A Novel Stabilization Technique for Cranial Cruciate Ligament Rupture in Cattle

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

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The Ohio State University

2018

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#### Abstract

Cranial cruciate rupture in cattle is a common cause of lameness referred to the stifle and results in gains, production, and long term use of the animal. Several techniques to stabilize the stifle joint in cattle suffering from cranial cruciate rupture have been developed, but are either not appropriate for mature cattle, or have a high complication rate, often leading to failure. The aim of this study was to develop a novel stifle stabilization technique that would be appropriate for large cattle and mitigate catastrophic complications, particularly septic arthritis, by placing an extracapsular prosthesis through bone tunnels in the femur and tibia. Isometric points in the femur and tibia were determined with lateral radiographs to determine the optimal location for placement of the prosthetic implant. Nylon leader line (800lb test) and stainless steel crimp configurations were distracted to rupture to determine the optimum prosthesis. Bone tunnels were created in the locations deemed optimal by the radiographic study, and the optimum prosthetic/crimp configurations were placed through the bone tunnels in 4 live cattle. Lameness was graded weekly for 3 months. Bone tunnel placement was determined to be distal and caudal on the femoral condyles, and cranial and proximal on the tibial tuberosity. Nylon leader line looped and crimped with 3 stainless steel crimps was found to be the optimum configuration. Lameness of cattle with stabilized stifles was not significantly different than lameness of cattle not stabilized. However, all 8 cattle had

medially luxating patellas at the time of slaughter. This technique needs to be implemented in patients with naturally occurring disease to determine its effectiveness for stabilizing cranial cruciate ligament deficient stifles in cattle.

# Acknowledgments

I wish to thank my Master's committee, Drs. Niehaus, Lakritz, Hinds, Durgam, and Jones. I thank Dr. Vanhoy, Sadie Strayer, Megan Lagatta with the Farm Animal section, and Carl O'Brian, and Amanda and Devin Heilman in anesthesia for their support throughout the project.

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## Publications

Chapel EC, Lozier J, Lakritz J, Schober KE. Interventional closure of a patent ductus arteriosus using an amplatz canine duct occluder in an alpaca cria. J Vet Intern Med. 2017;31(4):1221-1224.

Lozier JW, Niehaus AJ. Surgery of the forestomach. Vet Clin North Am Food Anim Pract. 2016;32(3):617-628.

### Fields of Study

Major Field: Comparative and Veterinary Medicine

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### Chapter 1. Introduction

Anterior cruciate or cranial cruciate ligament rupture is a condition observed in most, if not all, domestic veterinary species as well as people. There is considerable research resulting in an abundance of literature focusing on stifle/knee stabilization for both dogs and people with cranial cruciate ligament (CCL) deficiency. In contrast, there is considerably less research aimed at treatment of CCL disease in large animals. Unlike in horses, successful therapy for CCL rupture in cattle is possible. Horses carry a poor prognosis for return to function or salvage post CCL rupture, and no successful repair or replacement techniques have been reported.<sup>1-3</sup> This may be due to cattle's calm temperament and unlike the horse, spend a majority of the time recumbent during convalescence after orthopedic injuries. Additionally, cattle do not experience contralateral limb pathology as frequently as horses do. Finally, most cattle are not expected to perform at the same athletic level like many equine patients do.<sup>4</sup> Cranial cruciate ligament rupture in cattle is likely underdiagnosed as a cause of lameness.<sup>5,6</sup> One report suggests that it may account for 21% of bovine lameness localized to the stifle, with other causes such as septic arthritis, meniscal injury, and collateral ligament injury accounting for the rest.<sup>7</sup> Although the prevailing thought is that 80%-90% of lameness originates below the fetlock,<sup>8,9</sup> a recent paper suggests that upper

limb lameness in feedlots, including stifle injuries, may be a more significant cause of lameness than previously thought.<sup>10</sup>

Left untreated in cattle, CCL rupture and the resulting joint instability leads to severe osteoarthritis subsequent economic losses due to increased recumbency, decreased weight gain and milk production, and reluctance of bulls to mount cows during breeding.<sup>6,11</sup> A recent study suggests that joint disease may be a cause of infertility in bulls, with stifle disease accounting for 93% of these cases.<sup>12</sup>

Anatomy of the bovine stifle

The stifle is made up of femorotibial and femoropatellar joints. The cranial cruciate ligament is a ligament of the femorotibial joint, which is a condylar joint involving articulations between the tibial and femoral condyles. This joint functions mainly in flexion and extension is supported by collateral, cranial cruciate, caudal cruciate, transverse, meniscotibial, and meniscofemoral ligaments.<sup>13</sup> The stifle joint is actually composed of three joint compartments: the femoropatellar, lateral femorotibial and medial femorotibial. In cattle, the medial femorotibial and femorotibial and femoropatellar joints always communicate, and all three joints communicate in 57%.<sup>14</sup>

The distal femur consists of almost parallel medial and lateral condyles with prominent epicondyles on which the collateral ligaments of the stifle originate. The condyles are separated by a deep intercondylar fossa. Cranially on the distal femur, there is a trochlear groove with a larger medial and smaller lateral condylar ridge. Cranial to the lateral condyle is an extensor fossa. The long digital extensor and peroneus tertius have a common origin at the lateral aspect of the lateral trochlear ridge and run distally through the extensor groove on the craniolateral aspect of the tibia.<sup>15</sup>

The proximal tibia contains both a lateral and medial condyles separated by an intercondylar eminence. There is a broad tibial tuberosity and an extensor groove immediately lateral to the intercondylar eminence. The fibula is rudimentary and consists of the fibular head which fuses to the tibia proximolaterally. The lateral collateral ligament inserts at this site.<sup>15</sup>

There are 3 patellar ligaments in the bovine stifle continuous with the quadriceps muscles. The lateral patellar ligament is continuous with the gluteobiceps. The intermediate (middle) and medial patellar ligament along with the medial fibrocartilage and patella form a loop. When the stifle is extended, the proximal part of this loop courses over the proximal medial trochlear ridge of the femur. Similar to horses, this loop forms part of the bovine stay apparatus; by engaging the proximal medial trochlear ridge it will prevent the stifle from flexing without muscular exertion. Although this loop functions mechanically similar to the equine stay apparatus, it is not as robust as the locking mechanism present in the horse.<sup>16</sup> Additionally, medial and lateral femoropatellar ligaments secure the patella to the femur.<sup>13</sup> Both the cranial and caudal cruciate ligaments are intraarticular and extrasynovial.<sup>17</sup> The cranial cruciate ligament originates at the lateral aspect of the intercondylar wall of the femur and inserts lateral to the cranial aspect of the intercondylar eminence. At this point the CCL becomes two flattened bands which are separated by the cranial ligament of the lateral meniscus.<sup>18</sup> The CCL resists cranial displacement and internal rotation of the tibia relative to the femur and, when

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transected, results in about 3cm of cranial tibial movement.<sup>19</sup> The caudal cruciate ligament runs from the intercondylar fossa to the popliteal notch.<sup>18</sup> The caudal cruciate ligament resists caudal movement of the tibia relative to the femur, but when transected alone results in little joint laxity.<sup>19</sup>

Lastly, the bovine stifle has medial and lateral menisci that are crescent shaped. The lateral meniscus is attached to the tibia via the cranial and caudal meniscotibial ligaments, and femur via the meniscofemoral ligament. The medial meniscus is attached to the tibia via the cranial and caudal meniscotibial ligaments, but is also adhered to the medial collateral ligament and joint capsule, making it more susceptible to concurrent injury with joint capsule trauma.<sup>15,18</sup>

Presentation, Clinical Findings, Diagnostics

Cranial cruciate ligament rupture in cattle has two presentations: acute traumatic rupture, and rupture secondary to chronic osteoarthritis. Bulls more commonly present with cranial cruciate rupture secondary to severe osteoarthritis, while cows more commonly develop cranial cruciate rupture secondary to acute trauma.<sup>20</sup> It has been hypothesized that cattle, specifically bulls with a "straight hock" conformation are at an increased risk of developing osteoarthritis of the femorotibial joint, leading to cranial cruciate ligament rupture.<sup>11,19</sup>

Clinical signs of cranial cruciate ligament rupture include non-weight bearing lameness accompanied by effusion,<sup>9,17,19</sup> though in more chronic cases, the animal may be partially weight bearing in the affected limb.<sup>9</sup> Crepitus may be palpable and audible radiating from the affected limb as the patient shifts weight.<sup>19</sup> If the tibia displaces cranially to the

femur, the tibia is considered to be in a state of "cranial drawer" which is a sign of cranial cruciate ligament deficiency. An examiner can determine if the tibia is in cranial drawer: the examiner places his or her knee on the plantar aspect of the hock, placing both hands on the tibial crest, and sharply pulling in a caudal direction.<sup>17</sup> Alternatively, the examiner's hands may be placed on the tibial crest from a cranial direction and sharply pushing caudally. Both of these maneuvers functions to replace a cranially-displaced tibia. Increased internal rotation of the tibia is another sign of cranial cruciate ligament deficiency and can be evaluated by placing a hand on the hock and stifle, and outwardly rotating the hock.<sup>9</sup> Not all cattle with cranial cruciate ligament rupture will have a positive drawer test, highlighting the importance of radiographs for diagnosis.<sup>20</sup> If radiographs are taken, the tibia will be displaced cranially (cranial drawer) in a lateral to medial projection, with the intercondylar eminence cranial to the femoral condyles. In a normal stifle, there should be complete overlap of the tibial eminences by the femoral condyles. Caution needs to be used when interpreting these radiographs, as slight obliquity can artifactually create this sign and true lateral radiographic projections of the stifle in mature cattle can often be difficult to obtain due to the size of the patient and the muscle mass in this area. Additional avulsion fractures of the eminence, avulsion fractures of insertion sites of ligaments, mineralization of the cranial cruciate ligament, degenerative joint disease, or calcified meniscus may also be observed in cattle with cranial cruciate ligament rupture.<sup>9</sup>

If arthrocentesis is performed, fluid should be taken from the area of maximum distention and will reflect sterile inflammation (increased white blood cells and protein), but is

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typically not necessary if physical exam findings and radiographs are supportive of cranial cruciate ligament rupture.<sup>9,17</sup>

Arthroscopy may allow direct visualization of the cranial cruciate ligament in cattle permitting a definitive diagnosis of cranial cruciate injury. Bovine stifle arthroscopy presents a unique set of challenges compared to equine arthroscopy: 1.) significant periarticular fat, 2.) extensive proliferative synovitis frequently encountered in chronic cases, 3.) a thick fibrous joint capsule. These challenges may limit visualization and preclude accurate diagnosis. Besides arthroscopic debridement of a frayed ligament, arthroscopy is generally not regarded as therapeutic in cattle or horses with cranial cruciate ligament deficient stifles. Additionally, arthroscopy is not a necessary diagnostic if physical exam and radiographs are supportive of cranial cruciate ligament rupture.<sup>17,21,22</sup>

Conservative therapy for cranial cruciate rupture in cattle

Typically, cattle left untreated have a poor prognosis and will progressively worsen in clinical signs, lose condition, develop muscle atrophy of the effected limb, begin to breakdown in the contralateral limb, and may become unable to rise. Therefore, patients in which perceived value does not justify the expense of therapy should be salvaged.<sup>17,19</sup> Cattle with only a partial cranial cruciate rupture may be treated conservatively. Most importantly, the animal should be placed in a stall with good footing. A manure pack is ideal. A restricted diet to prevent weight gain or encourage weight loss may be indicated, as patients weighing less than 450kg have a better prognosis.<sup>19</sup> These animals should be provided with analgesics (NSAIDs), and may improve within 2-3 months.<sup>19</sup>

Surgical stabilization

Stabilization of the CCL deficient bovine stifle is challenging for several reasons. One study determined that the ultimate tensile load needed in cranial cruciate ligament prosthesis would need to be 1-1.5 times the body weight of the animal,<sup>23</sup> while another determined the mean rupture force of the native cranial cruciate ligament in cattle to be 4541N.<sup>24</sup> A more recent study found that the mean rupture force is independent of the weight of the animal, and that rupture begins at a mean of 3315N and is complete at a mean of 4372N.<sup>25</sup> There is considerable tension and motion over the surgical site during the recovery and convalescent period, creating a high risk of surgical site complications. Together, these challenges put the patient at a relatively high risk of catastrophic failure.<sup>9</sup> All of the stabilization procedures are generally performed with the patient under general anesthesia. Standard preoperative procedures including fasting the patient for 12-48 hours prior to the anesthetic episode is recommended. An NSAID and a broad-spectrum antibiotic is recommended preoperatively and continued as needed following surgery.<sup>9</sup> Owners should be made aware that pursuing surgery would likely prohibit short-term salvage due to drug residue in the tissues.

Currently, the only extracapsular technique that has been described in cattle is joint capsule imbrication. The aim of this technique is to tighten the peri-articular tissues surrounding the stifle joint. Ultimately this technique results in increased fibrous connective tissue around the joint providing stabilization to the joint which hopefully will delay the onset of DJD.<sup>17</sup> For this technique, the patient should be placed in dorsal or dorsolateral recumbency and an S-shaped incision is made over the stifle starting 5cm

proximally to the patella, extending first medially then moving laterally, and ending 5 cm distal to the tibial crest.<sup>17</sup> The skin and subcutaneous tissues are undermined until periarticular fascia is reached. Evacuation of the synovial fluid from the distended joint can be beneficial to allow the surgeon to more easily and effectively tighten the imbrication sutures during the procedure. The limb is placed in full extension prior to tightening of the sutures. Lembert sutures using large, non-absorable sutures are recommended for joint imbrication. Braided number 5 polyester is commonly used for the procedure. Imbrication sutures are placed from patella to tibial crest at the level of the lateral and medial patellar ligaments. 1-2 more rows are placed over the previous rows of sutures as the strength of the fascia permits. Lateral antirotating sutures have been described but are not recommended due to the proximity of the peroneal nerve at this location.<sup>9,26</sup> Advantages of the imbrication procedure include minimizing the potential for iatrogenic, intra-articular infection or cartilage damage and thus less risk of catastrophic failure. Additional advantages include less procedural time compared with implant or graft placement, thus requiring shorter anesthetic times, and it is less technically challenging to perform, without the need for special equipment or implants.<sup>17</sup> Prognosis for satisfactory outcome with this technique has been reported to be as high as 59%, and those animals presenting 2 months or more after the initial injury performed worse than those presenting acutely.<sup>26</sup> This procedure is likely less effective in heavier patients and should be reserved for cattle under 730kg.<sup>17</sup>

Current techniques in human surgery rely upon ligament replacement. Ligament replacement techniques can be divided into two categories:

- Anatomical, in which tunnels are drilled through the attachment of lateral collateral ligament to the intercondylar space in the lateral condyle of the femur, from cranial to the tibial imminences to the medial tibial tuberosity, and then across the tibial tuberosity for passage of the ACL prosthetic in a normal anatomic location.
- 2. Non-anatomical (over-the-top) method, in which a graft, attached distally on the tibia, is pulled caudally around the lateral condyle and secured.<sup>27,28</sup>

Ligament replacement in cattle was first reported in 1968 for ligament repair in a bull in which a non-anatomical technique was performed. The surgical procedure involved collecting a strip of fascia lata which was then passed through a hole drilled in the lateral femoral condyle, passed through the joint, and secured to the middle patellar ligament. The bull was able to mount and breed cows 5 months post-operatively and subjectively had minimal pain, but would not fully flex the limb when observed ambulating.<sup>29</sup> Another non-anatomical intra-articular method has since been developed for stifle stabilization after cranial cruciate rupture in cattle using a gluteobiceps graft that is used in a true "over-the-top" method. The surgical procedure is described below: An incision is made through the skin from the greater trochanter of the femur to the lateral aspect of the patella, at which point it is curved to be parallel with the lateral patellar ligament and extended to the tibial crest. The fascia lata is incised over the cleavage of the vastus lateralis and gluteobiceps muscles. A 2cm wide, full-thickness strip of fascia is then dissected from the cranio-medial margin of the gluteobiceps muscle, distally through the fibrocartilaginous thickening of the lateral epicondylar bursa, and then continued as the

lateral half of the lateral patellar ligament taking up to 50% of the width of the patella ligament as the graft. Distally, this fascial graft is attached to the tibial crest. The femorotibial joint is then approached via arthrotomy between the lateral and middle patellar ligaments and the patellar fat pad is reflected. Inspection of the CCL can be challenging due to the depth. A graft passer is used to pass umbilical tape through the joint and over the lateral condyle. The graft is then sutured to this tape and pulled through the joint by cycling the leg through extension and flexion movements. The graft should be placed immediately against the femur, and care should be taken not to allow any of the proximal portion of the gastrocnemius muscle to separate the graft from the femur. The stifle is placed in 140 degrees of flexion and the tibia externally rotated while the graft is pulled tight and attached to the lateral aspect of the femur.<sup>9</sup>

The ultimate tensile load of this graft in one study was determined to be only 26.8% of the native cranial cruciate ligament at 4-5 months post-operation.<sup>30</sup> Another study found the graft tensile strength to be 30% of the contralateral cruciate. Furthermore, this study found no damage to the menisci or articular cartilages also at 4-5 months postoperatively.<sup>6</sup> Reported success rates range from 61%-75%.<sup>6,30</sup> Another source reports a success rate of 43% in bulls over 900kg, and 85% in cows weighing less than this.<sup>9</sup> The poorer success in adult bulls is supported in other literature.<sup>6</sup> Reported complications with this technique include incisional failure often leading to septic arthritis and, less commonly, graft failure, as well as contralateral cruciate ligament rupture.<sup>6,9,30</sup> A

variation of this over the top method by using a portion of the intermediate patellar ligament through a femoral condylar tunnel is described in a calf with success.<sup>31</sup> Anatomical reconstruction has also been reported in cattle. Hamilton and Nelson reported on the initial technique in which bone tunnels were drilled through the lateral condyle of the femur to the intercondylar fossa.<sup>32</sup> A second tunnel was created from the medial aspect of the tibia to the tibial attachment of the cranial cruciate ligament. A third hole was then drilled across the tibial tuberosity. A graft was then passed through the holes and sutured to itself in a loop as well as sutured to the middle patellar ligament and incorporated in the closure. Fascia, skin, Dacron in silicone rubber, Teflon, fascia and Teflon, and skin and Teflon were used and compared. Osteoarthritis was a common finding in the stifles in which synthetic prosthesis were used, and synthetic prosthesis were found to be fractured and fragmented. Fascial grafts showed signs of fibroblastic activity and vascularization within the joint and bone tunnels. In short, the autoplastic materials were accepted by the tissues of the joint, became incorporated into the drill canals in the bone by new tissue growth and appeared to be maintained and nourished or were undergoing gradual replacement with fibrous connective tissue. The alloplastic materials elicited a mild inflammatory and possible antigenic response.<sup>32</sup> In a subsequent study, fascia stretched and decreased in thickness while skin remained the same size and maintained tensile strength over 12 to 14 months. Skin grafts were surrounded by fibrous connective tissue. This finding along with the ease of obtaining skin from the edge of the incision led the authors to conclude that skin was the ideal replacement tissue for this technique of stabilizing stifle joints following CCL rupture in cattle.<sup>33</sup> Subsequent

investigations have been performed investigating the use of nylon as a synthetic prosthesis. One in-vitro study found that 3-stranded cords of 450-lb test monofilament nylon fishing line most closely approximated the strength of the cranial cruciate ligament.<sup>24</sup> Large nylon cables passed through bone tunnels, and secured to the lateral femoral epicondyle with a 5-hole plate has been performed with success similar to that of the non-anatomical technique, with reported success in 6 of 9 cows.<sup>9</sup>

Studies investigating synthetic replacement materials in dogs haves shown mixed results. Park et al, investigated the use of Dacron as a replacement material with promising results,<sup>34</sup> however more recent work by Barnhart et al. investigated the utility of a modern composite material with poor results.<sup>35</sup> This work parallels findings in human medicine in which early synthetic grafts were inferior compared to autografts. Conversely the same human study found no difference in "new generation" synthetic ligaments compared to autografts for ACL replacement. Materials defined as "new generation" replacements were polyglycolic Acid-Dacron and polyethelene.<sup>36</sup> Interestingly, the graft material showing early promise in canine literature was Dacron.<sup>34</sup>

Despite a study in canine literature suggesting that the non-anatomical reconstruction method may result in superior outcomes to a bone tunnel technique with bovine xenografts,<sup>37</sup> a bovine study examining the two methods found a higher ultimate tensile load with a bone tunnel technique than that obtained via an "over the top" method.<sup>30</sup> The over-the-top method is frequently performed in humans with anterior cruciate ligament (ACL) ruptures in skeletally immature patients. Studies comparing bone tunnels and over

the top methods in humans have found little clinical significant difference between the two.<sup>27,28,38,39</sup>

It is recommended for all surgical techniques, patients remain in a box stall for 6-8 month, and skin suture removal be delayed to 3 weeks due to the tension during flexion of the stifle. Initially, patients are usually toe-touching lame, but will gradually increase weight bearing after a few days. Patients should be markedly improved by 2 months, and continue to improve until around 6 months.<sup>9</sup>

#### Extracapsular Stabilization

Extracapsular stabilization techniques have been performed in people and dogs alike. In people, this was initially performed via tenodesis of iliotibial band, with cast immobilization. These techniques have since been abandoned as full knee biomechanics were not restored and often resulted in degenerative changes in the knee.<sup>40</sup> More recently, researchers in people with ACL deficiency have attributed much of the internal rotation of the tibia to disruption of the iliotibial band and other lateral extracapsular structures likely damaged at the time of ACL damage. Therefore, internal repair combined with an extracapsular stabilization is becoming more popular.<sup>40</sup>

A common extracapsular stabilization technique in dogs is termed the lateral suture. After a craniolateral arthrotomy to assess and, if necessary, treat the menisci, the joint capsule is closed and imbricated. The lateral fabella is identified and a suture is placed proximally to the fabella in the region of the lateral fabello-femoral ligament. One to two holes are drilled in the tibial crest. The suture is passed through one hole and back under the patellar ligament or through both holes to complete the mattress suture. The limb is held in 135 degrees of extension and the suture is tightened to neutralize cranial drawer and excessive internal rotation, ensuring that stifle range of motion is not affected. Crimped nylon leader line is shown to be superior for this procedure in dogs. Different anchorage sites have been tested in canine patients. Increasing body weight is associated with increased complications with this procedure.<sup>41</sup>

Another technique, termed the tightrope technique has also been described. In this technique, a bone tunnel is drilled from a point on the caudal, lateral, and distal aspect of the femur to a point more proximally (approximately at the level of the proximal aspect of the trochelear ridge) on the medial side of the femur. Another bone tunnel is created from the proximal aspect of the tibia laterally to the proximal metaphysis of the tibia medially. A synthetic material is then passed through the tunnels, secured by buttons on the medial aspect of the limb. The material traverses the joint on the lateral aspect of the joint running from the emergence on the caudolateral femur to the proximocraniolateral tibial. Comparison of this technique to tibial plateau leveling osteotomy (TPLO) in dogs showed that the outcomes were not different in regards to radiographic findings or client evaluated lameness. The "Tightrope" technique was associated with a shorter anesthesia time compare to TPLO.<sup>42</sup> When compared to other extracapsular techniques, the "Tightrope" technique performed superiorly, with more resistance to elongation.<sup>43</sup> Studies examining materials have shown nylon to be a very promising material for extracapsular stabilization in dogs.<sup>44</sup> Additionally, ethylene oxide sterilization of nylon results in preserved strength and minimizes elongation of the material.<sup>45</sup> However,

several studies have shown that polyethylene suture is stronger, stiffer, and has less elongation than nylon leader material for the use in the dog.<sup>46,47</sup>

Additionally, there have been studies in dogs determining the best method of securing an extracapsular stabilization. In one study, less elongation of nylon leader line was observed when materials were crimped compared to being knotted.<sup>45</sup> Another study found that mode of failure with crimps includes both breakage at the crimp, or slippage through the crimp. The type of crimper used also made a significant difference regarding ultimate strength of the suture-crimp construct.<sup>48</sup> No such studies have been performed in cattle.

Ligament repair and other methods in dogs

Although recent studies have identified a promising ligament grafting technique using hamstring or deep digital flexor tendon allografts,<sup>49,50</sup> osteotomy techniques remain the mainstay for stifle stabilization after CCL rupture in dogs.<sup>41</sup> One study has shown that when compared to osteotomy or extracapsular stabilization, an intracapsular technique was inferior.<sup>51</sup> Osteotomy techniques in dogs are aimed at changing the dynamics of the stifle and reducing forces resulting in cranial translocation of the tibia. When comparing TPLO and tibial tuberosity advancement (TTA), TPLO results in better outcomes for patients at a trot than TTA. As canine patients increase in size, complications arising from these techniques may increase.<sup>52</sup> If tibial osteotomies behave similarly to tibial fractures in cattle, osteotomy techniques are not advisable because tibial fractures in cattle have a poor prognosis due to challenges with providing adequate stabilization.<sup>53</sup>

### Femorotibial Isometry

Regardless of the stabilization technique used, if a graft is to be implemented, finding the isometric points is paramount. If the fixed points for the graft become closer during range of motion, the graft will loosen, allowing cranial translation of the tibia in relation to the femur. If the distance between the two points lengthens, there will be increased load on the implants, potentially resulting in stretching or rupture of the implant, as well as restriction of motion of the joint.<sup>54</sup>

Several studies investigated femorotibial isometry in dogs for extracapsular stabilization with suture. In one study, lead spheres were driven into the fabella and 11 sites along the proximal tibia, as well as 6 distal femoral sites. Lateral radiographs were then taken with the limb in 150°, 130°, 110°, 90°, 70° and 50° of flexion. The most ideal femoral location was found to be to be on the very caudal edge of the lateral femoral condyle (named F2). Proximal tibial locations underwent elongation under flexion, while cranial and distal tibial locations shortened.<sup>54</sup> Subsequently, a study used laterally projected radiographs to identify the femoral origin and tibial insertion of the CCL in dogs determining that the location distal and caudal on the condyle or around the fabella are acceptable sites, while a very cranial and proximal site in the tibia is ideal.<sup>55</sup> Another study placed suture in different locations and then measured tension at 150°, 130°, 90° and 50° of flexion. This study found a similar site for isometry in the femur (F2), but a proximo-caudal (T3) tibial site to be the ideally matched locations.<sup>56</sup> A slightly more cranial (T2) site for the tibial anchorage location was supported by a study which employed the tightrope system and

tested cadaveric limbs for cranial drawer, cranial tibial thrust, internal/external, range of motion and varus/valgus.<sup>57</sup>

A recent study examined strain on a graft when placed in different locations in an anatomical replacement technique. A tibial tunnel was drilled and a graft passed through 1 of 3 different locations. Total, cranial, and caudal tibial translation and total, medial, and lateral angular displacement were measured at 30°, 60°, 90°, and 120°. The ideal femoral tunnel location was found to be immediately craniodistal to the fabella.<sup>58</sup> Many studies in human literature are aimed at finding the most isometric points for ligament replacement for ACL injury. One study found the optimum femoral attachment site to be similar to the site for the over-the-top method, close to the posterior end of "Blumensaat's line," or the roof of the intercondylar fossa.<sup>59</sup>

Lameness grading in cattle

There are several semi-quantitative systems which have been developed to grade lameness in cattle.<sup>8,60,61</sup> Only one published subjective lameness system in cattle has been validated in dairy cows with correlation of scoring and productivity of cattle, that is the Sprecher scale.<sup>62</sup> The Sprecher system uses a lameness score of 1-5. It has been shown that cows with scores of greater than 2/5 will have extended intervals from calving to first service and conception, spend more days in the breeding herd, require more services per pregnancy and are 8 times more likely to be culled.<sup>62</sup>

Static weight shifting has been shown to be a good measure of lameness in cattle. Systems using load cells measuring individual limb weight-bearing can be used to objectively measure lameness in cattle. These systems have shown that cattle tend to shift more from the lame leg to the sound contralateral limb, rather than shift weight from front to back or back to front limbs, particularly when lameness originates in a hind limb.<sup>63,64</sup>

#### Chapter 2. Hypothesis and Objectives

The objective of this study is to develop an extracapsular repair technique for CCL rupture in cattle that is able to effectively stabilize the stifle of adult cattle, including adult bulls, while avoiding a major complication associated with ligament replacement, whether anatomical or non-anatomical, septic arthritis.

#### Hypothesis 1

We hypothesize that 800-lb test monofilament nylon leader line crimped in a loop with 3 316 stainless steel crimps with an inner diameter of 1/8 inch will yield similar elongation patterns and have similar force to rupture compared to the CCL in mature cattle as measured from cadaveric bovine stifles.

#### Hypothesis 2

We hypothesize that bone tunnels through the distal femur and specifically, F2 - T2 sites will be ideal tunnel locations for an extracapsular cruciate prosthesis.

### Hypothesis 3

We hypothesize that adult cattle with surgically transected CCL and treated with extracapsular stabilization with a crimped loop of nylon will have significantly less lameness (scored semi-quantitatively) at the walk and improved weight-bearing (objectively measured with a load cell as a ratio of weight bearing to body weight) 2 months post-operatively.

### Subjects

Cadaver Study: Thirteen stifles were collected from 13 adult cattle euthanized for reasons unrelated to the stifle joint. The body weight of these cattle (prior to euthanasia) ranged between 450 - 800 kgs. An institutional animal care and use committee approval was not required as these cattle were euthanized for reasons unrelated to this study. The femurs and tibias were transected at the mid-diaphysis, and all soft tissues other than the ligamentous structures of the stifle and the femorotibial joint capsule were removed and the limbs were stored at -18°C. The soft tissue dissection was performed in a standard large animal operating suite (ambient temperature  $\approx 20^{\circ}$ C).

In-vivo Study: Eight, relatively healthy, mature Jersey cows (various ages) were obtained from another research protocol. They were apparently healthy and had no obvious lameness. They were housed in the research ward of the veterinary teaching hospital for the duration of the study. All procedures on these cows were approved by the Ohio State Institutional Animal Care and Use Committee.

### Cadaveric Isometric testing

One cadaveric stifle was randomly selected and thawed 36 hours prior to testing. A grid was created with points 2 cm by 2 cm apart on a section of light cardboard. This was used as a template to create depressions in the lateral aspect of the distal femur and proximal tibia. Steel 4.5 mm ball bearings were then inserted into the depressions, 7 in the femur, and 9 in the tibia. The ball bearings in the tibia were labelled T1 through T9 and those in femur were labelled F1 through F7 as shown in the figure below:





Radiographic evaluations of the stifle joint was conducted using lateral to medial projections in 135°, 90°, 65°, and 35° degrees of flexion. A type 1a external fixator applied to the femur and tibia, spanning the stifle joint, was used to maintain the joint in the various degrees of flexion for the radiographs.

All combinations of femoral and tibial points were measured on the lateral radiographs at each degree of flexion. If at any point the femoral point was cranial to the tibial point (a line drawn from femoral point to tibial point being beyond 90 degrees running caudally to the long axis of the tibia, this combination was disqualified, as this would allow for cranial displacement of the tibia. Maximum elongation was determined by taking the longest distance between the two (femoral and tibial) proposed locations and subtracting that from the shortest distance between the two proposed locations.

After identifying the most isometric points, tunnels were drilled through the femur and tibia at these locations. Suture was passed through the tunnels and the limb was taken through a range of motion to subjectively identify over-elongation of the suture. Materials testing

Measurements were made on a cadaveric limb to estimate the length of material needed to make a suture loop through the drilled tunnels in femur and tibia. The total length was estimated by summation of the following lengths: the width of distal femur from the lateral to the medial femoral condyle, the width of the proximal tibia measured from the lateral to medial tibial condyle, the length from the lateral femoral to the lateral tibial condyle and finally the length from the medial femoral to the medial tibial condyle. Monofilament nylon leader line, rated at 800 lb test, (Blue Ocean Tackle, Coconut Creek, Florida) was used to prepare 73 cm loops. The loops were created by securing the nylon to itself using one of the following four methods:

- 1. 2 square knots
- 2. 2 stainless steel crimps

- 3. 3 stainless steel crimps
- 4. 4 stainless steel crimps

All crimps stainless steel crimps (Blue Ocean Tackle, Coconut Creek, Florida)were compressed with a commercial, 18 inch fence-crimper (Agri-Supply, Garner, North Carolina) by the same investigator (JWL). The suture loops were fixed within a hydraulic load frame (LANDMARK, MTS Systems Corporation). One end of the load frame was equipped with a hydraulic ram, and the other end was equipped with a load cell (Model 661.20F-03, MTS Systems Corporation), which was used to measure the tensile force applied to the constructs. The constructs were loaded to ultimate failure by displacement at a constant rate, set at 1 mm/s. The distraction forces were measured at 100 measurements/sec. Twelve nylon loops were created and tested to failure for each of the knot/crimp configurations.

After identifying optimum tunnel placement and crimp configuration, 12 cadaveric stifles were instrumented with a construct. The limbs were allowed to thaw for 36 hours and the remaining soft tissue structures (ligaments of the stifle and joint capsule) were removed. Tunnels were drilled with a 5.5 mm drill bit. Nylon material was passed through the tunnels, manually pulled tight, and crimped with 3 stainless steel crimps performed by the same investigator who had previously compressed the crimps (JWL). An apparatus was created to fix the femorotibial joint and attach the limbs to the same hydraulic load frame. Two tunnels were drilled in the diaphyseal/metaphyseal regions of the femur and tibia of each limb and threaded rod was passed through the tunnels. An adjustable jig (Unistrut, Atkore, International, Harvey, Illinois) was constructed to hold the limb and

attach it to the load frame for distraction. The limbs, fitted with nylon stabilizing material, were then tested as previously described.

In vivo

All eight cows were randomly assigned to 1 of 2 groups. Group 1 consisted of cows with surgical transection of the CCL and stabilization of the stifle with extracapsular nylon material. Group 2 had surgical transection of the CCL alone i.e. without stabilization. One day prior to surgery, all cattle were weighed and scored for lameness using the Sprecher et al.k 1999, lameness scoring system. In addition weight that the animal was bearing on each pelvic limb was measured with a digital scale. These methods were repeated 1 day post operatively and 14 weeks post operatively. Lameness grading using the Sprecher scoring system was performed once per week for a total of 15 subjective scoring dates.

Subjective lameness scoring was performed by 3 blinded observers. All were provided the scoring chart prior to and during lameness scoring. All observers were large animal veterinarians and experts in farm animal medicine/surgery and had performed lameness evaluation in cattle prior to instruction.

Objective scoring was conducted with a load cell platform (Panther, Metler Toldedo). Subjects were loaded into cattle transporters (Shanks). The load platform was elevated to a level even to the floor of the cattle transporter. Side panels were removed and the subjects' pelvic limbs were shifted to the platform. Measurements were recorded when the cows stopped shifting weight and stood comfortably on the platform. Next, individual pelvic limb forces were measured by shifting just one limb to the platform which was placed either to the left or right of the cattle transporter, and recording force when the animal stood comfortably on the platform.

#### Peri-operatively

All cattle were fasted 12-24 hours prior to undergoing general anesthesia. Cattle were administered 6.6 mg/kg of ceftiofur crystaline free acid subcutaneously in the fat pad behind the ear 2-6 hours prior to surgery. This was chosen as a study demonstrated that serum ceftiofur derivatives are above the minimum inhibitory concentrations for *Treuperella pyogenes*, the most common pathogen causing septic arthritis in cattle beginning at two hours and lasting over a 7 day period.<sup>65,66</sup> Additionally, all cattle received 0.1 mg/kg of morphine with 30ml of sterile 0.9% saline in the sacrococcygeal epidural space. All cattle also received 1.1 mg/kg of flunixin meglumine IV as an analgesic for the procedure.<sup>67,68</sup> All cattle were sedated with 0.02 mg/kg of xylazine IV and induced with 1.5-3.5 mg/kg of ketamine and 70-110 mg/kg guaifenesin in a 5% dextrose solution IV. All cattle were orotracheally intubated and maintained on inhaled isoflurane for the duration of the procedure.

#### Surgical procedure

The subjects were placed in dorsal recumbency with the right hind limb suspended by a hoist. The limbs were clipped of hair, cleaned, and sterilely prepared circumferentially from the level of the hip to the level of the tarsus. The stifle was maintained at approximately 90° of flexion for the duration of the procedure. An "S" shaped incision was made starting 5 cm proximal to the patella. The incision extended distally and medially, crossing over the mid-patellar ligament region and then extended laterally

ending 5 cm distal to the tibial crest. The subcutaneous tissues were undermined with sharp dissection, exposing the periarticular fascia of the stifle.<sup>9</sup> The stifle joint was approached by first incising the lateral femoropatellar ligament midway between the patella and the femur. A 5 cm long linear arthrotomy was then performed between the lateral and middle patellar ligaments. The patella was medially luxated, to expose the CCL for transection as described by Moss to access the bovine stifle in a cruciate replacement procedure.<sup>30</sup> After the CCL transection, the patella was reduced and the joint capsule and periarticular tissues were apposed using horizontal mattress sutures with number 2 polydioxanone. The lateral femoropatellar ligament was apposed using number 2 polydioxanone in a horizontal mattress pattern.

Extracapsular Stabilization: In group 1, predetermined femoral and tibial tunnels were drilled lateral to medial using a universal aiming device (IMEX, Inc., Longview, Texas) and a 5.5 mm drill bit. Monofilament nylon leader line (800 lb test) was previously sterilized with ethylene oxide, and was used as the extra-capsular prosthesis. The leader line was passed through the tunnels and crimped with 3 stainless steel crimps on the lateral aspect of the stifle. The same crimps and crimper were used as previously described for the cadaveric specimens.

In both groups, the subcutaneous tissues were closed with 0 glycomer 631 with walking sutures and in runs of simple continuous. The skin was closed with number 2 polymerized caprolactam in an interrupted cruciate pattern. A stent bandage was used to cover all surgical sites.

Patients were recovered in a standard, padded recovery stall.

Post-operatively

Meloxicam was administered PO at a dose of 1 mg/kg the day following surgery, two days after surgery at a dose of 0.5 mg/kg, and continued every other day at a dose of 0.5 mg/kg for a total of 11 treatments.<sup>69</sup> Skin sutures were removed 21 days postoperatively. A post-mortem examination of each limb was performed following euthanasia at 3 months post-operatively or if cows were removed prior to the study before that time. Statistical analysis

All data was analyzed using a commercial software program (SPSS, IBM). Knot and crimp data, graded lameness, and force plate scores were checked for normality using the Shapiro-Wilk test with normality set at greater than 0.05.

Mean elongation and mean maximum force at failure of knotted, 2 crimp, 3 crimp, and 4 crimp constructs was compared using a one-way ANOVA with significance set at  $p \le 0.05$ . Post-hoc tests were performed using the Bonferroni and Sidak tests.

Mean lameness scores comparing stabilized and control stifles were compared using independent samples t-test with significance set at  $p \le 0.05$ .

Mean vertical impulse forces of both right and left limbs of cows with stabilized stifles and controls within groups, as well as the right limbs between groups at each time point were analyzed using an independent samples t-test with significance set at  $p \le 0.05$ . Mean crimp width between groups that failed by slipping and those that failed by rupture of material was analyzed with an independent samples t-test with significance set at  $p \le 0.05$ . If data were not found to be parametric, a Wilcoxon rank-sum test was used in place of the paired t-test, and a Kruskal Wallis test was used in place of a one-way ANOVA.

### Chapter 4. Results

# Isometric testing

The ideal combination of isometric points was determined to be F7 (the most caudal and distal point on the femur), and T3 (the proximal and cranial most point on the tibia).The points are shown below, connected by a red line:



Figure 2: The most isometric points of the femur (F7) and tibia (T3) relative to each other, connected by a red line.

This was also a location in which the femoral point was never cranial to the tibia point

using the long axis of the tibial shaft as reference. The table below shows the

measurements and calculations used to determine these isometric points:

Points		Degrees	180	135	90	75	35	Elongation	F point
		of							past T
		flexion							point
Femoral	Tibi	al Point							
Point									
F1	T1		7.8	8.8	10.	11.	11	3.7	No
					5	5			
	T2		8.5	9.2	10.	10.	9.6	2.1	No
					2	6			
	T3		10.	12.4	15.	16.	16.	5.5	Yes
			9		2	4	2		
	T4		10.	11.6	14.	15.	14.	4.8	Yes
			3		1	1	5		
	T5		10.	11.3	12.	13.	12	2.8	No
			3		7	1			

Table 1: Distances between femoral and tibial locations at different degrees of flexion.

	T5	10.	11.3	12.	13.	12	2.8	No
		3		7	1			
	T6	10.	11.6	12.	12.	11.	1.7	No
		9		4	6	3		
	T7	12.	14.2	16.	17.	17.	5.1	Yes
		7		7	8	3		
	T8	12.	13.5	15.	16.	15.	4.2	Yes
		2		6	4	6		
	Т9	12.	13.2	14.	15.	13.	3	No
		1		7	1	9		
F2	T1	7.7	8.4	9.6	10.	9.5	2.4	Yes
					1			
	T2	8	8.5	9.1	9.2	8.2	1.2	No
	T3	11.	12.4	14.	15.	14.	3.9	Yes
		2		4	1	6		
	T4	10.	11.4	13.	13.	13	3.1	Yes
		5		2	6			
	T5	10	10.6	11.	11.	10.	1.5	No
				5	5	4		

	T6	10.	10.8	11.	11	9.6	1.6	No
		5		2				
	T7	13	14.1	15.	16.	15.	3.3	Yes
				8	3	8		
	T8	12.	13.1	14.	14.	14	2.6	Yes
		3		7	9			
	T9	12	12.6	13.	13.	12.	1.5	No
				5	4	3		
F3	T1	5.2	6.1	8.1	9.5	10	4.8	Yes
	T2	5.7	6.6	8.1	9.1	9.1	3.4	No
	T3	8.6	9.7	12.	14.	15	6.4	Yes
				5	2			
	T4	7.8	8.9	11.	12.	13.	5.8	Yes
				5	8	6		
	T5	7.5	8.6	10.	11.	11.	4	Yes
				4	5	5		
	T6	8.1	9	10.	11.	11	3.2	No
				5	3			

	T7	10.	11.4	14.	15.	16.	6.1	Yes
		3		3	6	4		
	T8	9.6	10.7	13.	14.	14.	5.2	Yes
				2	4	8		
	T9	9.4	10.5	12.	13.	13.	4	Yes
				4	3	4		
F4	T1	5.6	5.9	6.8	7.5	7.8	2.2	Yes
	T2	5.6	5.8	6.3	7	7	1.4	No
	T3	9.6	10.3	11.	12.	12.	3.2	Yes
				6	4	8		
	T4	8.7	9.2	10.	11.	11.	2.6	Yes
				4	1	3		
	T5	7.9	8	8.8	9.4	9.3	1.5	Yes
	T6	7.9	8.2	8.6	9.1	8.8	1.2	No
	T7	11.	11.7	13.	13.	14.	3	Yes
		2		1	7	2		
	T8	10.	10.7	11.	12.	12.	2.3	Yes
		3		9	5	6		

	T9	9.7	9.9	10.	11.	11.	1.6	Yes
				8	3	3		
F5	T1	7.6	7.2	6.4	6	5.2	2.4	Yes
	T2	7	6.5	5.3	4.8	4.2	2.8	Yes
	T3	11.	11.8	11.	11	10.	1.7	Yes
		9		4		2		
	T4	10.	10.6	10	9.5	8.8	2.7	Yes
		7						
	T5	9.3	8.8	7.7	7.3	6.6	2.7	Yes
	T6	9.2	8.5	7.1	6.7	6.2	3	Yes
	T7	13.	13.1	12.	12.	11.	1.8	Yes
		2		7	2	4		
	T8	12.	11.9	11.	10.	10	2.1	Yes
		1		1	7			
	T9	11.	10.7	9.8	9.2	8.6	2.6	Yes
		2						
F6	T1	4.5	4.3	5	6	7.1	2.8	Yes
	T2	4.2	3.9	4.7	5.8	6.9	3	Yes

T	3	8.9	8.9	9.6	10.	11.	3	Yes
					6	9		
T	4	7.8	7.7	8.4	9.4	10.	3	Yes
						7		
T	5	6.4	6.2	7	8.1	9.2	3	Yes
T	6	6.4	6.2	7.1	8.1	9.1	2.9	No
Τ	7	10.	10.3	11.	12.	13.	3	Yes
		3		1	1	3		
T	8	9.2	9.1	10	10.	12.	3	Yes
					9	1		
T	9	8.3	8.2	9.1	10	11.	2.9	Yes
						1		
F7 T	1	6.2	5.3	4.4	4.5	5.3	1.8	Yes
T	2	5.2	4.4	3.5	4	5.1	1.7	No
T	3	10.	10.1	9.4	9.5	10.	1.1	Yes
		5				2		
T	4	9.3	8.7	8	8	8.7	1.3	Yes
T	5	7.5	6.9	6	6.3	7.4	1.5	Yes

Table 1 continued

T6	7.2	6.5	5.7	6.2	7.4	1.7	No
T7	11.	11.3	10.	10.	11.	1	Yes
	7		7	7	5		
T8	10.	10	9.3	9.5	10.	1.2	Yes
	5				3		
T9	9.3	8.6	8.1	8.3	9.2	1.2	Yes

When the nylon loop was secured through these proposed sites in a cadaveric limb and taken through a range of motion, the lateral epicondylar flair and the extensor groove of the tibia on the proximolateral aspect created a large step that the prosthesis was forced to traverse over during flexion and extension. This resulted in extensive elongation of the nylon loop. To mitigate this, the tibial tunnel was drilled from the lateral location at the same level as the proposed location, but directly distal to the femoral tunnel. It was aimed in a cranial direction to exit medially at the proposed tibial location. As the medial aspect of the tibia and femur in cattle are relatively flat, this prevented cranial displacement of the tibia without a step artifact resulting in elongation. As the suture was looped and secured at the lateral aspect, internal rotation which would otherwise be possible with a device only on the medial aspect, was eliminated. The image below illustrates the final tunnel positioning in the femur and tibia:



Figure 3: Final extracapsular prosthesis positioning.

Materials testing

All knotted constructs elongated to 70mm and thus were not included in the statistical analysis.

The mean elongation for 2 crimp, 3 crimp, and 4 crimp configurations were 36.4mm  $\pm$  1.8mm, 40.8mm  $\pm$  3.0mm, and 41.1mm  $\pm$  8.1mm respectively. Elongation data was normally distributed for 2-, and 3-crimp configurations (p values of the Shapiro-Wilk test being 0.086, 0.208); but not the 4-crimp configuration (p=0.006). Therefore, elongation

was compared using a Kruskal-Wallis H test. A Kruskal-Wallis H test showed that there was a statistically significant difference in elongation score between the different configurations,  $\chi^2(2) = 6.066$ , p = 0.048, with a mean elongation score of 12.46 for 2-crimps, 22.17 for 3-crimps, and 20.88 for 4-crimps.

The mean force to failure of 2-, 3-, and 4-crimp configurations was  $2472.8N \pm 448.9N$ ,  $3636.2N \pm 594.2N$ , and  $3806.8N \pm 1084.7N$ , respectively. Within the 2-crimp constructs, 9 failed at the crimps and 3 slipped at the crimps. Mode of failure in the 3-crimp constructs consisted of 8 breakages and 4 slippages. Eleven of the constructs with 4crimps failed by breaking at the crimps, while 1 slipped. Failure force with 2, 3, and 4 crimps were normally distributed per the Shapiro-Wilk test with p-values of 0.737, 0.665, and 0.067, respectively. There was a statistically significant difference between groups as determined by one-way ANOVA (F = 3.175, p < .001). A Sidak post hoc test revealed that the force at failure was significantly lower in 2-crimp construct compared to 3crimps (-1163.4  $\pm$  310.1 N, p = .002) and 4 crimps (1334.0N  $\pm$  310.1N, p = .000). There was no statistically significant difference between 3- and 4-crimps (p=0.929). Based on these findings, a 3-crimp configuration was selected for the in-vivo study. This provided higher force to failure to the 2-crimp configuration, and no additional force was added with a 4<sup>th</sup> crimp. Further, additional implants increased the chance for reaction or infections in the live patient.

The mean diameter of the crimps which failed due to slippage of the nylon loop was  $4.4 \pm 0.3$  mm and  $4.0 \pm 0.3$  mm for those that failed due to breaking. Crimp width of constructs which slipped were normally distributed (p=0.289) but not for those that broke (p<0.001).

A Wilcoxon signed-rank test showed that the crimped width of constructs that slipped versus those that broke were significantly different (Z = -2.717, p = 0.007).

Ex vivo

All constructs placed in the cadaveric limb failed via slippage.

In vivo

Of the 8 cattle enrolled, 3 were removed before the end of the study. One patient in the stabilized group developed an arrhythmia characterized by an accelerated ventricular junction rhythm intraoperatively which was treated with a lidocaine constant rate infusion at a rate of 82mg/hr. This patient was noted to have ex-opthalmos in recovery which was not noted pre-operatively. The patient was unable to stand for a 24 hour period and was euthanized with a presumptive diagnosis of multicentric lymphoma, though this could not be confirmed on necropsy. Two additional patients, one from the stabilized group and one from the control group were removed from the study due to development of septic arthritis in the operated stifle resulting in non-weight bearing lameness. T. pyogenes and E. coli were cultured from the joint. Despite therapy consisting of daily saline lavage, flunixin meglumine, and procaine penicillin G, the lameness persisted and the subjects were euthanized for humane reasons.

The mean graded lameness score prior to surgery was  $1/5 \pm 0.0$  for the stabilized group, and  $1.11/5 \pm 0.6$  for the control group. The mean lameness score of all of the lameness grades for the stabilized group following surgery was  $3.6/5 \pm 0.3$  and  $3.4/5 \pm 0.5$ . Data for both stabilized and control groups following surgery was normally distributed (p=0.489 and p=0.078, respectively). An independent t-test found no significant difference between the lameness scores of the two groups (t = 1.68, p = 0.150). The mean percent of total body weight-bearing on the right (operated) pelvic limb prior to surgery was 17.7 ± 2.9%, and 19.5 ± 3.8% in the left (non-operated) pelvic limb. An independent t-test found no significant difference between weight bearing in the right and left pelvic limbs prior to surgery (t= -0.862, p=0.414). Additionally, the right and left pelvic limbs of each independent group were not significant from one another within groups "stable" (right mean= 16.9 ± 4.4%, left mean= 18.1±2.6%, t= -0.343, p=0.764) and "control" (right mean = 18.2 ±2.5%, left mean= 20.4 ±4.7%, t= -0.730, p=0.506). However, the right pelvic limb bore significantly less weight, as a percentage of total body weight at both time points following surgery within the groups, as demonstrated in the table below:

Table 2: Total percent of body-weight bearing of right (operated) leg compared to left (non-operated) leg at each time point.

Group	Time	Mean weight bearing right leg (%)	Mean weight bearing left leg (%)	t-value	p-value
Stable	1 day post- op	4.6±6.4	33.7±6.1	-4.627	0.044
Control	1 day post- op	9.8±4.5	32.5±5.6	-5.479	0.005
Stable	3 months post-op	7.4±3.5	33.0±1.8	-9.313	0.011
Control	3 months post-op	9.9±3.1	32.4±2.2	-10.237	0.001

Independent samples t-test found no statistically significant difference between the right (operated) pelvic limbs of the stabilized group and control group at one day post-operatively (mean weight bearing stabilized=  $4.6\pm6.4\%$ , mean weight bearing control=  $9.8\pm4.5\%$ , t= -1.09, p= 0.355) and no statistically significant difference between groups ("stabilized" and "control") at 3 months post-operatively (mean weight bearing stabilized=  $7.4\pm3.5\%$ , mean weight bearing control=  $9.9\pm3.1$ , t= -0.849, p= 0.458). At post-mortem examination, one additional patient from the stabilized group had developed septic arthritis. Culture results indicated a heavy growth of T. pyogenes from the joint. All nylon loops were intact, but very minimal force was needed to break them. Unlike the single force to rupture, all loops had worn and broke at sharp turns exiting from the bone tunnels. Additionally, the apposition of the lateral femoropatellar ligament had failed, and all limbs had medially luxated patellas.

#### Chapter 5. Discussion and Conclusions

Currently established techniques for surgical treatment of cranial cruciate ligament rupture, both intra- and extra-capsular, in adult cattle, particularly bulls, do not provide satisfactory outcomes. Major complications of intra-capsular repair consist of but are not limited to implant failure and septic arthritis secondary to the surgical procedure.<sup>9</sup> Our study evaluates a novel technique for stifle stabilization with an extracapsular prosthesis, therefore potentially eliminating at least one of these complications (septic arthritis). First, optimal tunnel placement was determined based on lateral to medial projected radiographs in adult bovine cadaveric stifles. Secondly, a previously evaluated prosthetic material<sup>44,45,48</sup> was placed as a loop at the predetermined locations and secured in place with 2, 3, or 4 crimps. Biomechanical evaluation of these constructs demonstrated that 800-lb nylon monofilament loop secured with 3 crimps was most ideal at single load to failure. Lastly, this construct was evaluated in-vivo in a randomized, controlled study involving 8 adult cows with surgically-transected cranial cruciate ligament. At 90-days post-operatively, our results indicate that this extra-capsular technique did not result in improved lameness score or weight bearing when compared to control animals. Isometric points in distal femur and proximal tibia for prosthesis placement were first determined via multiple lateral to medial radiographic projections using metallic markers placed on the stifle at various joint (stifle) angles. This method has been used in the past in canine studies.<sup>54,55</sup> However, lateral to medial projections alone do not account for true isometry in the stifle and are often determined by measuring tension and elongation of sutures implanted in the stifle itself, as performed in human knees.<sup>59,70</sup> In this study, when implants were placed in the proposed isometric locations, I.e. most distal and caudal aspect of the femur and most cranial and proximal aspect of the tibia, lateral motion caused by the lateral condylar flare and extensor groove of the tibia resulted in, subjectively, extensive elongation of the prosthesis. This necessitated moving the tibial tunnel so that the medial aspect was very proximal and cranial on the tibia, while the lateral opening was still most proximal but caudal. This eliminated the need for the material to interact with the lateral condylar flare and extensor groove completely. Additional kinematic studies and suture tension studies through a range of motion may be necessary to completely confirm the ideal location for femoral and tibial bone tunnels. Three crimp construct was determined to be optimum for the following reasons. Three and four crimp constructs were found to have a significantly greater force to failure than a two-crimp configuration, and not significantly different from each other. Therefore, 3 crimps were used in the in-vivo study. This also meant decreased foreign material implanted in the stifle, decreased surgical time and consequent potential for foreign matter reaction or infection. Commercially available crimps and crimpers were used in this study. Specialized surgical equipment manufactured with implant-grade stainless steel would be ideal for future use. This will potentially reduce risk of foreign matter reaction, and more importantly, normalize the crimping width. Although one investigator applied all the crimps, a significant difference was found in the crimp width of those that failed via slippage and those that failed by the monofilament nylon breaking at the crimp. Further investigation is warranted to determine the optimum crimp width and its relationship to mode of suture failure. Development of biological implant-grade crimps

and surgical crimper that delivers a consistent crimp dimension also warrants further investigation.

The elongation and tensile force to failure of adult bovine cranial cruciate ligament have been investigated. One study suggests that the ultimate tensile strength is correlated with the body weight (ratio of ultimate tensile strength/body weight = 1.4).<sup>23</sup> A previous study in this lab determined that the CCL of cattle ruptured at a mean  $\pm$  SD force of 4,541  $\pm$ 1,417 N with an elongation of  $2.0 \pm 0.3$  cm.<sup>24</sup> Another study concluded similar results, with a mean ultimate tensile load of  $4,372 \pm 1,485$  N and a mean load at first physical signs of tearing of  $3,315 \pm 1,336$  N. No correlation between load to failure of CCL and body weight could be made.<sup>25</sup> Several studies have also attempted to determine the optimum prosthetic material for functional replacement of bovine CCL. In this study, a single strand of 800-lb test monofilament nylon and 3 stainless steel crimps had a mean elongation of  $40.8 \pm 3.0$  mm, roughly 2 cm longer than the native CCL,<sup>24</sup> and force to failure of  $3,636.2 \pm 594.2$  N, roughly 80% of the force needed to completely rupture native CCL.<sup>24</sup> This force is lower than the previously tested 3 parallel strands of 450-lb test nylon (6,260  $\pm$  239N) and resulted in greater elongation (3.3  $\pm$  0.1mm).<sup>24</sup> Further, this stabilization achieved greater force to failure than the established and tested over-thetop method which approximately reached only  $29.7 \pm 1.67\%$  of native CCL strength.<sup>6</sup> However, 800-lb monofilament nylon material evaluated in this study was providing minimal to no support at the time of necropsy, 3 months postoperatively. This calls into question the usefulness of nylon as a prosthetic material. To corroborate our finding, human and canine literature have found poor success with most synthetic materials with

the exception of polyglycolic Acid-Dacron and polyethylene.<sup>34,36</sup> Despite extensive physical degradation of the nylon prosthesis, most significantly due wearing at the entry and exit bone tunnel locations, being noted 90-days post-implantation, the stability offered by the prosthesis during the initial period may facilitate sufficient fibrosis to provide long-term stability. This was not evaluated in this study. In addition, the entry and exit points of the bone tunnels may be smoothened to decrease wearing of the nylon at the entry and exit locations. Currently, there is limited evidence in cattle evaluating autogenous grafts as functional CCL prosthesis. In cattle, one study demonstrated that autogenous skin used as a prosthesis in an anatomical CCL repair maintained its size and subsequently incorporated with the bone tunnels.<sup>33</sup> This limited evidence warrants further evaluation of autogenous skin or similar decellularized autogenous grafts in an extracapsular repair technique using the bone tunnel locations determined in this study. There was no significant difference in the lameness evaluted by blinded graders or weight bearing between the stabilized and control cows. There are several explanations for this. Three cows in this study were excluded from the analyses (one suspect lymphoma and 2 septic arthritis) which left 3 control cows and were statistically compared with 2 stabilized cows. The consequent low number of cattle left in the stabilized and control groups may not have been sufficient to find a significant difference, even if there was one (type II statistical error). Additionally, one cow in the stabilized group had septic arthritis diagnosed at necropsy. This may have increased the lameness in the stabilized group falsely. It is possible that the prostheses restricted movement of the stifle, or were overly irritating to the tissues, also resulting in increased lameness in the stabilized group. The

remaining cows had a medially luxated patella at necropsy which was likely a significant confounding factor in this study. We attribute medial luxation of the patella in all cows likely due to dehiscence of the apposed lateral femoropatellar ligament. This sequela/complication has been reported previously with a 9% incidence (2 bulls out of 22 total cattle) when the lateral femoropatellar ligament was transected to place the over-the-top graft.<sup>6</sup>

This method of extracapsular stifle stabilization requires further investigation to determine its clinical utility. In addition, the surgical exposure required for this extracapsular repair technique allows imbrication of the stifle, a technique already described for addressing partial CCL deficiency.<sup>26</sup> The major advantage of extracapsular CCL repair techniques is mitigating post-surgical septic arthritis that can occur with anatomic CCL reconstruction. However, all subjects in this study underwent an arthrotomy and transection of the lateral femoropatellar ligament to gain access to the CCL prior to experimental transection. Consequently, 37.5% of animals developed septic arthritis, and all 5 cattle evaluated 90-days post-operatively had a medially luxated patella. These complications noted in this study are major confounding factors in determining the credibility of this novel technique. Evaluating this technique in a non-arthrotomy CCL transection model and/or naturally occurring disease must be considered. <sup>6,30,32,33</sup>In light of our findings, this technique should be avoided until further evaluation.

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