic gel was used to allow good contact of the probe with the skin surface. Both the medial and lateral menisci were evaluated in the cranial, middle, and caudal regions with the probe in a different position for each region. For evaluation of the cranial region, the probe was

placed cranial to the medial or lateral collateral ligament with caudal angulation of the transducer face. For the central region, the probe was placed on the respective collateral ligament with the probe angled medial to lateral or lateral to medial. To evaluate the caudal region, the probe was placed on the stifle caudal to the collateral ligament with cranial angulation of the transducer face. Images were recorded in the sagittal plane. Sonographic inspection of the menisci included evaluation for displacement, echogenicity, and shape, as well as presence of joint effusion, synovial thickening, and osteophytosis.

e. Surgery and gross inspection

Immediately following humane euthanasia, a second look arthroscopy was performed with the instrumentation and portals as described above. Both hindlimbs were disarticulated at the stifle joint and the menisci were carefully removed for thorough gross inspection and further processing.

f. Histologic evaluation

The menisci were sectioned into cranial, middle, and caudal regions (Figure 12). Five micron sections were cut from each region and stained with hematoxylin and eosin (H&E) and toluidine blue (T blue). Each section was subjectively assessed by one investigator blinded to the sample group using the meniscal scoring system (Appendix 3). For statistical analysis, only the caudal section of the medial meniscus was used based on the scoring for tissue architecture alone.

g. Scoring

Gross and arthroscopic meniscal inspection and scoring were performed by one surgeon and sonographic and MR examination and scoring were performed by one radiologist, both of whom were blinded to dog number and treatment group. For all outcome measures, menisci were subjectively scored for presence and severity of pathology in the cranial horn, body, and caudal horn. Scoring schematics were similar based on a four point scale, but tailored to the imaging modality (Appendices 4, 5 and 6). Gross examination served as the reference standard to which all diagnostic modalities were compared.

h. Statistical analysis

All statistical analyses were performed using a computer software program (Sigma Stat®, San Rafael, CA). The Mann-Whitney Rank Sum test was used to perform statistical analysis for significant differences between groups. The Spearman Rank test was used to determine strength of correlations among the outcome measures. The Chi-Square Odds ratio was used to compare imaging techniques with gross evaluation as the clinical standard. All statistical analyses for comparison of diagnostic techniques were performed on the caudal aspect of the medial meniscus, as this is the region most commonly damaged in clinical meniscal disease of the canine as well as in the canine CrCLX model. Statistical significance was set at $p<0.05$.

3. Results

Tables 8, 9 and 10

a. Gross evaluation

There was a wide range of medial meniscal pathology, with scores across the scoring spectrum from zero to four. Bucket handle tearing of the medial meniscus was the most common configuration noted (Figure13). By both gross and arthroscopic evaluation, medial menisci had significantly more frequent and severe damage than lateral menisci ($p<0.001$). There were no significant differences with respect to presence or severity of meniscal pathology between treatment (proprietary compound) and control groups (placebo) for any outcome measure ($p>0.37$); however this finding must be interpreted with caution based on the relatively low power for this analysis (power$\leq$0.1)

b. MRI

Average acquisition time for the MRI study was 58 minutes. MRI scoring of the caudal medial meniscus ranged from zero to four, with the majority of the menisci (20/21) showing at least mild pathology of the region (Figure 14). There were both medial and lateral menisci that showed degenerative changes, as evidenced by areas of hyperintensity without signs of tearing. Unless additional pathologic changes were noted, these menisci were given a score of zero. The

sensitivity and negative predictive value of MRI for detection of meniscal pathology were 100%. The specificity was 33.3% and the positive predictive value was 80%.

c. Ultrasonography

Approximate acquisition time per stifle examination was ten minutes. All medial menisci in the caudal region scored from one to four, showing at least mild pathologic changes via ultrasound (Figure 15). The most common findings in the stifle joint were mild to moderate effusion and soft tissue thickening. The sensitivity was 92.9% and specificity was 71.4%. The positive and negative predictive values were 86.7% and 83.3%, respectively. Ultrasound had strong and significant positive correlations with both arthroscopy ($r=0.777$; $p<0.001$) and gross inspection ($r=0.748$; $p<0.001$).

d. Arthroscopy

Via arthroscopic evaluation, there were medial meniscal pathology scores from zero to four (Figure 16). Arthroscopy had the strongest positive correlation to gross inspection ($r=0.918$; $p<0.001$) among all of the significant correlations. The sensitivity was 100% and specificity was 83.3%, and the positive and negative predictive values were 93.9% and 100%, respectively. Arthroscopy was 3.3 times more likely than ultrasound and 4.7 times more likely than MRI to correctly diagnose the presence or absence of gross pathology of the medial meniscus of dogs 8 weeks post CrCLX; however, these differences were not statistically significant using Chi-Square test ($p=0.34$; power=0.23).

e. Histology

There were strong and significant correlations between histologic scoring of the caudal medial meniscus and MRI, arthroscopy, ultrasound and gross evaluation of that region ($r>0.6$, $p<0.005$; Figure 17).

4. Discussion

To the author's knowledge, this is the first study to compare ultrasound, MRI, arthroscopy, and histology for diagnosis of meniscal pathology using gross examination as the

reference standard in a canine CrCLX model. In this CrCLX model, there was greater pathology in the medial meniscus compared to the lateral meniscus, which is consistent with previous reports.[1,112,113,144,157] Additionally, the wide spectrum of pathologic changes in the medial meniscus and the common bucket handle configuration of tearing are both consistent with the clinical picture of canine meniscal disease and supportive of the use of this model and the outcome measures included. Gross evaluation was used as the standard for comparison in this study, as the menisci were able to be thoroughly inspected both in situ and after removal.

The appearance of menisci on MRI and ultrasound has been described for the normal and pathologic canine meniscus.[36,78,89,92,93,95,96,158-160] On MR images, the normal menisci appear in the sagittal and dorsal planes as wedge-shaped, homogenous, and hypointense. The signal intensity is often slightly increased in the center surrounded by a small border of lower signal.[89] Meniscal pathology appears as a heterogenous signal, often a hyperintense focal area extending to the tibial or femoral surface of the meniscus. On ultrasound, the normal menisci are homogenous, echogenic, wedge-shaped structures visualized between the hyperechoic bone of the femoral condyles and the tibial plateau. The meniscus should be flush with the surface of the condyle and tibia. An abnormal medial meniscus can be seen as an anechoic void between the femur and tibia, anechoic fluid within the region of the medial meniscus, or displacement of the medial meniscus from its normal position. Oftentimes with bucket handle or peripheral tears, the primary abnormality noted on ultrasound may be an abnormal shape, or flattening, of the meniscus.

Using gross examination as the gold standard, arthroscopy was more sensitive than ultrasonography and had higher specificity and positive and negative predictive values than both MRI and ultrasonography. Arthroscopy is currently used as the clinical standard for most human meniscal studies evaluating accuracy of imaging modalities. There is controversy in the human medical literature regarding the accuracy of arthroscopy when compared to MRI. It has been argued that MRI is more accurate due to the ability to pick up degenerative meniscal changes as

well as increased sensitivity for tibial meniscal surface changes.[89] Currently in veterinary surgery, clinical decision-making is largely based on meniscal inspection and palpation at the time of surgery. Perhaps with increasing use of MRI and ultrasound pre-operatively, the images can provide information regarding location and degree of meniscal parenchymal changes in order to guide arthroscopic treatment. Regardless, the accuracy of arthroscopy shown here is consistent with previous reports and supports the growing use of this minimally invasive technique for diagnosis and treatment of canine meniscal disease.

There were strong and significant positive correlations between gross, histologic, arthroscopic, MRI and sonographic imaging of the caudomedial meniscus in this study (Table 9). This bodes well for the scoring systems utilized for these outcome measures. One of the strongest correlations was between MRI and histology (r=0.900). Multiple studies of human pathologic menisci have shown a correlation between MRI signal intensity and degree of histologic changes.[161,162] This correlation is not surprising based on the innate ability of both techniques to detect subtle intraparenchymal changes. The detection of very subtle architectural changes is excellent for attempting to characterize the spectrum of meniscal pathology, but the clinical relevance of these changes is as yet unknown and likely contributes to the lack of specificity (33.3%) of MRI seen in this study. This problem of low specificity of MRI is not unique to canine meniscal pathology. A recent study by Van Dyck et al investigated the accuracy of images from a 3.0 Tesla magnet to correctly identify mensical tears in people.[163] The authors concluded that, when findings are subtle, using the criteria of two or more abnormal images increase accuracy in diagnosing meniscal tears. It is a fine line between accurate and over-interpretation in very subtle cases, which can decrease the specificity of any imaging modality.

Ultrasonography more consistently correctly diagnosed the presence of caudal meniscal pathology than MRI when compared to gross examination. In previous work ultrasound and macroscopic examination of the CrCLX rabbit medial meniscus showed a strong, positive correlation, as we showed in the present study.[71] Similar to our results, a clinical study

investigating internal derangement of the human knee by Kahn et al showed greater accuracy of ultrasound than MRI for diagnosis of traumatic meniscal pathology.[101]

Interpretation of images via all diagnostic techniques described is greatly affected by skill level and experience. Direct comparisons between observers have been made for human specialists specifically for the evaluation of knee pathology on MR images.[164-166] Two of these reports specifically evaluated accuracy of diagnosis of meniscal pathology and found that experience level of the radiologist affected the reliability.[164,166] One study even found that the experience of the interpreter was more important than the magnet field strength (1.0T to 3.0T).[164] A similar study could not be found on comparison of user experience for ultrasound of the knee joint; however, it is widely accepted that musculoskeletal ultrasonography is operator dependent for both acquisition and interpretation of images. Despite this belief, one study compared the accuracy of musculoskeletal ultrasound performed by human rheumatologists considered international experts in the field with those rheumatologists who had self-directed ultrasound training only.[167] There were only slight differences in the reliability of the technique attributed to experience. There is also no known comparison of experienced versus non-experiences arthroscopists in the literature. However, stifle arthroscopy takes advanced skill and may also be considered operator dependent for evaluation of meniscal pathology.[45] It is certainly possible that experience may play a role in our results, and in comparing the results of this current study with other imaging studies available.[93]

The techniques, hardware and software involved in image acquisition also have tremendous effects on the accuracy of diagnosis. For example the probe size and subtleties of probe positioning greatly affect sonographic imaging. Some technical differences exist between our sonographic technique and others previously described. In previous studies, a 7.5 MHz mechanical sector probe or 7.5 MHz linear transducer were used, whereas we used a 10-14MHz linear transducer.[78,95,158] Additionally, a standoff pad was used in at least two of the previous studies in order to improve visualization of superficial structures, whereas we do not use the pad.

A 7.5 MHz transducer has relatively poor resolution for menisci compared to a 10-14 MHz high resolution probe and the standoff pad can lead to artifacts due to doubling of interfaces. Reed et al and Kramer et al reported that the caudal half of the medial and lateral menisci cannot be visualized via ultrasound in dogs.[78,158] However, we have previously described the appearance of the abnormal meniscus in clinical canine patients, and were able to accurately visualize the entire medial and lateral menisci.[36] This difference may be due to technique or equipment. Regardless, the importance of visualization of the caudal aspect of the medial meniscus is illustrated in the current study, as most of the pathology was located in this region, and we have shown the repeatability of imaging this region.

Perhaps the most variability in technique is with MRI in which sequences, coil, magnet strength, slice thickness, relaxation times, software, and other variables can all play a part in the quality of images, and thus, accuracy of diagnosis. These variables are not consistently standardized for studies as described in the veterinary literature.[159] Multiple papers suggest that the best combination for diagnosis of meniscal lesions on MRI are dorsal and sagittal images using a proton density-weighted sequence.[92,94] We only performed sagittal images due to limitations by the larger parent study from which the meniscal imaging study branched. Despite this limitation, it has been shown that sagittal proton density images had the best agreement with surgical findings of meniscal pathology, while the dorsal and transverse images had poorer agreement.[92] While the transverse view is commonly used in imaging the human meniscus, it is reportedly difficult to image the canine meniscus in this plane due to its shape and the small slice thickness needed.[92,94,95] The magnet strength reported in the literature for imaging canine stifles ranges from 0.06T to 3.0T. Advantages to low-field (<0.15T) MRI include improved tissue contrast, decreased chemical shift artifact, decreased sensitivity to motion, less radiofrequency power deposition, less danger of projectiles, lower start-up and operating costs, easier siting, ability to accommodate larger patients due to gantry opening, and there is no need for specialized anesthesia equipment that is MRI compatible.[160,168] Disadvantages to low-field imaging include

longer acquisition times, poor signal-to-noise ratio, inability to obtain thin slices, and poor spatial resolution.[93,160] It is speculated that increased magnet strength may provide the resolution needed to detect earlier and more subtle degenerative meniscal changes.[159] The magnet used for this research was 1.5T and provided the resolution necessary for the excellent sensitivity we found in the current study. A variety of coil types have been reported for use for MRI of the canine stifle including human extremity coils, human knee coils, and flexible surface coils. We utilized a custom-designed canine stifle coil that is not available on the market, but appears to be appropriate for use, again, based on the sensitivity noted in this study.

Scoring systems for MRI meniscal pathology have been reported, and several attempts have been made to utilize human meniscal scoring systems for the canine model. The authors of one report speculated that a scoring system differentiating grading of tears is likely clinically more important for dogs than attempting to separate degrees of mucoid degeneration.[89] Mucoid or myxoid degeneration (MD) is a degenerative lesion of human menisci characterized by an increase in the mucoid ground substance in the connective tissue containing glycoprotein and mucoprotein.[169] Proposed mechanisms for MD include trauma, infection, intrameniscal hemorrhage, and developmental cyst formation.[170] These lesions generally appear as focal or diffuse hyperintensities on MRI that do not extend to either the tibial or femoral meniscal surface. While we did see hyperintense lesions that did not extend to the meniscal margins in the current study it is unclear what these signal changes truly mean for the canine meniscus, as previous reports suggest that most menisci in CCL deficient stifles show some degree of degenerative change.[88,92-94,171] However, histology had a strong positive correlation to MRI, suggesting that the hyperintense lesions represented a histologic disruption of the meniscal tissue architecture. Because rotational instability likely plays a major role in canine cranial cruciate disease, the resultant shear forces on the medial meniscus likely lead to disruption of the meniscal tissue rather than mucoid degeneration.[89] Additionally, a histologic study of menisci from dogs with various stages of CCL disease found no signs of histologic mucoid degeneration in any

meniscus.[172] We took note of increased signal intensity but did not use this as a pathologic change on our scoring system, following the lead of some other canine and human studies.[92,173] It has been reported that the presence of a high signal extending through the meniscus to intersect with one or two meniscal margins is the most reliable criterion for meniscal tearing on MR images.[89,174] Our scoring systems for all outcome measures were modeled after whole organ imaging system (WORMS) of Peterfy et al, which includes regional scoring and combined total scoring for pathologic changes.[175] Utilizing a four point scale with similar degrees of meniscal damage, we were able to tailor the criteria to each diagnostic modality, but keep the numbering consistent. The scoring schematics used appear to be appropriate for use in the CrCLX model of meniscal disease and could also be used for clinical studies. However, as we only compared the diagnostic modalities in the caudal aspect of the medial meniscus, further work must be performed to validate the use of this method in the scoring of canine meniscal pathology.

In this model of canine CrCLX, we found that ultrasound was more accurate than MRI in the diagnosis of pathology of the caudal medial meniscus. Technical expertise is necessary for performance and interpretation of both ultrasound and MRI; however, availability, noninvasiveness, and cost are notable advantages of ultrasound for aid in diagnosis of meniscal tears in dogs. Further investigation including randomized controlled trials imaging clinical patients is warranted to increase our understanding of how these imaging techniques perform in the natural disease state and to optimize imaging protocols.

CHAPTER 4

STUDY 3: MENISCAL RELEASE IN CRUCIATE LIGAMENT INTACT STIFLES CAUSES LAMENESS AND MEDIAL COMPARTMENT CARTILAGE PATHOLOGY IN DOGS 12 WEEKS POST OPERATIVELY

1. Study purposes and hypothesis

Despite growing evidence questioning the value and appropriateness of meniscal release (MR) as a prophylactic treatment of the medial meniscus, objective clinical data to substantiate this evidence for optimizing clinical decision making is lacking. It is difficult to assess the true clinical effects of MR itself due to the confounding problem of accompanying cranial cruciate ligament deficiency in dogs studied to date. The important clinical question that remains is whether the well-defined biomechanical alterations caused by meniscal release alone translate into clinically relevant effects on stifle joint health and function. Therefore, the objective of this study was to evaluate the effects of MR in the CCL-intact canine stifle. We hypothesized that there would be significant differences in presence and severity of osteoarthritis between sham operated stifles and stifles in which MR had been performed.

2. **Materials and methods**

a. Animals

All procedures were approved by the University Animal Care and Use Committee. Ten purpose-bred, adult (2-4 years of age) hound dogs (mean weight = 20kg) were used for a blinded prospective study comparing models of osteoarthritis[176] in which either MR (n=5) or a sham (SH) surgery (n=5) was performed on the right stifle of each dog. All dogs included were judged healthy and free of orthopaedic disease based on complete physical and orthopaedic

examinations, complete blood count, serum biochemistry profile, heartworm test, and evaluation of orthogonal radiographic views of the hips and stifles.

b. Surgery

On the day of surgery, dogs were premedicated (glycopyrrolate @ 0.005 mg/kg (IM), xylazine @ 0.5 mg/kg (IM), morphine @ 0.5 mg/kg (IM)), anesthetized (thiopental @ 10-20 mg/kg (IV), isoflurane inhaled @ 1-4% in O_2), and prepared for aseptic arthroscopic surgery of one stifle. Craniolateral arthroscope and craniomedial instrument portals were established by making a 5 mm stab incision in the respective location to allow cannula placement.[36]

To perform meniscal release, a sterile meniscal push knife (Smith+Nephew) was inserted through the instrument portal. The knife was used to create a complete radial transection of the meniscus in the caudal horn near its junction with the caudal meniscotibial ligament. Care was taken to avoid iatrogenic injury to ligaments and articular cartilage. Complete radial transection was confirmed by arthroscopic inspection. To perform the sham procedure, a blunt obturator was inserted through the instrument portal. The medial meniscus was palpated with the probe at the caudal horn near its junction with the caudal meniscotibial ligament. Incisions were apposed with stainless steel staples. Recovery from anesthesia and surgery was monitored until extubation. Analgesics (morphine 0.5 mg/kg SQ) were administered to the dogs at the time of extubation, and then as necessary to control signs of pain for up to 48 hours postoperatively. The dogs were returned to their individual kennels. The dogs were allowed unrestricted use of the affected limb in an 18-25 square foot kennel. In addition, the dogs were walked on a leash 5 days each week for 15 minutes at a pace to ensure use of all four limbs.

c. Orthopedic examination and limb function

One investigator blinded to treatment performed complete orthopaedic examinations in each dog preoperatively and at 4, 8, and 12 weeks after surgery and recorded any abnormalities detected. Two investigators evaluated limb function in the dogs preoperatively and at 4, 8, and 12 weeks after surgery using two different methods. Visual Analog Scale (VAS) function

evaluations were assessed and recorded using a 10 cm VAS with 0 being poor and 10 being excellent hindlimb function. Clinical lameness scores were assigned based on visual examination of gait at a walk and trot using a scoring system previously validated to force plate analysis of dogs with meniscal pathology.[134] (Appendix 1)

d. Ultrasonography

Twelve weeks post-operatively, sonographic examination (Logiq 9, General Electric, Fairfield, CT; with a 12-14 MHz high-resolution linear transducer) of the operated stifle of each dog was performed by one veterinary radiologist unaware of the surgical procedure undergone by each dog. The dogs were positioned in lateral recumbency with the limb of interest uppermost for ultrasound examination which included assessment and scoring (Appendix 7) for meniscal displacement, echogenicity, and shape as well as presence of joint effusion, synovial thickening, and osteophytosis.[36]

e. Radiography

Immediately following sonographic evaluation, the dogs were humanely euthanatized. Craniocaudal and mediolateral radiographic views of the stifle were obtained. The radiographs were scored by one investigator blinded to grouping and clinical signs, utilizing a subjective scoring system previously described.[177]

f. Arthroscopy

Arthroscopic evaluation of all operated stifles was performed using craniolateral and craniomedial portals. All articular surfaces of the patella, femur and tibia were examined and scored with respect to degree of articular cartilage damage using the International Cartilage Repair Society (ICRS) cartilage injury classification system (Appendix 7).[178] Meniscal pathology was arthroscopically assessed and described in terms of nature, extent, and location (Appendix 4).

g. Gross evaluation

Both stifles from each dog were carefully dissected to assess gross pathology of the articular cartilage, cruciate ligaments, and menisci. Disarticulation of the stifle was performed.

Photographs were taken of tibias with menisci in place and of femurs. Menisci were carefully removed and photographed and then processed for subsequent analyses as part of a separate study.

h. Percent area of cartilage damage (%ACD)

The femoral and tibial articular surfaces were painted with India ink, washed after 60 seconds with tap water, and photographed. Unexposed radiographic film was placed over each joint surface and the articular cartilage circumferences traced with a permanent marker. The areas of India ink staining were outlined using a permanent marker. Tracings of the India ink-stained areas were evaluated without knowledge of dog number or treatment group. The tracings were scanned using a computer software program (Image-Pro Plus, Media Cybernetics, Carlsbad, CA) and percentage of the total area of the articular cartilage stained calculated and recorded %ACD. The %ACD were determined for the medial and lateral femoral and tibial articular surfaces, separately and together, for each dog.[179-181]

i. Data analysis

All statistical analyses were performed using a computer software program (Sigma Stat®, San Rafael, CA). Means and medians were calculated for each outcome measure. Data were compared for statistically significant ($P < .05$) differences using *t*-test (VAS scores, %ACD) or rank sum test (lameness scores, radiographic scores, gross scoring, ultrasonographic scoring, articular cartilage scoring). Correlations among assessments (VAS vs lameness scores, gross vs ultrasonographic scores) were determined using Spearman Rank Order Correlation test ($r > 0.7$ for strong correlations, $r > 0.4$ for moderately strong correlations).

3. Results

a. Orthopedic examination and limb function

All dogs were judged to be orthopaedically sound on the hindlimbs preoperatively. Examinations performed 12 weeks after surgery revealed palpable joint effusion in 4 of 5 MR dogs and 1 of 5 SH dogs. One MR dog also had a palpable medial buttress. All outcome

measures for MR and SH dogs are reported in Table 11. All dogs had no detectable hindlimb lameness prior to study initiation based on both lameness grading and VAS. All of the dogs except two (one MR, one SH) were judged to have detectable lameness 4 weeks after surgery using both evaluation methods. At 8 and 12 week assessments, no MR dogs were judged to be completely sound while 3 dogs were considered completely sound in the SH group. Accordingly, MR dogs had numerically more severe lameness than SH dogs by both methods at 8 and 12 weeks after surgery, however, these differences were only statistically significant ($p=0.02$) for VAS scoring at 8 weeks postoperatively. Lameness grading and VAS evaluation showed a strong ($r = -0.976$) and significant ($p < 0.0001$) negative correlation.

b. Radiographic evaluation

All dogs were judged to have normal radiographic appearances of both stifles prior to study initiation. All stifles undergoing MR had some evidence of radiographic signs of osteoarthritis 12 weeks after surgery, and the degree of radiographic OA was significantly higher than seen in SH stifles ($p=0.007$) (Figure 18).

c. Ultrasonographic and gross meniscal assessments

Meniscal pathology was judged to be more severe in MR stifles compared to SH stifles by both ultrasonographic and gross assessments (Figures 19 and 20). However, these differences were only statistically significant for gross assessment ($p=0.008$). Ultrasonographic and gross assessments of meniscal pathology showed a moderately strong ($r = 0.591$) and significant ($p = 0.005$) positive correlation. Meniscal transection sites could be readily identified in each MR meniscus. No evidence of bridging tissue was noted in the axial 2/3 of the transection site, while partial bridging of the abaxial portion of the meniscus with yellowish connective tissue was noted in some specimens.

d. Articular cartilage evaluation

Based on arthroscopic and gross evaluations, MR stifles had significantly ($p < 0.001$) more severe articular cartilage pathology compared to SH stifles 12 weeks after surgery (Figures

21 and 22). No articular cartilage pathology was seen arthroscopically or by calculation of %ACD using the India ink staining technique in any SH stifles (0.0 ± 0.0%). In contrast, all MR stifles had detectable articular cartilage pathology based on both arthroscopic evaluation and India ink staining (8.1 ± 3.2%). Articular cartilage pathology in MR stifles was predominantly confined to the medial compartment and involved the tibial plateau and femoral condyle, and ranged from grade 1 to grade 4.

4. Discussion

Medial meniscal release consistently results in clinically significant osteoarthritis and dysfunction 12 weeks post-operatively in the CCL-intact canine stifle based on the presence of joint effusion, radiographic findings, numerically higher lameness scores, arthroscopic evidence of cartilage damage including full-thickness lesions, objective measurement of amount of cartilage damage, and gross evaluation of meniscal pathology. The lameness scoring system and visual assessment scale correlated well and the subjective and objective outcome measures utilized were all in agreement in this *in vivo* model. The nature and extent of meniscal healing in the present study was consistent with previous reports.[60,63,64,129,130] We noted minimal visual healing of the radial meniscal transection site. When present, bridging tissue was isolated to the abaxial portion of the meniscus only, corresponding to the vascular zone of the meniscus.[4] Even when meniscal tears are bridged with reparative tissue, mechanical function is not typically restored.[63,64] This is especially true with respect to radial tears, which meniscal release most closely resembles, in which functionally inferior fibrovascular scar tissue may partially bridge the defect.[58,63,129,182]

The intended purpose of MR is to allow the caudal pole of the medial meniscus to translate abaxially during stifle joint motion and load-bearing, thereby protecting the medial meniscus from potentially pathologic compressive and shear forces in an unstable stifle.[117] This abolishes the load transmission, congruity, stabilization, and shock absorption functions of the tissue, and the *ex vivo* biomechanical alterations of MR have been elegantly elucidated.[33,47] In the

study presented here, focal articular cartilage pathology was consistently seen on the medial tibial plateau and medial femoral condyle in the areas of contact shown to be overloaded after meniscal release in cadaveric limb testing, thereby validating the *ex vivo* work by Pozzi, et al.[47] We also observed small articular cartilage lesions of the lateral tibial plateau and lateral femoral condyle in some MR stifles. Although further work is needed to delineate the nature of these lateral compartment lesions, it is possible that the effects of meniscal release on joint kinematics are not limited to the medial compartment and may have deleterious effects on the lateral compartment and even patellofemoral compartment with time.

The model used in this study was designed to eliminate confounding variables including CCL-related stifle instability, method of stifle stabilization, and effects of arthrotomy, so as to determine the effects of MR alone for clinical decision making. Previous *in vivo* studies have examined the effects of meniscal pathology of various types on stifle joint health and function, but have not addressed the confounding variables or included informative controls.[104,129-131] In an experimental canine model of meniscal injury, several medial meniscal tear configurations were created via open arthrotomy with medial collateral ligament transection.[130] Gross and histologic evaluation 3-15 months post-operatively showed the most severe degenerative changes occurred in association with meniscal insults that allowed for free-floating or entrapped segments, especially caudally, similar to those created by MR. After radial transection of the medial meniscus in sheep, severe OA developed within six months and was significantly worse than in controls.[129] Two clinical studies evaluating dogs after stifle stabilization with tibial plateau leveling osteotomy (TPLO) with and without meniscal release reported that more severe progression of radiographic signs of OA occurred following meniscal release,[131] and that meniscal release was neither protective of the meniscus, nor beneficial for decreasing the risk for late meniscal tears requiring additional surgery.[104,131] The data presented here provide further evidence to this effect. Ultrasound has been reported in both human and veterinary medicine to be a sensitive, specific, consistent, and cost effective measure of meniscal disease in the clinical

and research settings.[36,72,73,77,183] On both sonographic and gross evaluation in the present study, meniscal pathology beyond the direct surgical insult was noted in MR dogs suggesting that the medial meniscus may be subject to biomechanical and biologic insults after release that can contribute to symptomatic stifle pathology.

The development of symptomatic osteoarthritis secondary to MR is likely multifaceted. Evidence suggests that both biomechanical and biologic factors play roles in this disease process. The medial meniscus has been established as a secondary stabilizer of the stifle joint[32,184-187] and plays an even greater role in stabilization of CCL deficient stifles.[33] Loss of this secondary stabilizing function along with the detrimental changes in contact areas and pressures brought about by abolishment of meniscal functions of congruity, load transmission, and shock absorption set up a biomechanical imbalance that certainly has the potential for severe consequences for joint health and function. Direct mechanical trauma caused by the free caudal pole of the meniscus may be another biomechanical factor in the initiation of focal cartilage lesions for which there is some anecdotal clinical evidence. Direct iatrogenic trauma to the articular cartilage of the femoral condyle during MR has been previously reported[111] and cannot be entirely ruled out as a potential biomechanical factor in this study, however, it is considered unlikely based on thorough arthroscopic documentation of the articular surfaces before and after performing MR and the location of the articular cartilage lesions in reference to the surgical instrumentation and technique used for MR in this study. Inflammatory and degradative mediators produced by meniscal tissue playing key roles in the development and progression of OA is a current theory gaining credence as a biological-based explanation for joint pathology.[49-52,150] Data from all outcome measures employed in the present study support these mechanisms and provide translational data with important clinical applicability for veterinary surgeons. Biomechanical imbalance is supported by the location and nature of the articular cartilage damage consistently noted in MR stifles. Inflammation of the operated joint in the MR group was evident based on palpation and radiographic signs of effusion, and ultrasonographic and arthroscopic evidence of

synovitis. Radiographic, ultrasonographic, and arthroscopic findings also suggest ongoing degradation. Importantly, the clinical measures of symptomatic joint disease, including orthopedic examination, gait assessment, radiographic and ultrasonographic imaging, and arthroscopy, consistently correlated to the quantitative "basic science" measures of disease. Simply stated, the data confirm that MR causes damage to the medial meniscus and articular cartilage that results in the development and progression of stifle osteoarthritis and lameness.

Several possible limitations in the study design should be taken into consideration. While this *in vivo* design was optimal for delineating the pathologic changes associated with MR, the model may not accurately mimic the naturally occurring disease state, and it may be more directly clinically relevant to look at concurrent CCL disease with MR. However, instability in the CCL deficient model or canine patient acts as a significant confounding factor and it is therefore impossible to determine the effects of MR alone on stifle joint health and function without first isolating this technique from the others that are performed in clinical cases of CCL disease. The length of the study, timing and nature of outcome measures, and number of dogs included also present limitations which must be considered. A study duration of 12 weeks with the majority of outcome measures being scoring systems performed only at time 0 and endpoint does not allow for modeling or assessing the full spectrum of changes associated with stifle joint pathology. Both methods of lameness assessment revealed more dogs showing lameness with numerically worse mean lameness scores in the MR dogs than the SH dogs, which we considered most clinically relevant. However, the only statistically significant difference was noted in VAS scoring at the 8 week time point. The lack of other statistically significant differences in lameness scoring may be due to a true lack of differences or due to the relatively small number of subjects studied, the nature of the scoring systems, and a type II error. While adding study subjects, outcome measures, and additional assessment time points and extending the length of the study would be informative and beneficial, that was not possible based on the scope of the

study. However, the experimental design did allow us to accomplish our stated objective and test our hypothesis.

Because of the well-established association of CCL disease with meniscal injury in dogs, it has been difficult to discern the relative contribution of *meniscal injury alone* to presence and severity of stifle pathology and limb dysfunction in clinical studies or previously considered animal models (e.g., CCL transection). Likewise, it is difficult to establish from previous studies how much, if any, stifle degeneration may be attributable to *MR alone*. Previously reported evidence is clear in showing that 1) meniscal release causes detrimental biomechanical alterations in the canine stifle with or without treatment for CCL deficiency[33,47], and 2) meniscal release does not completely prevent subsequent symptomatic meniscal pathology.[104,131] The current data provide additional clinically-relevant validity by showing that meniscal release alone results in further meniscal pathology, articular cartilage loss, degenerative joint disease, and lameness. These factors should be seriously considered when making decisions about performing meniscal release in dogs.

The major clinical questions which then arise are whether meniscal release is ever indicated and how we can best "protect" the integrity and function of the menisci. Numerous techniques for stabilization of the CCL deficient stifle, with and without MR, have been reported, but none has been proven superior for return to function, halting progression of disease, or preventing late meniscal damage.[112,113] In fact, for at least two of the major techniques employed for stabilization of CCL deficient stifles, MR is strongly recommended because of the documented inability of the techniques to address abnormal joint biomechanics and kinematics in such a way as to maintain an acceptable risk for subsequent symptomatic meniscal damage.[41,126,127] In 2006, deRooster, et al. suggested that "The goal of reconstructive methods [for CCL deficiency in dogs] should not only be to alleviate the existing instability of the unstable stifle joint, but also to mimic normal joint kinematics as closely as possible."[188] The authors strongly support this philosophy and contend that this goal cannot be attained without including

maintenance and protection of meniscal structure and function in the clinical approach to treatment. The data from the current study provide evidence to support this contention.

CHAPTER 5

FUTURE DIRECTIONS

Findings in the current studies can be directly compared with parallel research on human meniscal disease from our laboratory. In one study using similar outcome measures and scoring systems, menisci from 27 knees undergoing total knee arthroplasty were compared to menisci from six aged normal knees.[189] The same dissection technique was used to take sections of both medial and lateral menisci and perform histology, biochemical assays and gene expression analysis. Affected knees had significantly more severe gross and histologic pathology, especially in the medial menisci in the middle and posterior regions. Collagen, GAG, and percent water content were all greater for affected knee menisci than controls. Water content and GAG concentration were also greater for medial than lateral menisci in affected knees. Gene expression analysis revealed upregulation of Col-1, -2, -3, -6, and MMP-13 and downregulation of MMP-3 and VEGF in affected menisci. Significant correlations were seen between the various basic science measures of disease and these also correlated well with radiographic signs of osteoarthritis, supporting the outcome measures and scoring systems utilized.

As presented, the first two experimental studies in this thesis produced similar results. CrCLX menisci showed increased water content, higher proliferation using the same scoring system, and similar alterations in gene expression including MMP-3, -13, and Col-3 and -6. Clinical imaging identified greater changes in affected medial menisci than controls. We also found strong correlations between histologic and clinical measures of disease.

The similarities seen between the human and canine species is encouraging, as animal models of disease are commonly used in osteoarthritis research. Likewise, that research on human menisci may be extrapolated to the canine is important for clinical veterinary patients.

The overall purpose of this work was to provide information toward the comprehensive characterization of pathology of the canine meniscus, both for the basic science applications and to be translated into practical application in the clinical setting. Toward that end, an informal email survey was performed of practicing veterinary general practitioners, veterinary surgical residents, and boarded veterinary surgeons (ACVS, ECVS).[190] The questions asked were:

1) How do you manage the torn medial meniscus during CCL surgery? If torn? If not torn?

2) How do you diagnose a suspected meniscal tear post stifle stabilization?

3) How do you treat a meniscal tear post stifle stabilization? If truly torn? If not torn based on exploratory surgery?

There were 45 responders – nine general practitioners, 18 private practice surgeons, seven academic surgeons, seven surgery residents, and four residents speaking on behalf of their surgical mentors. 31% of responders perform arthrotomy, 20% perform mini arthrotomy, and 13% perform arthroscopy for evaluation of the stifle joint. If the meniscus is torn at the time of stabilization surgery, 96% perform a partial meniscectomy and 4% perform a complete meniscectomy. If the meniscus is normal upon initial exploration, 47% will "sometimes" perform a meniscal release, while 11% "always" perform a meniscal release. The other 42% do not perform meniscal release. For diagnosis of post-operative meniscal tears, 80% use history and clinical signs, 18% attempt medical management and suspect a tear if it fails, 18% evaluate for radiographic effusion, and 13% perform MRI (4%) or ultrasound (9%) prior to surgical exploration. Treatment for the post-operative tear includes arthroscopy for 44%, arthrotomy for 24%, or mini arthrotomy for 6.6%. 71% of responders will perform a partial meniscectomy of the torn portion, 6.6% will perform a complete meniscectomy, 15.5% will also perform a meniscal release if the meniscus is torn, and 15.5% will perform a meniscal release if the meniscus is *not* torn.

The survey indicated that the standard of care is toward meniscal preservation with 96% performing partial meniscectomy instead of complete. However, 58% of surgeons at least "sometimes" perform a meniscal release, which is now known to be detrimental to meniscal and joint function. Arthroscopy appears to be more common for addressing secondary tears than during the initial surgery, which indicates a trend toward minimally invasive surgery. Only 13% of surgeons perform advanced imaging prior to surgery for suspected post-operative meniscal tears. Whether this is due to cost, availability, or simply surgeon preference is unknown. Perhaps the trend toward less invasive surgery lessens the concern of misdiagnosis for some, since the morbidity is lower for arthroscopic procedures than arthrotomy.[102] It continues to be a pervasive attitude that MR protects the meniscus, although recent studies prove otherwise.[104,131,191] It takes time and good communication among investigators and clinicians in order to make major shifts in paradigms.

The current clinical protocols for diagnosis and treatment of meniscal disease at the Orthopedic Surgery Service at the University of Missouri have been affected, in part, by the work presented here. Ultrasound for suspected meniscal disease is commonly performed prior to stifle surgery. Additionally, an overall shift toward avoiding meniscal release and preservation of the meniscal tissue is the prevailing standard.

Future studies must be aimed at continuing the line of parallel animal and human research to gain understanding of the similarities and differences of the pathophysiology of meniscal disease. Until such time as a true understanding occurs, treatment strategies such as meniscal repair, replacement, and regeneration may not be optimized. Studies using larger numbers of subjects are needed. While there were limitations to each of the three aforementioned research projects, one interesting point is that all three projects were branches off of larger studies, decreasing cost of these projects and terminal use of animals. While osteoarthritis research traditionally placed the focus on articular cartilage, the joint as an organ can be valuable for this research utilizing all tissues including menisci, ligament, and synovium as well. Finally, the use

of randomized, blinded studies in clinical patients with objective outcome measures is lacking in veterinary medicine. This will be a necessary next step in order to include a wide spectrum of disease and to minimize use of animals as research subjects.

TABLES

Table 1: Reported accuracy of sonography for diagnosis of medial meniscal pathology in the literature. PPV – positive predictive value and NPV – negative predictive value

	Sensitivity	**Specificity**	**PPV**	**NPV**
Human[192]	83%	90%		
Human[193]	92%	83%	58%	98%
Human[76]	60%	21%		
Human[101]	93%	92.8%		
Human[194]	100%	95%	95%	100%
Human[70]	86.4%	69.2%	82.6%	75%
Canine[75]	82%	93%	90%	88%
Canine[36]	90%	92.9%	90%	92.9%
Lapine[71]	92%	87.5%	92%	87.5%

Table 2: Reported accuracy of CT arthrography for diagnosis of meniscal pathology in the literature.

	Sensitivity	**Specificity**
Human[81]	88.5%	95.5%
Human[195]	92%	88%
Human[80]	91.7-100%	98.1%
Canine cadavers[196]	90%	100%
Canine cadavers[85]	13.3-73.3%	57.1-100%
Canine[79]	71%	100%

Table 3: Reported accuracy for MRI for diagnosis of canine meniscal pathology in the literature.

	Sensitivity	**Specificity**
Blond 2008[94]	100%	94%
Barrett 2008[92]	90%	96%
Bottcher 2010[93]	64%	90%

Table 4: Water content of menisci in the CrCLX, CdCLX, sham-operated, and control groups. The data from the cranial, middle and caudal sections of the menisci were pooled to obtain a mean percentage of water content per meniscus.

	CrCLX	CdCLX	Sham	Control
Medial menisci	67.97%	63.7%	68.5%	61.64%
Lateral menisci	63.6%	61.39%	59.3%	59.24%

Table 5: Total sulfated GAG content of menisci in the CrCLX, CdCLX, sham-operated, and control groups. Values reported are µg GAG/mg tissue dry weight.

	CrCLX	CdCLX	Sham	Control
Medial menisci	80.4	116.1	77.2	72.8
Lateral menisci	89.5	69.1	61.8	79.2

Table 6: Total collagen content of menisci in the CrCLX, CdCLX, sham-operated, and control groups. Values reported are µg HP/mg tissue dry weight.

	CrCLX	CdCLX	Sham	Control
Medial menisci	81.17	44.53	94.46	95.95
Lateral menisci	103.46	130.24	98.87	68.06

Table 7: Total PGE2 content of menisci in the CrCLX, CdCLX, sham-operated, and control groups. Values reported are pg PGE2 /ml of sample used for purification.

	CrCLX	CdCLX	Sham	Control
Medial menisci	1.02	0.79	0.82	0.47
Lateral menisci	0.67	0.50	0.55	0.52

Table 8: Raw scoring values for gross, magnetic resonance imaging (MRI) ultrasound (US), arthroscopy (scope), and histopathology (histo). Signal hyperintensities indicative of degenerative lesions = Deg; histologic sections that were unscorable for tissue architecture = X.

Dog	Gross	MRI	US	Scope	Histo
1	0	0 (Deg)	1	0	X
2	3	2	1	3	2
3	0	1	2	1	1
4	3	3	1	3	1
5	0	2	1	0	0
6	4	3	3	4	X
7	4	4	2	4	3
8	0	0 (Deg)	3	0	0
9	4	3	3	4	1
10	4	2	3	3	3
11	2	2	1	3	3
12	0	3	2	0	0
13	3	3	2	3	2
14	0	3	2	0	2
15	3	2	4	4	1
16	1	3	1	1	2
17	2	2	2	3	3
18	4	2	3	4	2
19	4	3	4	4	2
20	2	2	4	3	3
21	3	4	4	3	3

Table 9: Correlations of imaging techniques with strength and significance, listed from strongest to weakest

Correlations: caudomedial meniscus	**Strength and significance**
Gross and arthroscopy	r=0.918; p<0.001
Histopathology and MRI	r=0.900; p<0.001
Arthroscopy and ultrasound	r=0.777; p<0.001
Gross and ultrasound	r=0.748; p<0.001
Arthroscopy and histopathology	r=0.723; p<0.001
Gross and MRI	r=0.693; p<0.001
Arthroscopy and MRI	r=0.685; p<0.001
Ultrasound and histopathology	r=0.676; p<0.001
Gross and histopathology	r=0.654; p<0.001
Ultrasound and MRI	r=0.592; p<0.001

Table 10: Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of arthroscopy, ultrasound, and MRI as compared to gross evaluation as the reference standard

Diagnostic modality	Sensitivity	Specificity	PPV	NPV
Arthroscopy	100%	83.3%	93.3%	100%
Ultrasound	92.9%	71.4%	86.7%	83.3%
MRI	100%	33.3%	80.0%	100%

Table 11: Outcome measures at 4, 8, and 12 weeks post meniscal release or sham surgery. Bolded are statistically significantly different, p-values in text

	VAS			LAMENESS			RAD	US	GROSS	%ACD
Time point	4	8	12	4	8	12	12	12	12	12
MR	8.6 ± 2.1	**7.3 ± 1.8**	7.8 ± 2.0	1.1 ± 0.7	1.8 ± 0.8	1.9 ± 1.1	**3.4 ± 1.6**	2.0 ± 1.4	**4 ± 0**	**8.1 ± 3.2**
SHAM	9.2 ± 1.8	**9.1 ± 1.0**	9.0 ± 2.0	1.0 ± 1.1	0.9 ± 1.0	0.6 ± 0.8	**0.6 ± 0.5**	1.2 ± 0.4	**0 ± 0**	**0.0 ± 0.0**

FIGURES

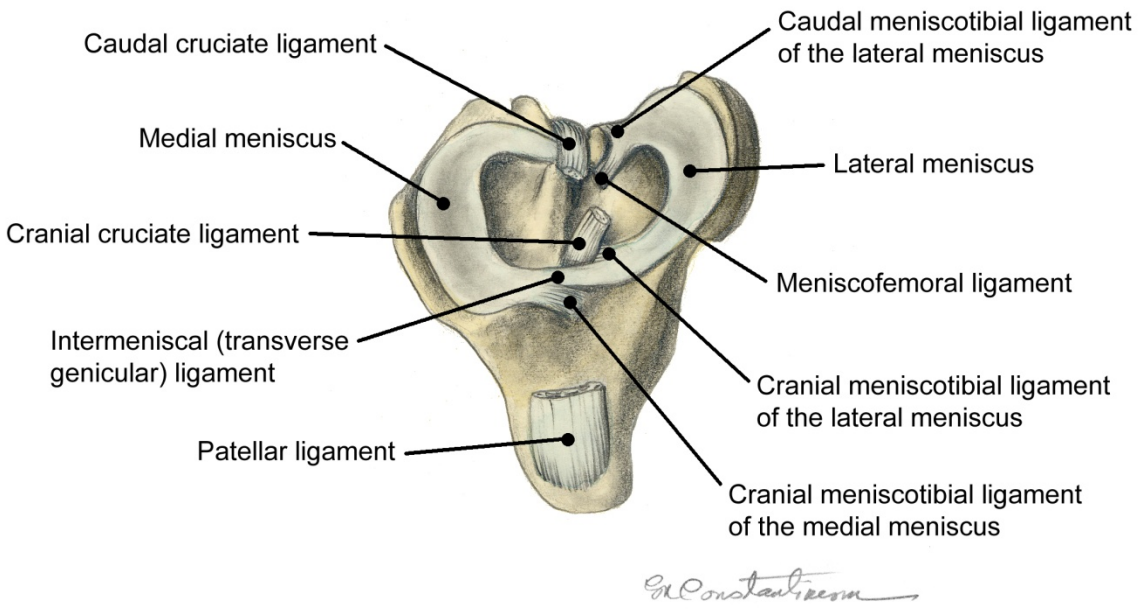


Figure 1: Anatomy of the canine menisci in situ on the tibial plateau

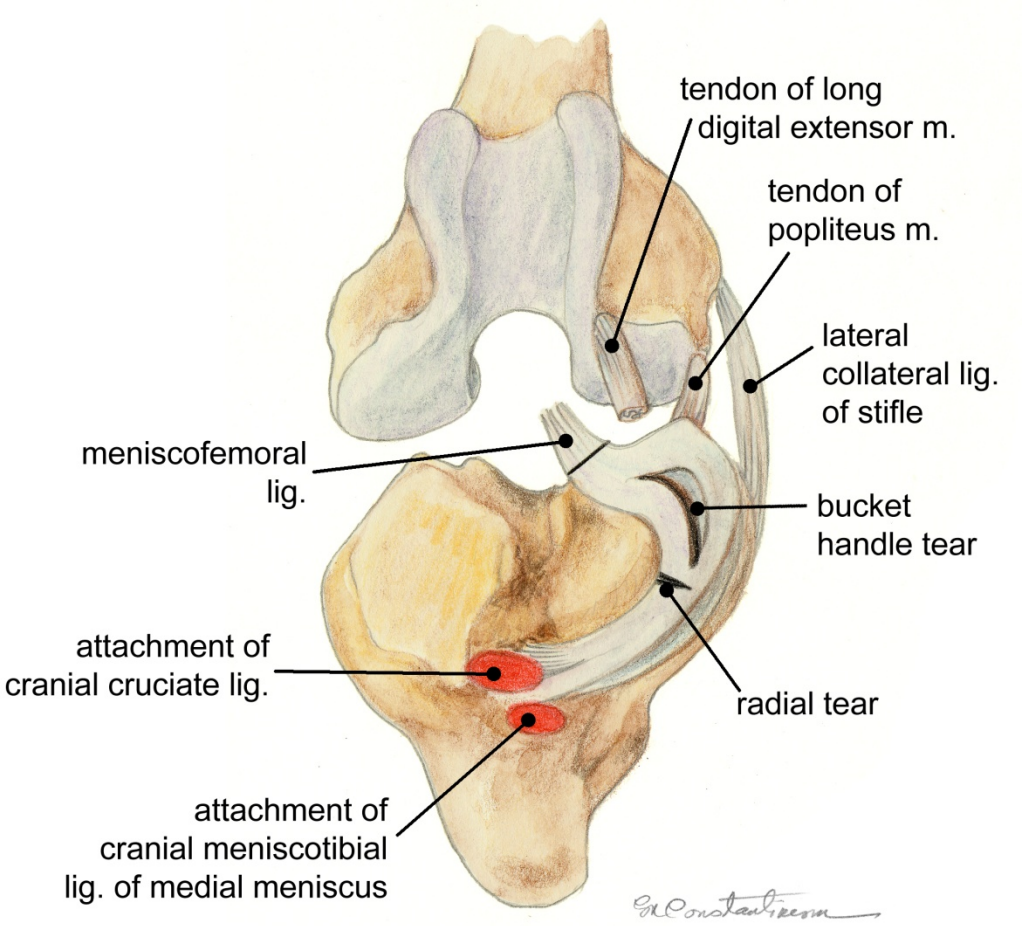


Figure 2: Illustration of the canine medial meniscus in situ with a bucket handle tear

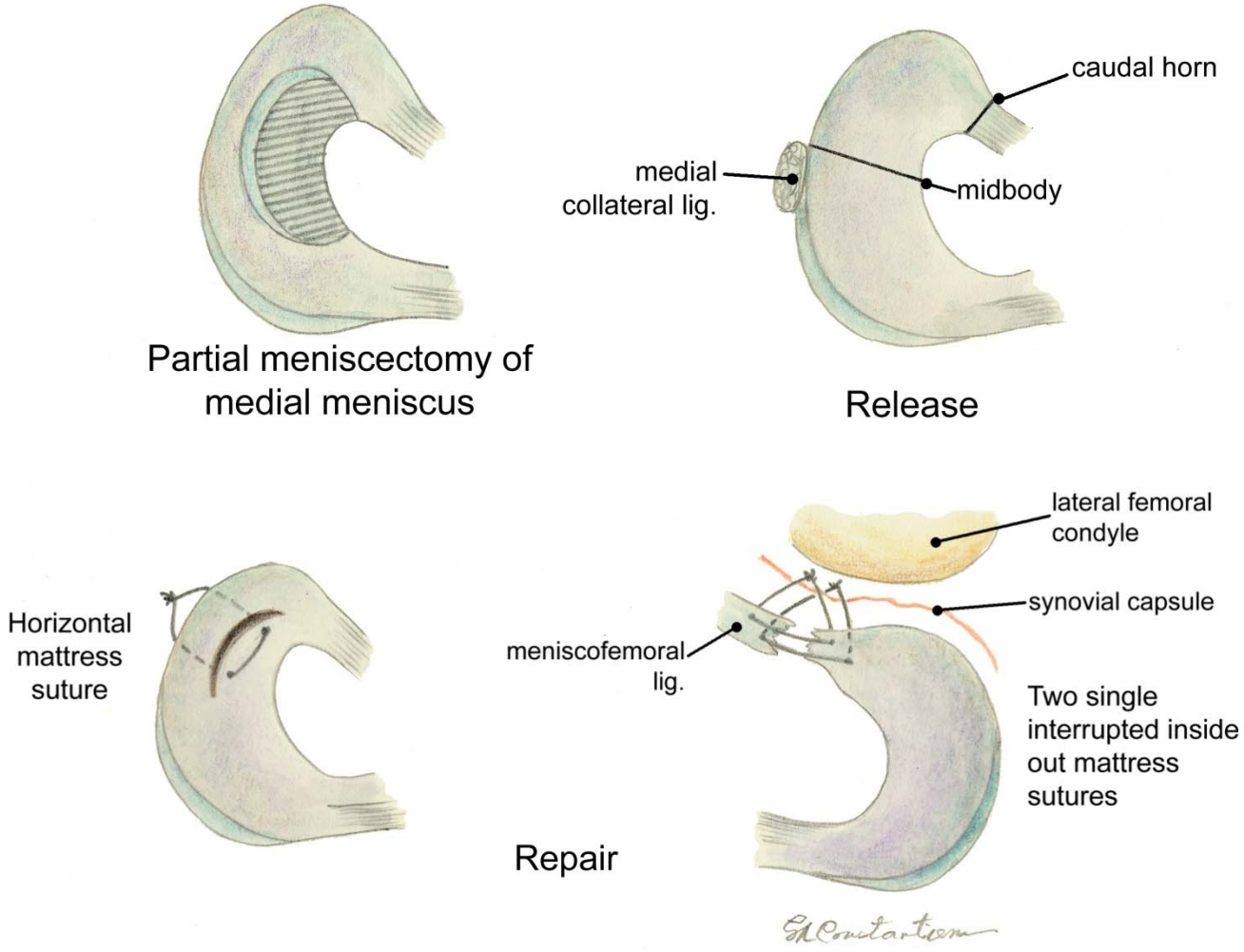
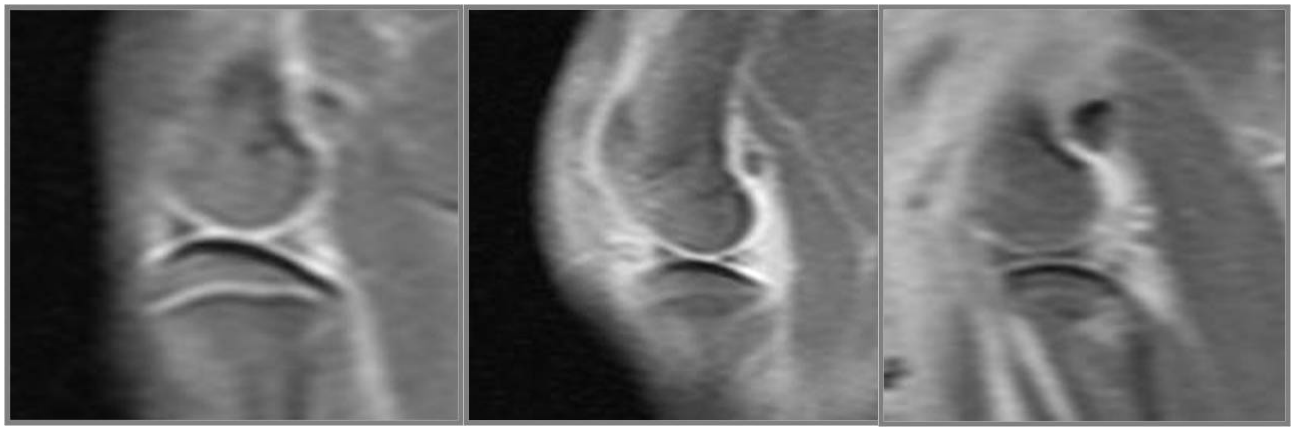


Figure 3: Illustration of therapeutic and prophylactic surgical options for the canine meniscus



Control Sham CrCLX

Figure 4: 5 week post-operative MR images of stifles from one control, sham, and CrCLX dog

Control

CrCLX

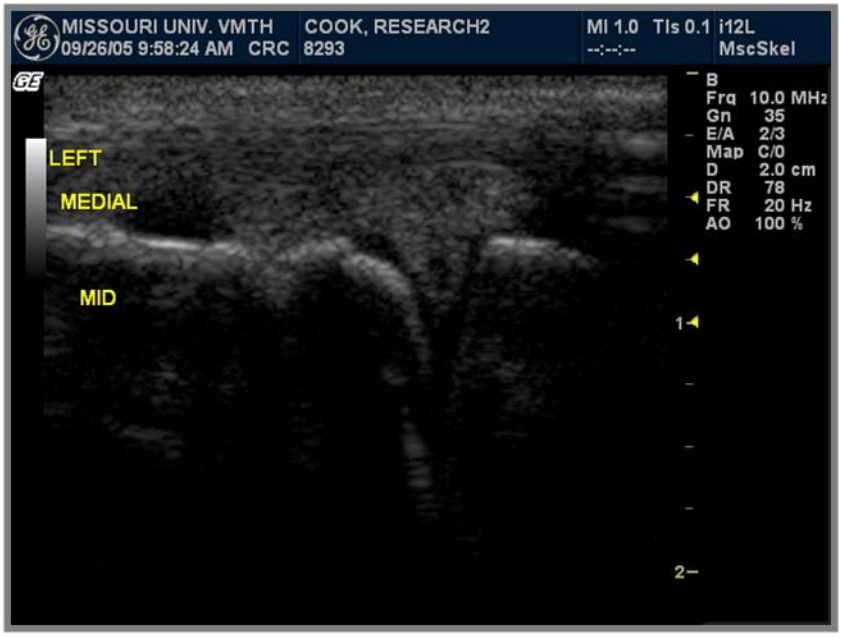


Figure 5: Ultrasound images of one control and one CrCLX stifle joint; the control meniscus is normal, while the CrCLX meniscus is surrounded by effusion and is mildly displaced

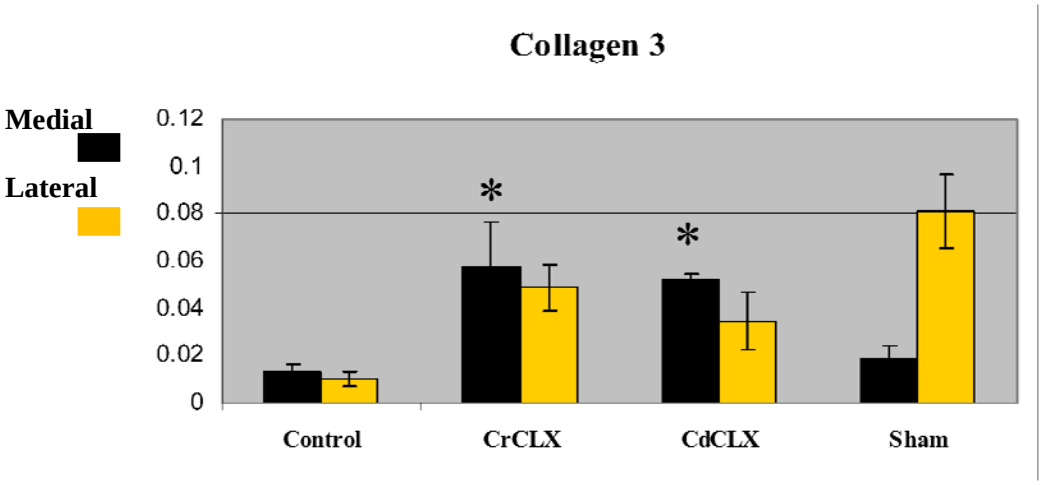


Figure 6: Significant gene expression results for Collagen 3 comparing CrCLX, CdCLX, Sham and Control dogs ($p<0.05$).

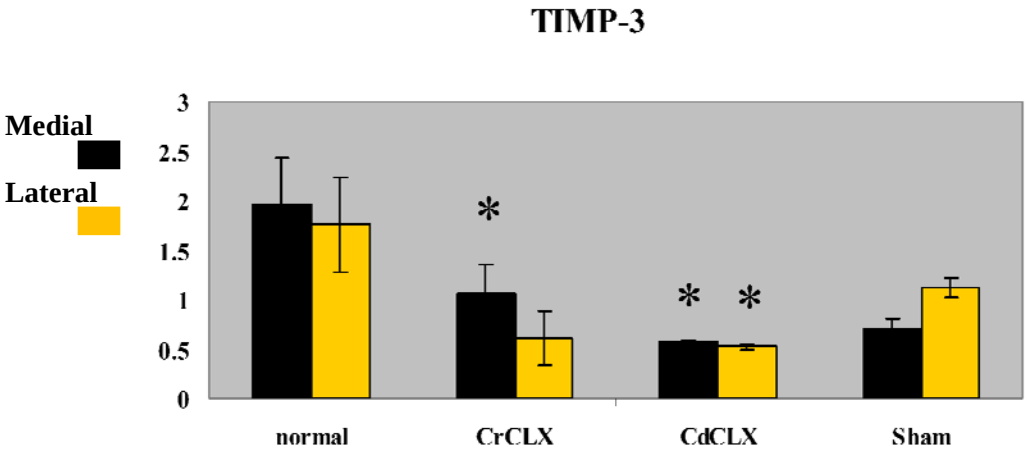


Figure 7: Significant gene expression results for TIMP-3 comparing CrCLX, CdCLX, Sham and Control dogs ($p<0.05$).

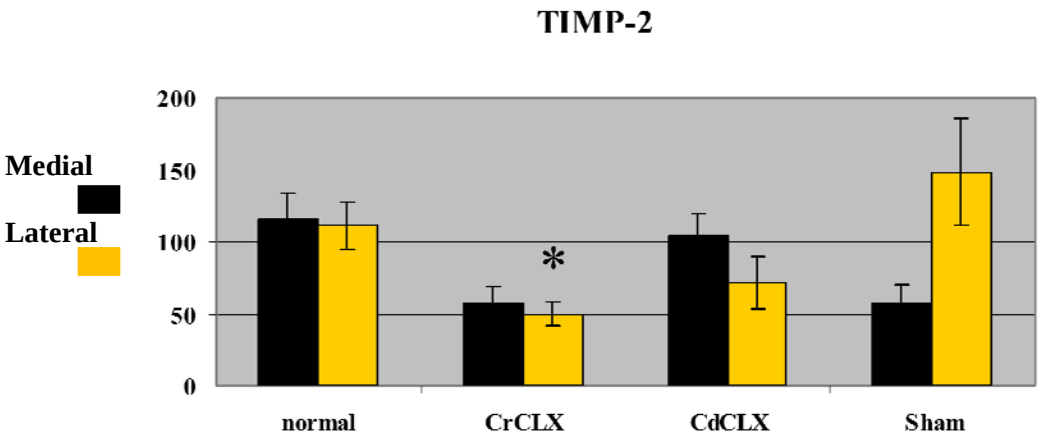


Figure 8: Significant gene expression results for TIMP-2 comparing CrCLX, CdCLX, Sham and Control dogs ($p<0.05$).

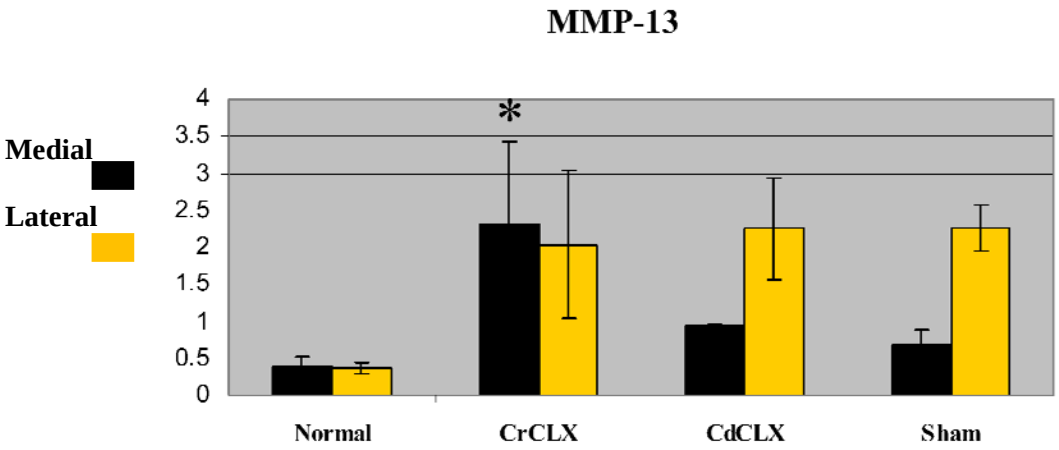


Figure 9: Significant gene expression results for MMP-13 comparing CrCLX, CdCLX, Sham and Control dogs ($p<0.05$).

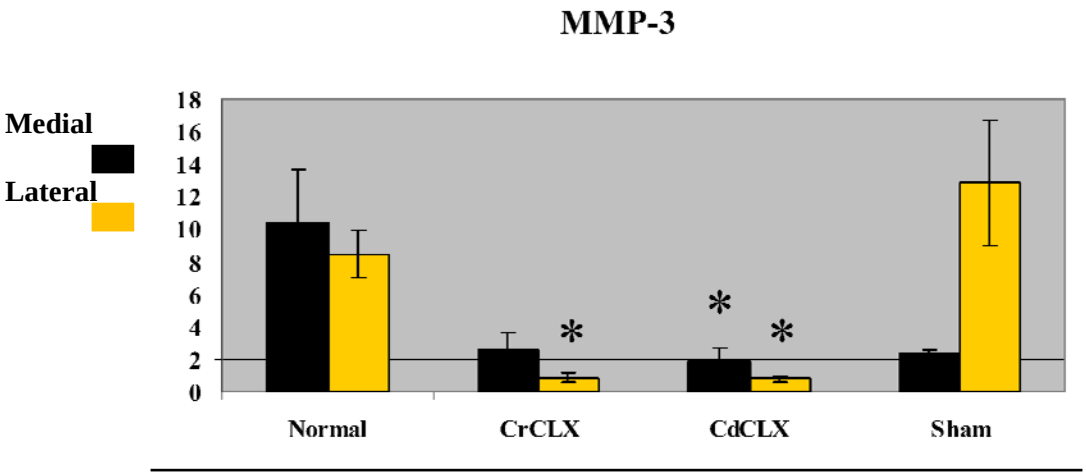


Figure 10: Significant gene expression results for MMP-3 comparing CrCLX, CdCLX, Sham and Control dogs ($p<0.05$).

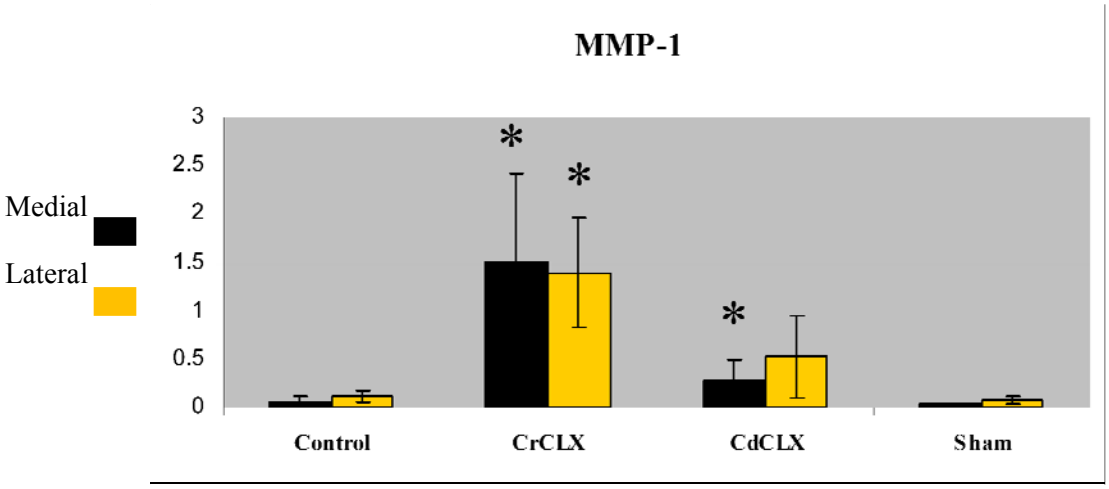


Figure 11: Significant gene expression results for MMP-1 comparing CrCLX, CdCLX, Sham and Control dogs ($p<0.05$).

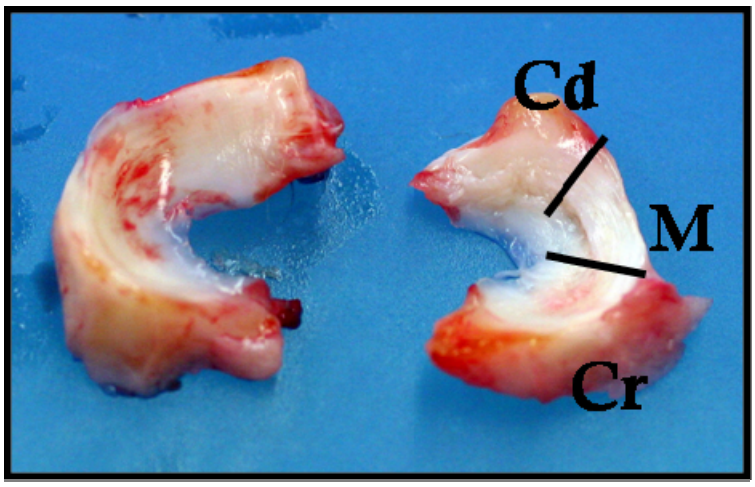
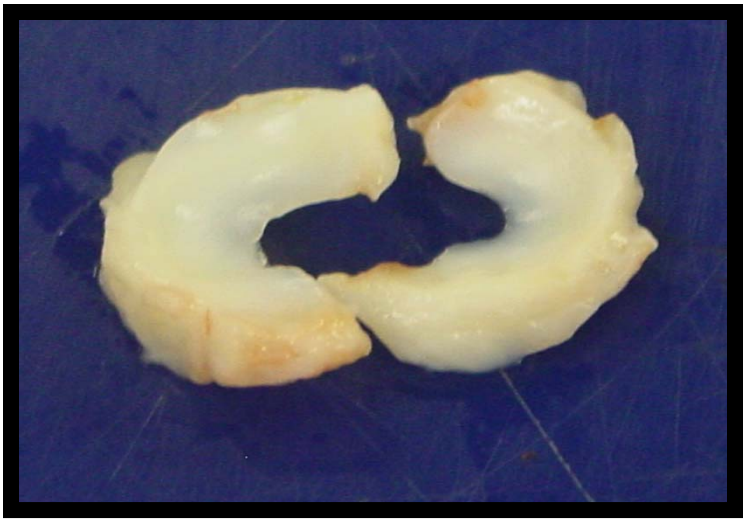


Figure 12: Photographs of medial and lateral menisci undergoing sectioning into cranial, middle, and caudal regions for histologic processing

a)



b)

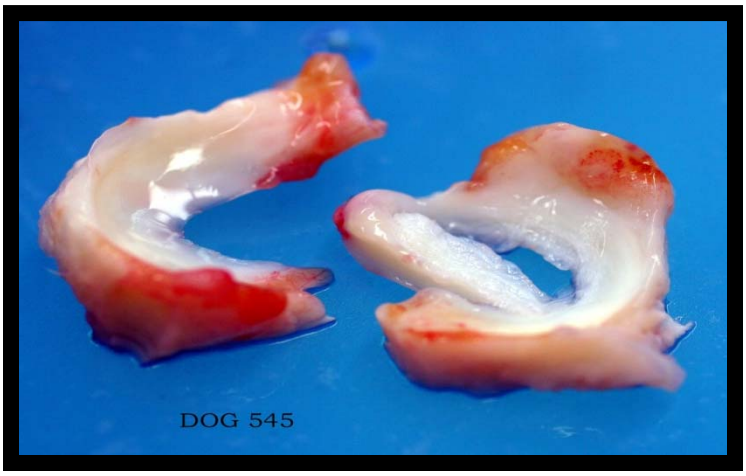
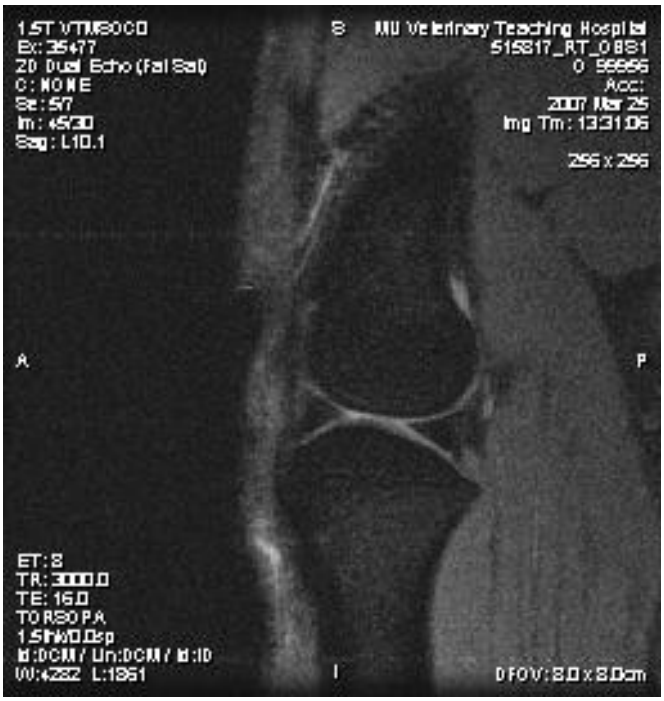


Figure 13: Representative photographs of normal menisci (a), and a grade 4 tear in a medial meniscus (b)

a)



b)

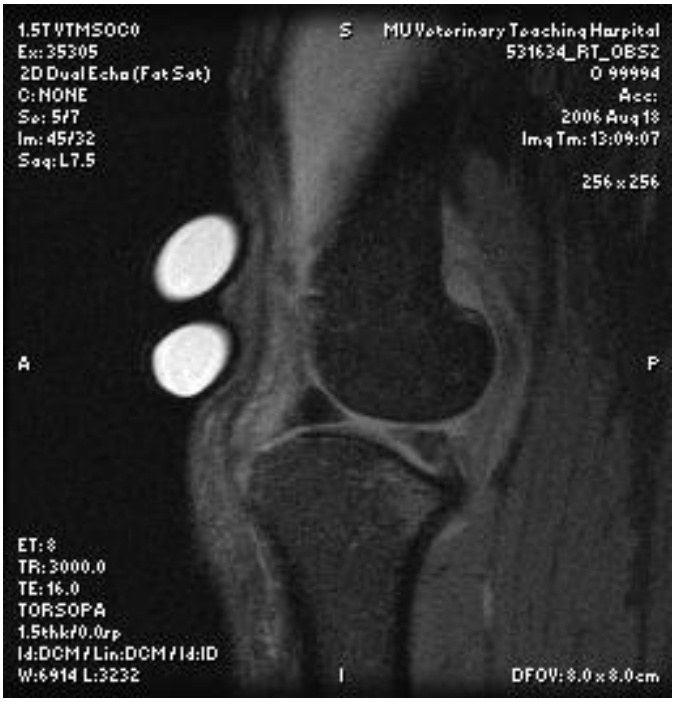
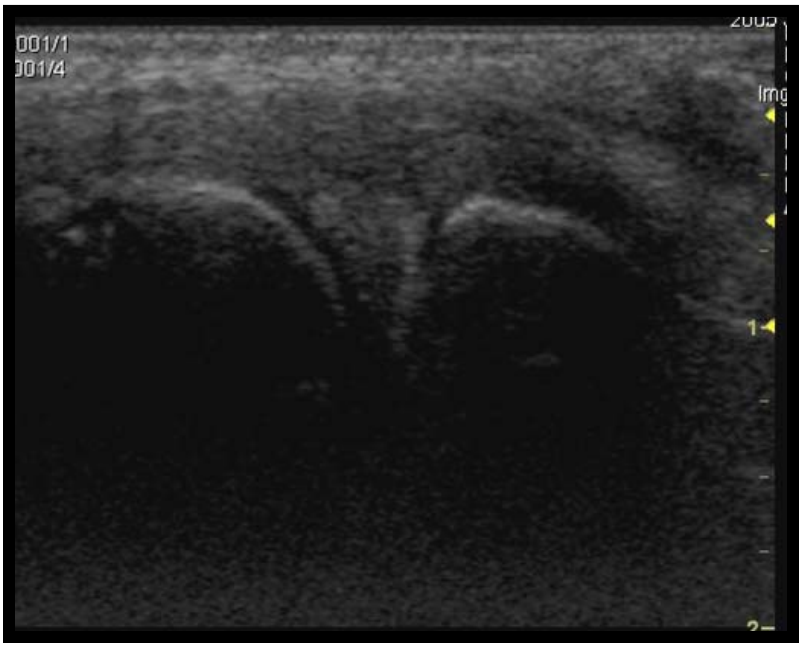


Figure 14: Representative MR images from a dog with a normal medial meniscus(a) and from a dog (b) with a hyperintense signal and bone marrow edema

a)



b)

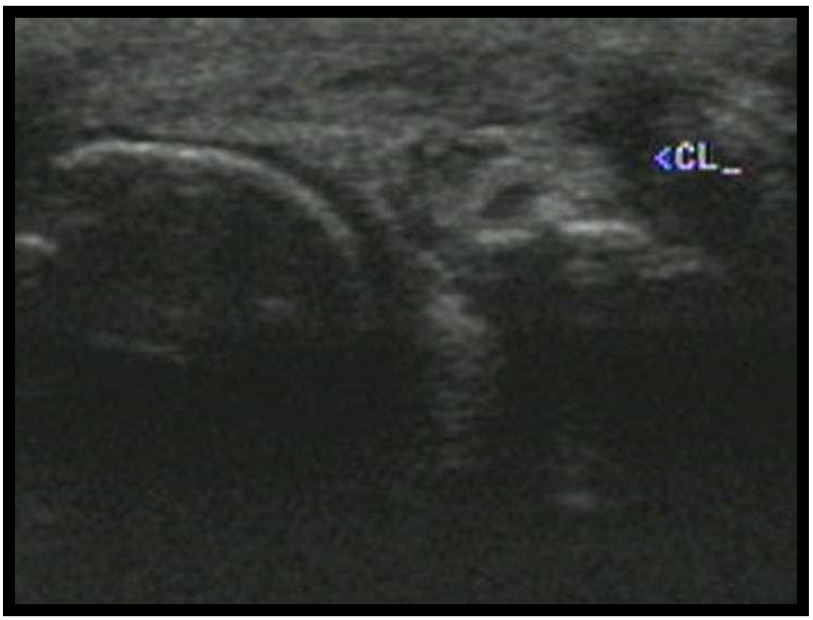
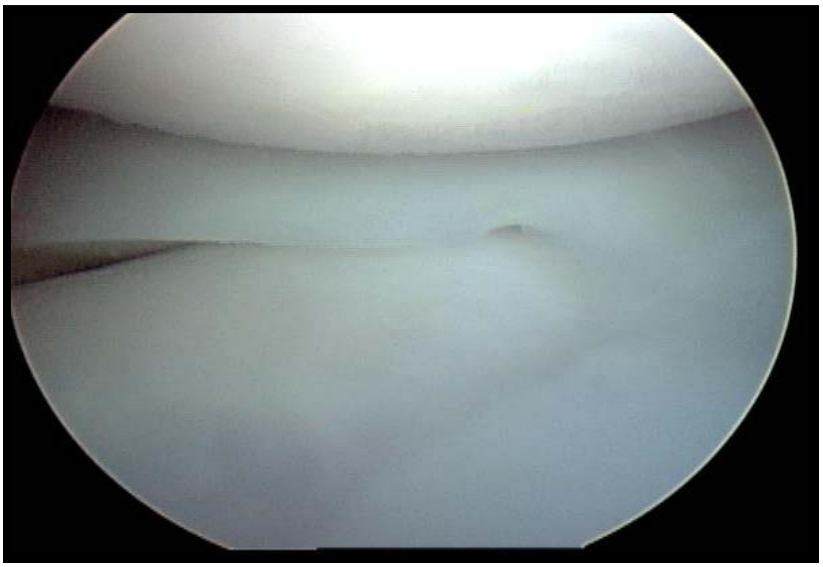


Figure 15: Representative sonographic images from a dog (a) with a normal medial meniscus, and a (b) with a medial meniscus showing a heterogenous echogenicity, flattening and perimeniscal effusion

a)



b)

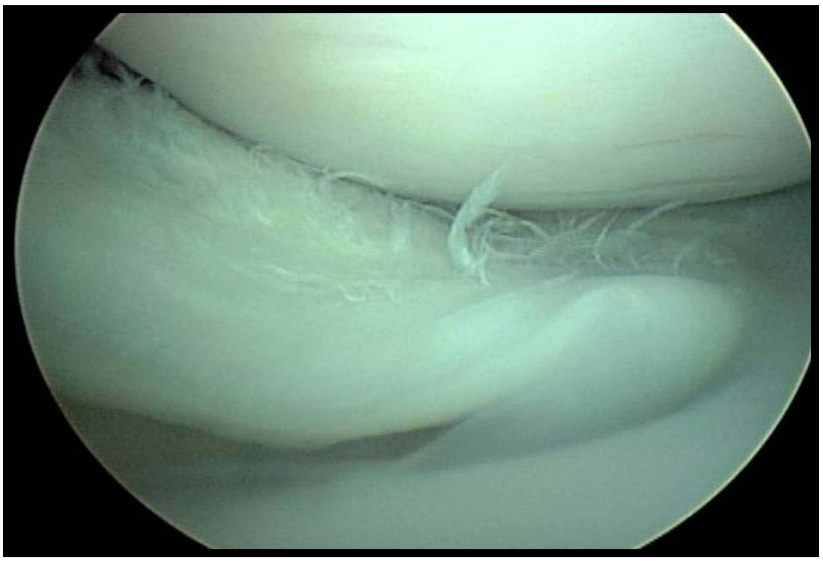
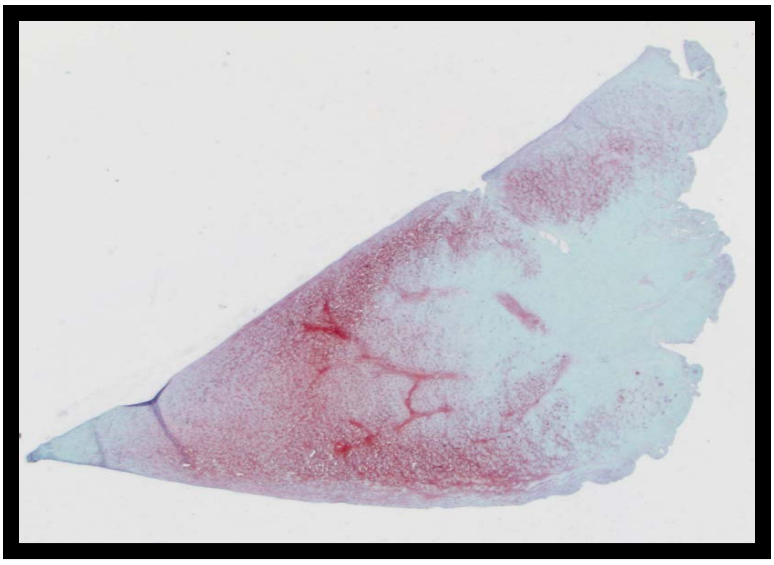


Figure 16: Representative arthroscopic images from a dog (a) with a normal medial meniscus, and a dog (b) with a bucket handle tear of the medial meniscus

(a)



(b)

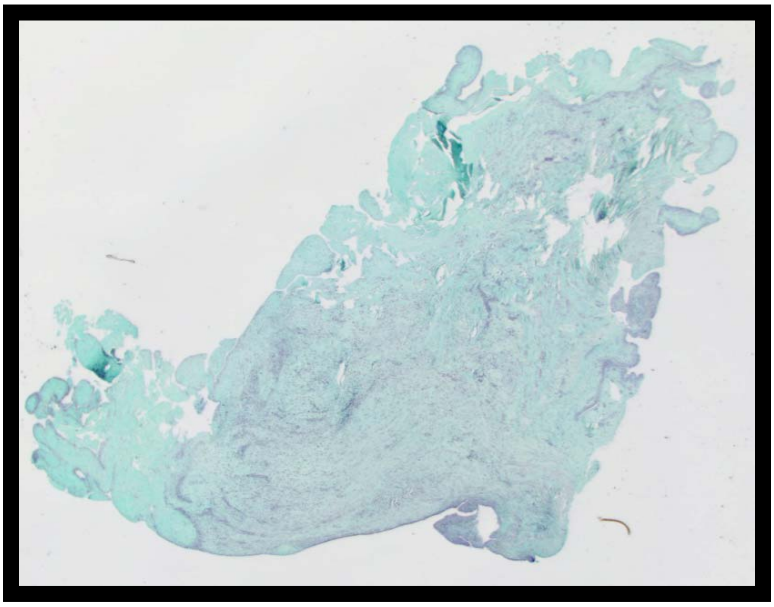
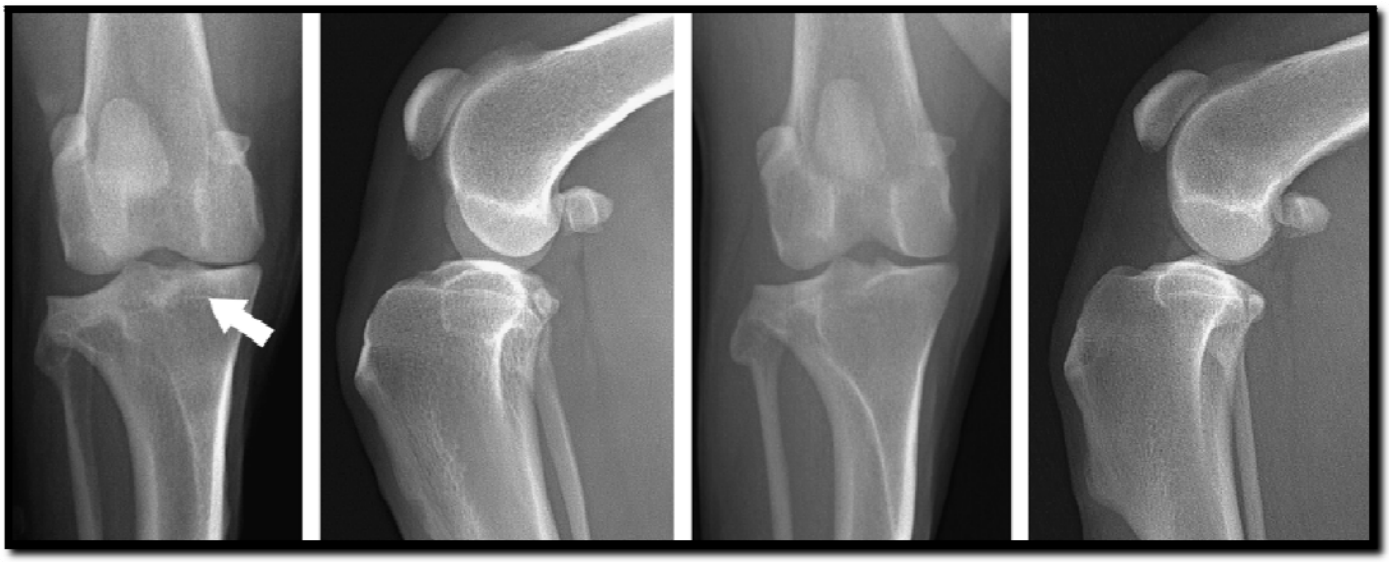


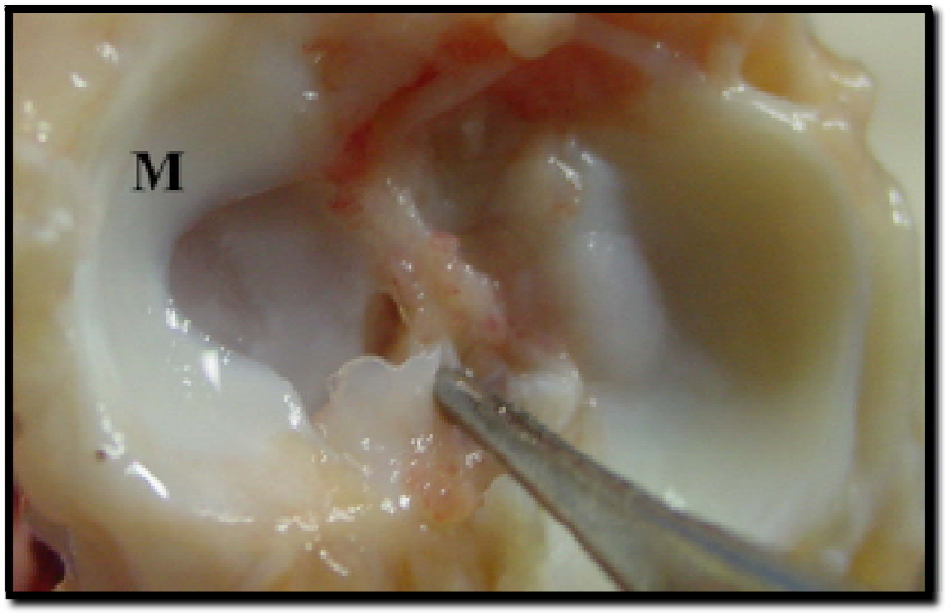
Figure 17: Representative images (H and E staining) of normal medial meniscal histology (a) and a section of medial meniscus (b) with severe changes including architectural disruption and cellular destruction



(a) (b)

Figure 18: Craniocaudal and mediolateral views of stifles 12 weeks after meniscal release (MR; a) or sham (SH; b) surgery. MR stifles had more effusion and degenerative changes, including subchondral bone sclerosis (arrow) and osteophytosis, than SH stifles

(a)



(b)

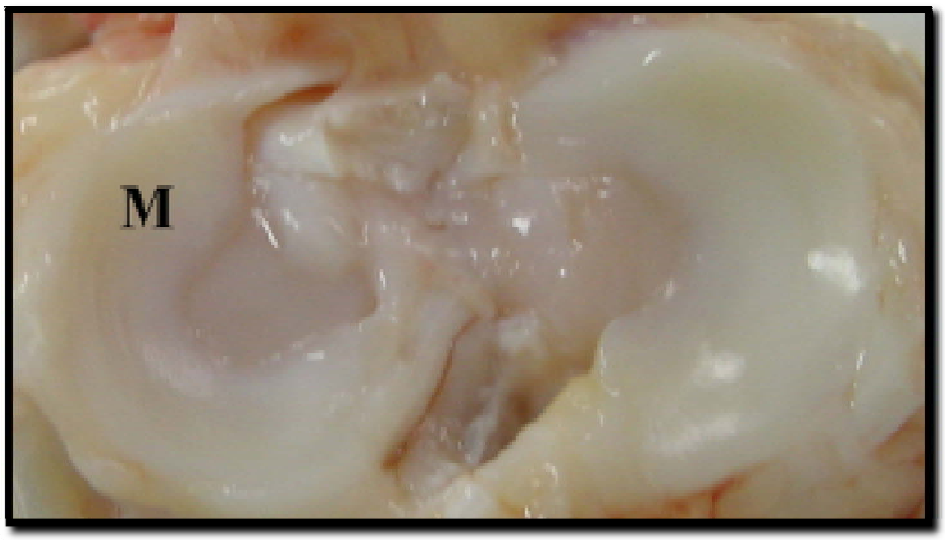
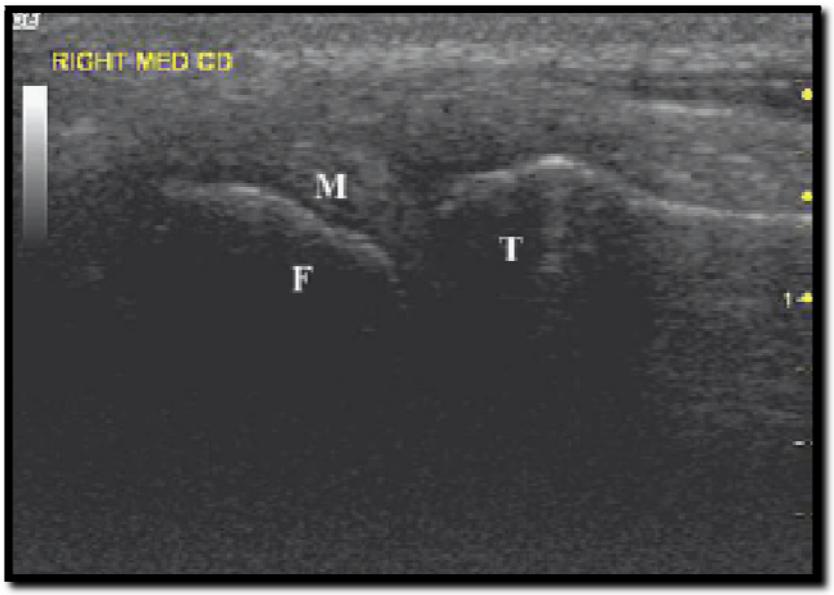


Figure 19: Gross appearance of the femoral surface of medial and lateral menisci 12 weeks after meniscal release (MR; a) and sham (b) surgery. The metallic probe indicates the junction of the caudal horn and caudal meniscotibial ligament of the medial meniscus where the MR was performed. M, medial meniscus

(a)



(b)

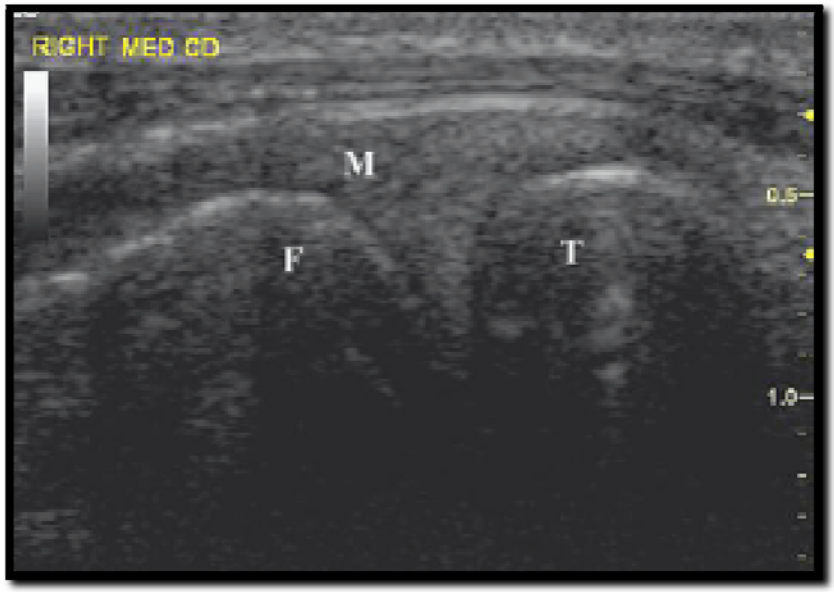
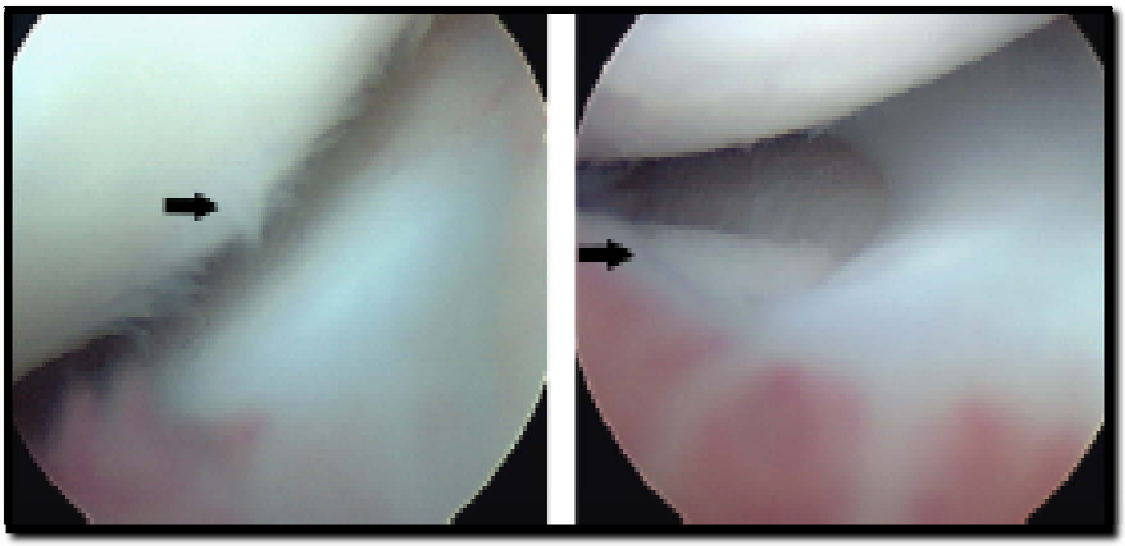


Figure 20: Medial sagittal sonographic views of meniscal release (MR; a) and sham (SH; b) operated stifles 12 weeks post-operatively. The caudal horn of the MR medial meniscus shows abnormal shape (flattening) and mottled echogenicity with moderate stifle effusion, while the same region of the SH medial meniscus is normal. F, femur; M, meniscus; T, tibia

(a)



(b)

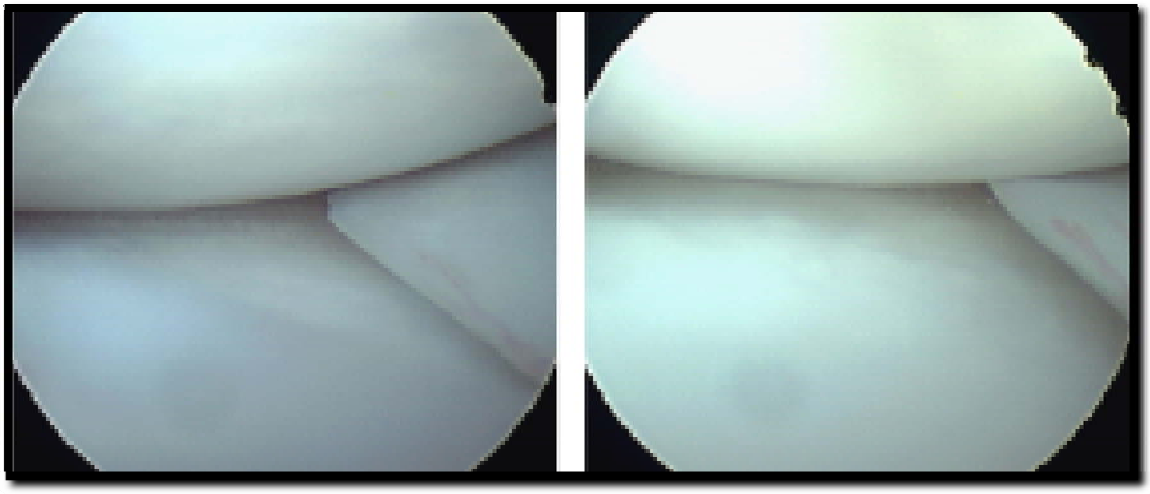
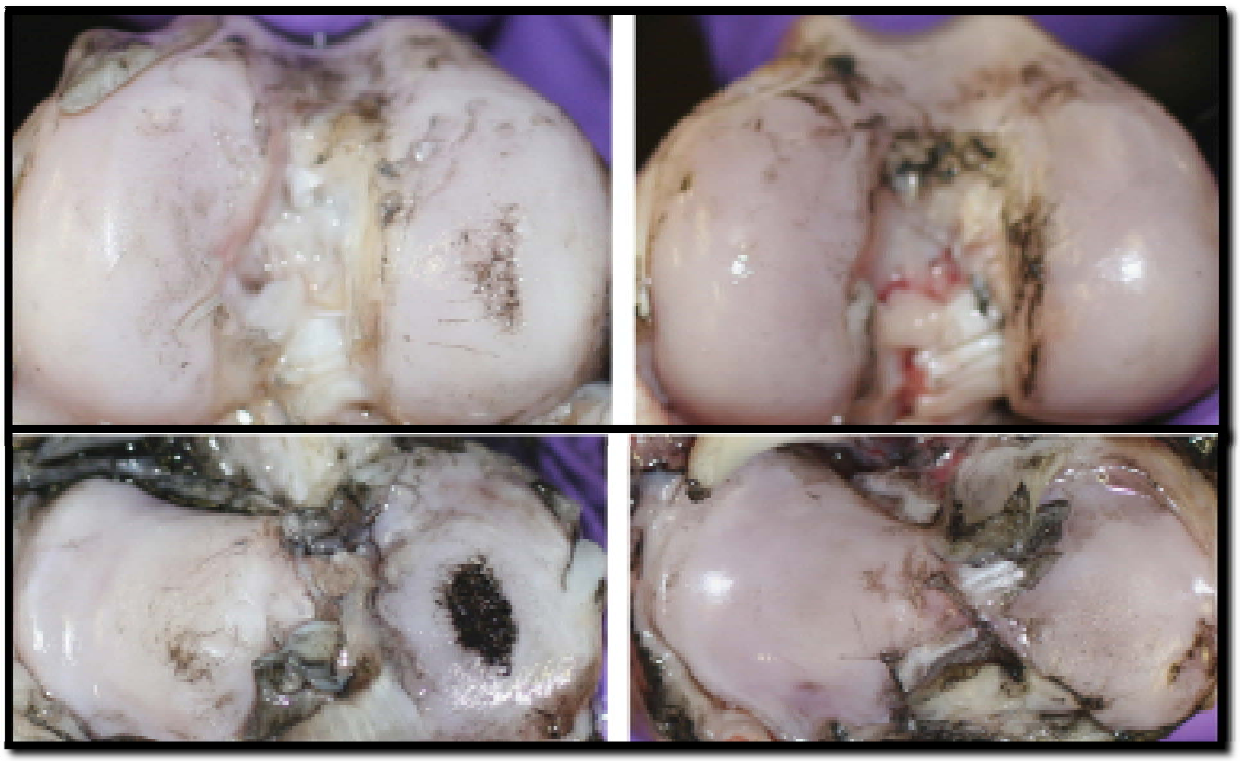


Figure 21: Arthroscopic appearance of the medial compartment of meniscal release (MR; a) and sham (b) operated stifle joints 12 weeks postoperatively. Cartilage lesions (arrows) ranged from ICRS grade 1-4 and were present on both the tibial plateaus and femoral condyles of the medial compartment in MR stifles



(a) (b)

Figure 22: Representative gross appearances of India ink-stained articular cartilage surfaces in meniscal release (MR; a) and sham (SH; b) operated stifle joints. The photographs were taken immediately after tracings of the articular surfaces and lesions were made. Percent area of cartilage damage was significantly greater in MR than SH stifles as indicated here by the darkly stained regions on the MR medial femoral condyle and tibial plateau.

APPENDICES

Appendix 1: Limb function scoring system, previously validated to force plate analysis[134]

0- no observable lameness

1- intermittent, mild weightbearing lameness with little if any change in gait

2- consistent, mild weightbearing lameness with little change in gait

3- moderate weightbearing lameness – obvious lameness with noticeable "head bob" and change in gait

4- severe weightbearing lameness – "toe-touching" only

5- non-weightbearing

Appendix 2: Gross scoring of medial and lateral menisci

0 – normal

1 – all 3 zones present

high total weight

2 – all 3 zones present

low total weight

3 – 1 or more zones missing

Appendix 3: Histologic scoring schematic developed for use in human meniscal tissue[189] and adapted for canine meniscal tissue

Tissue architecture-Tissue loss

0 – normal

1 – minimal disruption or loss

2 – moderate disruption with loss

3 – complete loss of tissue architecture, >50% loss

Cell and Matrix (PG and collagen) content and morphology

0 – normal

1 – minimal alterations of cell and matrix content and morphology

2 – moderate alterations of cell and matrix content and morphology

3 – severe loss/disruption of cells, PG, and collagen

Proliferative response

0 – none

1 – minimal proliferation of cells at synovial junction

2 – proliferation of cells at synovial junction extending into tissue or along surfaces

3 – marked proliferation of cells involving majority of remaining tissue

Appendix 4: Gross and arthroscopic scoring schematic

0 – normal

1 – minimal or minor fraying or tearing (usually restricted to inside edge of meniscus)

2 – mild fraying or tearing (extends deeper into the meniscus)

3 – moderate fraying or tearing (definite structural damage)

4 – severe fraying or tearing (with fibrous tissue proliferation, often bridging between torn areas)

Appendix 5: Sonographic Meniscal Scoring (adapted from Peterfy et al. WORMS scoring)[59]

Region scoring (cranial horn, body, caudal horn)

0 – normal

1 – +/- effusion, normal echogenicity, slight abnormal shape/flattening, no displacement

2 – mild effusion, normal or hypoechoic, abnormal shape /flattening of tibial side of meniscus, mild abaxial displacement

3 – mild to moderate effusion, mottled or hypoechoic meniscus, abnormal shape/ flattening of meniscus, moderate abaxial displacement

4 – moderate to severe effusion, irregular or no meniscal tissue within joint and/or severe displacement of meniscal tissue

Total scoring

0 – all 0

1 – at least one 1, but no>1

2 – 2 in only one region

3 – 2 in more than one region

4 – 3 in one or more regions

5 – 4 in only one region

6 – 4 in more than one region

Appendix 6: MRI Meniscal Scoring (adapted from Peterfy et al. WORMS scoring)[59]

0 – intact

1 – minor radial tear

2 – non-displaced tear or bucket handle tear

3 – displaced tear

4 – complete maceration/destruction

Appendix 7: Articular cartilage scoring

ICRS cartilage injury classification system

0 – normal

1 – malacia, superficial fibrillation

2 – fibrillation, fissures <50% depth

3 – erosion, >50% fissures

4 – full thickness

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