EVALUATION OF A RELATIVELY HYPEROSMOLAR IRRIGATION SOLUTION FOR USE IN EQUINE FEMOROTIBIAL ARTHROSCOPY

A Thesis presented to the Faculty of the Graduate School

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of the Requirements for the Degree

Master of Biomedical Sciences

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by

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

EVALUATION OF A RELATIVELY HYPEROSMOLAR IRRIGATION SOLUTION FOR USE IN EQUINE FEMOROTIBIAL ARTHROSCOPY

presented by Lyndsey Hayden, DVM,

a candidate for the degree of Master of Biomedical Sciences,

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

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ABBREVIATIONS

US	Ultrasound
HYPER	Hyperosmolar
LRS	Lactated Ringer's Solution
ACUC	Animal Care and Use Committee
MFTJ	Medial Femoral Tibial Joint
LFTJ	Lateral Femoral Tibial Joint
TTJ	Tibiotarsal Joint
GAG	Glycosaminoglycan
SAA	Serum Amyloid A

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Lyndsey Hayden, DVM

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Hyperosmolar irrigation solutions have been shown to decrease fluid extravasation during arthroscopic procedures and safety has been demonstrated in canine and human studies. Prospective, blinded, randomized controlled trial was performed to compare lactated Ringer's solution (273 mOsm/L) and a hyperosmolar (600 mOsm/L) irrigation solution for an exploratory medial femorotibial joint arthroscopy. Primary outcomes focused on the amount of periarticular fluid retention based on change in stifle girth and ultrasonographic (US) measurements. The water content of the tissue samples was assessed, and viability of the cartilage tissue was determined. The objectives of this study were to determine: (1) whether an irrigation solution that is hyperosmolar relative to synovial fluid decreases tissue extravasation during an arthroscopic protocol when compared to a relatively hypoosmolar solution, (2) the safety of a hyperosmolar solution by assessing the viability of joint tissues after joint irrigation, (3) if the use of a hyperosmolar solution decreases water content in stifle tissue. There was not a significant difference in joint swelling between hyperosmolar (Hyper) and lactated Ringer's solution (LRS) treatment groups. Percent increments in femorotibial joint dimensions (mean ± S.D.) were seen in both treatment groups based on US (LRS, 83.9 ± 84.6%; HYPER, 131.2 ± 144.9%) and caliper measurements (LRS 5.5 ± 4.3%; HYPER 7.5 ± 5.8%) ($p \leq 0.05$). Chondrocyte viability and tissue water content were well maintained in both

treatment groups, and differences were not statistically significant. These findings suggest that approximately doubling the osmolarity of the standard irrigation solution used for arthroscopy did not result in detrimental effects on chondrocyte viability or tissue water content, it did not significantly reduce tissue swelling.

CHAPTER 1: INTRODUCTION

a. Joint Environment

The function of a healthy synovial joint is dependent on the proper function and integrity of all its components.[1] The health of articular cartilage, joint capsule, and collateral ligaments are of utmost importance in all athletes, and more specifically in the equine athlete, as the transfer of load in the distal limb occurs primarily across the hyaline cartilage as there is limited muscle mass in the equine distal limb to help dissipate energy and stabilize the joint.[2] The synovial lining of the joint produces synovial fluid, which is an ultrafiltrate of plasma and serves as the lubricant in the joint.[1] The type B synoviocytes add hyaluronic acid and lubricin to the synovial fluid, allowing for nearly frictionless movement between the cartilage surfaces of the synovial joint.[3] Hyaluronic acid is important for maintaining the viscosity of the synovial fluid and controls tissue hydration.[4] Tissue hydration is achieved as hyaluronic acid is one of the most hydrophilic substances in the body and the polysaccharide chains bind to water molecules.[4] Concentrations of electrolytes in the synovial fluid mirror the plasma and chondrocytes are dependent on synovial fluid for nutritional supply.[1,3]

Chondrocytes make up a relatively small volume of articular cartilage (1-12%) with the remainder consisting of the extracellular matrix.[5] The extracellular matrix is made up of water, collagen, and proteoglycan. Chondrocytes are responsible for the maintenance of the extracellular matrix of the tissue through the production collagens, primarily type II collagen, and proteoglycans.[3] Type II collagen makes up 90 to 95% of articular cartilage collagens and forms fibrils that make up a network that provides strength to the tissues

and entraps proteoglycans.[3] Proteoglycans are an important component of the articular cartilage, and account for approximately 7-10% of the tissue's extracellular matrix.[3] Proteoglycans are proteins that are bound to sugar chains creating bottle brush structures called glycosaminoglycans.[3] The most prominent proteoglycan in the extracellular matrix of articular cartilage is aggrecan, which creates large aggregating structures by binding multiple aggrecan proteins to hyaluronic acid through link protein.[3] These large aggregating structures fill in the spaces of the collagen network of the extracellular matrix of articular cartilage.[3] Proteoglycans are also negatively charged, which draws water into the cartilage tissue.[3] The large structure of the proteoglycan, and ability to draw water into the tissue, contribute to the compressive stiffness of the articular cartilage and the ability of the tissue to resist and recover from compressive loads during movement.[3] Normal equine synovial fluid has an osmolarity of 350-450 mOsm/L.[2,6,7] The osmolarity of the articular cartilage and other joint tissues can change in response to changes in synovial fluid osmolarity, joint health, and static loading.[7]

A previous study in humans found that following exercise, hyaluronic acid concentrations and osmolality are decreased, and synovial fluid volume is increased, compared to unexercised controls.[8] Additionally, it has been shown that synovial fluid osmolality in traumatic or mechanically abnormal knees is decreased in comparison to normal knees.[9] The osmolality of the synovial fluid has a significant impact on chondrocytes specifically following mechanical injury. Decreased extracellular osmolality has been associated with increased chondrocyte death, while an increased osmolality has a chondroprotective effect.[8,10-12] The integrity of the chondrocytes, and the superficial zone of the cartilage is

important for maintaining the biomechanical properties and preventing degradative changes.[13] The superficial zone of articular cartilage protects the deeper layers from shear stress as it interacts with the synovial fluid and is responsible for most of the tensile properties of cartilage.[14]

b. Equine Arthroscopy

Arthroscopic surgical procedures have been widely adopted for the treatment of many conditions in the equine patient including, removal of osteochondral fragments, removal of osteochondritis dissecans lesions, treatment of subchondral bone cysts, and lavage of septic joints.[15] In addition to treatment, arthroscopic procedures are also useful in diagnosis of disease, specifically regarding articular structures for which diagnostic imagining modalities are limited. Arthroscopic procedures in the horse are generally accepted to be a low risk procedure, and the principal risks are often associated with the need for general anesthesia. When compared to arthrotomy, arthroscopy offers improved visualization of the joint and all its components, decreased post-operative complications, improved cosmetic outcomes, and more rapid return to function.[15]

Arthroscopic procedures require a methodology to distend the joint for visualization of the joint surfaces. The most commonly used method for arthroscopic procedures is fluid irrigation, but there are reports of using gas insufflation. Continuous irrigation is an essential component of arthroscopic surgery to maintain adequate joint distention for visualization of intraarticular structures. This is commonly achieved using 3- to 5- liter fluid bags and motorized roller pumps with adjustable rates. Effective intraarticular distention necessitates a fine-tuned balance of ensuring adequate visualization without

causing capsular damage or excessive peri-articular swelling (leakage). Presently pressure settings up to 150 mmHg and flow rates of up to 1.5L/min are recommended.[16] However, constant irrigation can result in localized joint inflammation and fluid extravasation of the capsule and other joint tissues.[17-20] Inevitable intra-procedural fluid extravasation compresses the synovial compartment precluding arthroscopic visualization, prolonging surgical time, and increasing pain following surgery.[20,21]

c. Adverse Effects of Arthroscopy

Potential adverse effects following arthroscopic lavage can be divided into perioperative and post-operative complication. The main perioperative complications that are commonly encountered include fluid extravasation, hemarthrosis, obstruction of view, iatrogenic damage, and instrument breakage.[22] As currently performed post-operative complications include synovitis, capsulitis, or articular cartilage damage/chondrocyte death, highlighting the need for safety studies prior to implementing any novel lavage fluid protocols in clinical practice. As in all orthopedic procedures for equine patients, pain control is of utmost importance and strategies to improve patient comfort are continually sought out.

Measurable synovial fluid parameters examined for diagnosing and monitoring synovitis in the horse include total protein, total nucleated cell count, percentage of neutrophils, and serum amyloid A (SAA). Jones et al found that arthroscopic lavage of healthy joints resulted in mild elevations in total protein up to 14 days post arthroscopic lavage.[23] It was found that the total protein and total nucleated cell counts (>20 x 10^3 cells/ μL) peak at 24

hours and 12 hours, respectively, following simple arthroscopic irrigation with LRS.[18] Sanchez-Teran et al found that arthroscopic lavage with LRS did not cause significant increases in synovial SAA concentration in healthy joints and it has been found that in septic models the synovial SAA concentration does significantly increase in comparison to healthy controls.[18,24] Increased synovial fluid nucleated cell counts and total protein concentrations following irrigation may indicate increased joint inflammation.[18,19]

Extra-synovial extravasation occurs when fluid accumulates between the joint capsule and the skin or fascial planes. Commonly this complication is encountered with relatively inexperienced arthroscopists or during difficult surgical procedures (especially prolonged procedures). Factors associated with increased likelihood of extravasation include the shape of the instrument portals, excessive intra-articular pressure, and excessive instrument manipulation through the portals and length of procedure. Joints that are commonly associated with extravasation in the horse include the scapulohumeral joint, the stifle, and the tibiotarsal joints.[22] Surgical visualization is precluded as fluid accumulates extra-articularly and locally compresses the intra-articular compartment. This problem prolongs surgical time and can complicate procedures. In equine patients compartment syndrome, excessive pressure resulting in decreased blood flow secondary to periarticular fluid extravasation, is less likely to occur when compared to human patients due to the fewer tissue planes between the joint capsule and the skin.[22] A degree of extravasation of fluid is inevitable and should be considered in the surgical planning as once it has begun surgical time becomes limited. Instrument entry through surgical portals becomes difficult with extravasation, prolonging surgical time. If large quantities

of fluid have accumulated excessive tension may be placed on skin sutures compromising closure.[22] Intraoperatively surgeons can pause fluid delivery and massage the periarticular area to disperse the accumulated fluid. In most cases the accumulated peri-articular fluid dissipates within 24 hours post-operatively.[25] Extensive fascial planes are present adjacent to the femoropatellar joint, making fluid accumulation common in this location. Dissipation of excess fluid associated with this joint can often take a longer period.[25]

Additionally, previous in vitro studies have indicated that saline irrigation solutions may have detrimental effects on cartilage health and hinder cartilage repair. Chondrocytes exposed to a low osmolarity environment (2273 – 200 mOsm/L) during monolayer in vitro culture are more susceptible to chondrocyte death, which can lead to compositional changes of the extracellular matrix. A study by Gulihar et al. found that in vitro proteoglycan synthesis was inhibited by 35% in saline solutions and 10% by LRS.[17] Irrigation of human osteochondral explant with balanced electrolyte solutions after injury resulted in significantly less superficial zone cell death, proteoglycan elution, and inhibition of collagen II expression.[26] Changes to the extracellular matrix detrimentally affects cell volume, cytoskeleton organization, gene expression, protein synthesis, and calcium signaling.[7] Previous studies have indicated that hyperosmolar irrigation solutions may mitigate these responses and have the potential to be an alternative to the accepted standard of care solutions currently in use.

d. Hyperosmolar Irrigation

Arthroscopic irrigation is presently routinely achieved using a balanced electrolyte solution such as LRS (273 mOsm/L), which is hypoosmolar relative to normal equine synovial fluid (350-450 mOsm/L).[2,6,7] This (surgically induced and temporary) hypoosmolar environment persists within the joint until the normal synovial fluid can be re-established, restoring normal joint homeostasis. In human arthroscopy studies, chondroprotective effects have been demonstrated with hyperosmolar solutions as high as 600 mOsm/L.[10]

Besides close monitoring of pump flow rates, minimizing surgical time and ensuring proper portal placement, fluid extravasation may be controlled to some extent through the osmolarity of the irrigation solution. Based on the principles of osmosis, utilizing a relatively hyperosmolar irrigation solution may inhibit fluid extravasation and the problems associated with an irrigation solution that is relatively hypoosmolar in nature. Recently published work using a canine arthroscopy model, the mean percentage change in shoulder girth was higher in the control (isosmolar) group than the hyperosmolar group (600 mOsm/L), and there was no significant change in water content or chondrocyte viability.[27] The study was translated to a human clinical trial, which demonstrated that a hyperosmolar group (593 mOsm/L) experienced significantly less mean weight gain, less change in shoulder girth, and a lower immediate postoperative visual analog scale pain score.[28] Therefore, the use of a hyperosmolar irrigation solution has the potential to improve the welfare of horses undergoing common arthroscopic procedures by reduction of surgical time and procedural inflammation.

When traditional irrigation solutions are utilized, chondrocytes are exposed to a relatively low osmolarity environment both during the procedure and post-operatively until the synovial fluid is re-established. In vitro cartilage explants that are incubated in low osmolarity media show increased proteoglycan elution out of the cartilage network and this ultimately effects the overall chondrocyte survival in these explants[26]. This process primarily occurs in the superficial zone (closer proximity to the irrigated fluid compartment) and is exacerbated in injured tissues.[26] It has been shown that cartilage ECM proteoglycan elution is decreased when hyperosmolar irrigation solutions are utilized.[26]

e. Summary

Arthroscopic surgical procedures are commonly performed in equine practice. Maintaining optimal joint health and patient comfort are important when developing a new surgical technique. A hyperosmolar irrigation solution has potential benefit in arthroscopic surgery with respect to chondrocyte protection and decreased fluid extravasation. An irrigation solution that is hyperosmolar relative to plasma and the synovial environment may decrease fluid extravasation during arthroscopic procedures according to the principles of osmosis. The use of a hyperosmolar irrigation solution may have important clinical benefits in equine arthroscopic procedures if proven safe for use.

CHAPTER TWO: EXPERIMENTAL PURPOSE AND HYPOTHESIS

Demonstrating the safety of a hyperosmolar irrigation solution with respect to chondrocyte viability and cartilage water content are deemed necessary before clinical use as these are the principal parameters by which the safety of an irrigation solution is determined. The specific aims of this study were to determine: (1) whether an irrigation solution that is hyperosmolar relative to synovial fluid decreases fluid extravasation from the femorotibial joint during an arthroscopic protocol when compared to a relatively hypoosmolar solution, (2) the safety of a hyperosmolar solution by assessing the viability of joint tissues after joint irrigation, and (3) if the use of a hyperosmolar solution decreases water content in femorotibial joint tissue. It was hypothesized that joint irrigation using a relatively hyperosmolar irrigation solution leads to decreased fluid extravasation and periarticular joint swelling, without compromising the viability of joint tissues.

CHAPTER THREE: MATERIALS AND METHODS

a. Horses

Results of a pre-study power analysis using data from previous studies[27] indicated that a minimum of 6 individuals would be needed to ensure sufficient power of significance. To ensure the power of significance was attained, eight healthy, adult horses were enrolled. Basing our approach on a previous canine study[27], and with ACUC approval (Protocol # 19306), eight horses of various ages and breeds owned by the University of Missouri's Veterinary Teaching Hospital were utilized for this study. All enrolled horses were determined to be healthy and free of stifle disease based on results of physical and lameness examinations as well as ultrasonographic examinations of both stifle joints undertaken prior to the study.

b. Surgical Procedure

Following sedation with xylazine hydrochloride (XylaMed™, MWI, Boise, ID, USA) (1.1 mg/kg IV), general anesthesia was induced using ketamine hydrochloride (Zetamine™, MWI, Boise, ID, USA) (2.2 mg/kg IV) and midazolam (Midazolam™,, Akorn, Lake Forest, IL, USA) (0.05 mg/kg IV), and anesthesia was maintained using isoflurane (IsoSol, Vedco, Saint Joseph MO, USA) in 100% oxygen. All horses were then placed in dorsal recumbency with both hind limbs positioned for routine arthroscopy of the MFTJ.[20] The stifle joint was specifically chosen in this model to determine the safety of the hyperosmolar solution in a situation in which different types of tissues could be affected (synovial membrane, meniscus, and articular cartilage). Proposed surgical sites were aseptically prepared for surgery. Standard cranial approaches to both the left

and right MFTJs were employed, and 4 mm 30-degree arthroscopes (Stryker™, Kalamazoo, MI) were introduced into the joints simultaneously through arthroscopic cannulas.[20] An approach to each MFTJ was used to create instrument portals. Three-millimeter egress cannulas were placed into the instrument portals. All joints were evaluated arthroscopically to confirm appropriate portal placement and to inspect each MFTJ for evidence of disease.

c. **Stifle Joint Irrigation**

Following establishment of both portals, irrigation was initiated with either LRS, (Lactated Ringer's Irrigation, Baxter, Deerfield IL, USA) which served as the standard of care control, or the experimental HYPER solution (1.8%, 600 mOsm/L) saline solution. The hyperosmolar solution was created by adding either 23.4% saline (APP Pharmaceuticals, Schaumburg, IL, USA) or 7.2% saline (HYPER Saline Solution 7.2%, Aspen Veterinary Resources, Liberty, MO) to a 3-L bag of 0.9% saline (0.9% Sodium Chloride, Baxter, Deerfield IL, USA). One MFTJ was irrigated using LRS, and the other with the HYPER solution. The surgeons (LRH and MJM) were blinded to which solution was randomly assigned to each stifle. Solution assignment was determined through a coin toss. Twelve liters of irrigation solution were delivered to each stifle at a constant rate (25 mL/min) through a double headed irrigation pump (Cole-Parmer®, Vernon Hills, IL, USA).

d. **Stifle Morphometry**

Morphometric evaluations of each stifle joint were undertaken by a single researcher (LRH) who was blinded as to which solution was used in the respective joints (Table 1).

Stifle caliper, tape measure and ultrasonographic measurements were obtained before instrumentation (time 0) and immediately after discontinuation of irrigation and portal removal (time completion).[27] Caliper and tape measurements were used to measure the medial-to-lateral width of the proximal aspect of the femorotibial joints (Figure 1). The distances between the origin and insertion of both the medial and lateral collateral ligaments were also measured using the calipers (Figure 1). Cutaneous staples were placed at points of measurement at time 0 to assure consistency for measurement points at conclusion of the irrigation procedure. The distance between the surface of the skin and the medial femoral condyle was ascertained using a 10 MHz linear ultrasound transceiver (MyLab™, Esaote, Fishers, IN, USA) prior to and at the conclusion of the irrigation procedure. Three separate measurements were performed for each stifle at each time point; the mean of these measurements was used for statistical comparisons. Percentage of change in the stifle measurements (caliper, taper measure, and ultrasound measurements) was calculated using the following formula: [(Time 0 caliper distance – Time completion caliper distance)/Time 0 caliper distance] x 100.[27]

e. Sample Collection

After the post-procedural measurements had been obtained, horses were euthanatized while under general anesthesia using a super saturated potassium chloride solution, and both stifle joints were disarticulated immediately post-mortem. Control samples were obtained from the LFTJ as, under normal conditions, communication between the LFTJ and MFTJ joints does not occur[29]. Tissue samples (articular cartilage, synovial membrane, and meniscus) for determination of water content, cell viability, and glycosaminoglycan (GAG) content were obtained from each irrigated MFTJ. Samples

were also obtained from the unirrigated LFTJ and the TTJ, as controls. Full thickness samples were obtained through sharp dissection. Cartilage samples from both the MFTJ and LFTJ were obtained from the femoral condyles and meniscal samples were obtained from the abaxial surfaces. Cartilage samples from the tibiotarsal joints were obtained from the lateral trochlear ridge of the talus.

f. Laboratory Analysis

Following dissection, tissue samples were immediately weighed, lyophilized (to remove water), and weighed again to determine the dry weight of the tissue. The percentage of water content was determined with the following equation: (wet weight – dry weight)/wet weight[27]. Lyophilized tissues were subsequently digested in papain digestion buffer (300 µg/ml Dithioreitol; 300 µg/ml papain, 20 mM Sodium phosphate pH 6.8; 1 mM EDTA) overnight at 65°C. Digested tissues were tested for tissue GAG content using the dimethylmethylene blue (DMMB) assay, as previously described.[30] The GAG content of the digest was standardized to the dry weight of the tissue for analysis.

To determine tissue viability, a second tissue sample from each site was stained using the Invitrogen (Waltham, MA) microscopic fluorescent cell viability staining system according to the manufacturer's protocol. The tissue samples were placed in phosphate buffered saline (PBS) containing the live cell (calcein am) and dead cell (ethidium homodimer) stains. Tissues were incubated for 30 minutes at 37℃ to allow stain penetration and processing[27]. After 30 minutes, the tissues were washed for ten minutes with plain PBS, and images were obtained of the staining using a fluorescent microscope. The area of tissue section used for counting was measured using the MicoSuite Basic

edition software program (Olympus American Inc, Melville, NY, USA)[27]. Viable cell density was determined using the formula: viable cell count/measured tissue area (mm^2).

g. Statistics

Data for each group were compiled and mean and standard deviation were determined for each variable assessed. Data were compared for statistically significant ($P < 0.05$) differences in both water and GAG content between groups with a Kruskal-Wallis test. Further, significant differences in fluid extravasation were determined by comparing pre and post irrigation measurements for the hypoosmolar and hyperosmolar treated groups using a paired t-test analysis with significance set at $p<0.05$.

CHAPTER FOUR: RESULTS

a. Horses

The breeds represented were American Quarter Horses (3/8 [37.5%]), American Quarter Horse crossbreds (3/8 [37.5%]), Thoroughbred (1/8 [12.5%]), and Westphalian (1/8 [12.5%]). There were 4 geldings and 4 mares and ranged in age from six to twenty-three years of age. All horses were free of evident stifle pathology based on palpation, ultrasonographic examination, and arthroscopic examination of the MFTJ and overt lameness was not present.

b. Periarticular Joint Swelling

Based on results of both external caliper measurements and measurements obtained using US, joint swelling developed in all treated joints. Percentage increase in joint dimensions based on caliper measurements for the LRS group (5.5 ± 4.3%) was not significantly different from the HYPER group (7.5 ± 5.8%). Similarly, the percentage increase in joint dimensions based on US measurement in the LRS group (83.9 ± 84.6%) was not significantly different from the HYPER group (131.2 ± 144.9%)

c. Chondrocyte Viability

The viable chondrocyte density of cartilage obtained from the medial femoral condyle after arthroscopic irrigation with either the HYPER or LRS solutions was not significantly different than the TTJ control samples, indicating that the arthroscopic solutions used in this study did not cause a significant reduction in cartilage tissue viability (Figure 2). There was not a significant difference in viable chondrocyte density

between samples in the LRS and HYPER groups, indicating that both solutions had similar effects on cartilage tissue (Table 2).The viable chondrocyte density of cartilage obtained from the medial femoral condyle following arthroscopic irrigation with either the LRS or HYPER solutions was not significantly different than the TTJ control samples, indicating that the arthroscopic irrigation solutions used in this study did not cause a significant reduction in cartilage tissue viability (Figure 2).

d. Tissue Water Content

The water content of tissues collected from the meniscus, synovial membrane, and articular cartilage after arthroscopic surgery using either the HYPER or LRS solutions was not significantly different from control samples, or from each other ($P>0.05$).

e. GAG Content

The GAG content in the meniscal and articular cartilage samples were considered normal for both groups and were not significantly different from one another or untreated control samples ($P = 0.977$ for meniscal and $P= 0.505$ for articular cartilage).

CHAPTER FIVE: DISCUSSION

The results of this study support the hypothesis that hyperosmolar arthroscopy irrigation fluid was not detrimental to chondrocyte viability or articular cartilage water content when compared with the standard-of-care solution (LRS). Additionally, there was not a significant decrease in tissue viability when compared to tissues from a non-operated control joint (TTJ). However, the results of this study did not support the hypothesis that swelling associated with intra-procedural fluid extravasation would be decreased in the hyperosmolar group compared to the current standard of care solution in the femorotibial joint.

High volume and pressure irrigation during arthroscopic procedures leads to fluid extravasation. This accumulation of irrigation fluid within the periarticular tissues may create technical challenges, especially in lengthy arthroscopic procedures. Extravasated fluid additionally may exert negative effects on local wound healing and promote infection in the post-operative period.[16] Extravasation can be limited through good anatomic placement of the portals, minimizing surgical time, and carefully monitoring the irrigation pump flow and pressure rates. It was shown in both human and canine studies that the osmotic effect of a hyperosmolar irrigation solution decreased fluid extravasation during standard arthroscopic procedures.[7,8] Although this observation was not supported by the findings from the present study, further research examining other joints (fetlock, carpus), for which the joint circumference is more easily measured, may provide further insight into the osmotic effect of a hyperosmolar irrigation solution at other locations.

Lactated Ringer's solution (273 mOsm/L) is the current standard-of-care irrigation solution used for equine arthroscopic surgery.[9-11] Normal saline (0.9% NaCl solution, 300 mOsm/L) has been shown to inhibit synthesis of GAG by chondrocytes and has therefore fallen out of favor in recent years.[17] The proteoglycan content of the extracellular matrix dictates the osmolality of the extracellular environment to which cartilage is exposed. By maintaining a relatively hyperosmolar environment, chondrocyte swelling is prevented.[18,19] While this study did not investigate the effect of hyperosmolar irrigation on responses in damaged cartilage surface (as would be encountered during clinical arthroscopy), it has been demonstrated in other species that irrigation fluid with higher osmolarity prevents fluid imbibition at damaged surfaces and prevents cell lysis secondary to chondrocyte swelling in diseased tissues with a damaged extracellular matrix.[12,20,21] Therefore, further studies assessing the effect of hyperosmolar arthroscopic fluid on damaged cartilage and the other tissues of diseased equine joints are warranted.

a. Limitations

Several limitations that should be considered when assessing the data from this study. First, the model used in this study does not provide recovery or long-term outcome measures, which are two important concerns associated with tissue extravasation in a clinical setting. Second, data from this study were obtained from a relatively small number of animals. However, the sample number used in this study was based on previously published animal model studies in this area of research, and the number of animals used in this study is in line with the principles of ethical use of animals for

research. Further, the number of animals used in this study did provide evidence for the safety of the arthroscopic solutions assessed, providing impetus for more comprehensive studies using clinical patients and evaluating long-term outcome measures. Third, because these data were obtained from normal stifle joints without observable pathology, these results may not be applicable to diseased joints that are typically encountered in a clinical situation. Therefore, further investigation is required to determine how tissues from disease joints respond to hyperosmolar irrigation solution. Previous studies have indicated chondroprotective effects of hyperosmolar irrigation solutions when used for arthroscopic surgery in human patients, indicating the need to investigate these solutions in horses. [12,20,21]

b. Conclusions

With these limitations in mind, it was concluded that the use of a hyperosmolar irrigation solution is as safe as LRS to use as an arthroscopic irrigation solution in equine patients. Assurance of safety is supported by the facts that both chondrocyte viability and articular cartilage water content were not significantly different between irrigation treatments under these experimental conditions. In contrast to previous studies, the data from this study did not indicate that a hyperosmolar solution resulted in a decrease in tissue extravasation during an arthroscopic procedure of the equine stifle joint. Since data from this study indicates that use of a hyperosmolar irrigation solution is as safe as LRS, and that positive effects of using a hyperosmolar solution have been demonstrated in other animals and human clinical patients, further studies investigating the use of this solution in other joints, such as the fetlock or carpus, as well as diseased joints are warranted. In

addition to the measures assessed in this study (joint extravasation, tissue water content, and tissue viability), the effect of a hyperosmolar irrigation solution on post-surgical joint inflammation should be investigated, as a previous study indicated potential anti-inflammatory effects of a hyperosmolar solution on diseased tissues using an *ex vivo* model.[22] The goal of this work is to improve the performance and outcomes of arthroscopic treatments for equine orthopedic patients.

Manufacturer's Details

xylazine (XylaMed,, MWI, Boise, ID, USA)

ketamine (Zetamine, MWI, Boise, ID, USA)

midazolam (Midazolam, Akorn, Lake Forest, IL, USA)

isoflurane (IsoSol, Vedco, Saint Joseph MO, USA)

4 mm 30-degree arthroscopes (Stryker™, Kalamazoo, MI)

Lactated Ringers Irrigation (Baxter, Deerfield IL, USA)

Hyperosmolar 0.9% Sodium Chloride, Baxter, Deerfield IL, USA

Hyper Saline Solution 7.2%, Aspen Veterinary Resources, Liberty, MO

Double headed irrigation pump (Cole-Parmer®, Vernon Hills, IL, USA).

10 MHz linear ultrasound transceiver (MyLab™, Esaote, Fishers, IN, USA

MicoSuite Basic edition software program (Olympus American Inc, Melville, NY, USA)

ILLUSTRATIONS

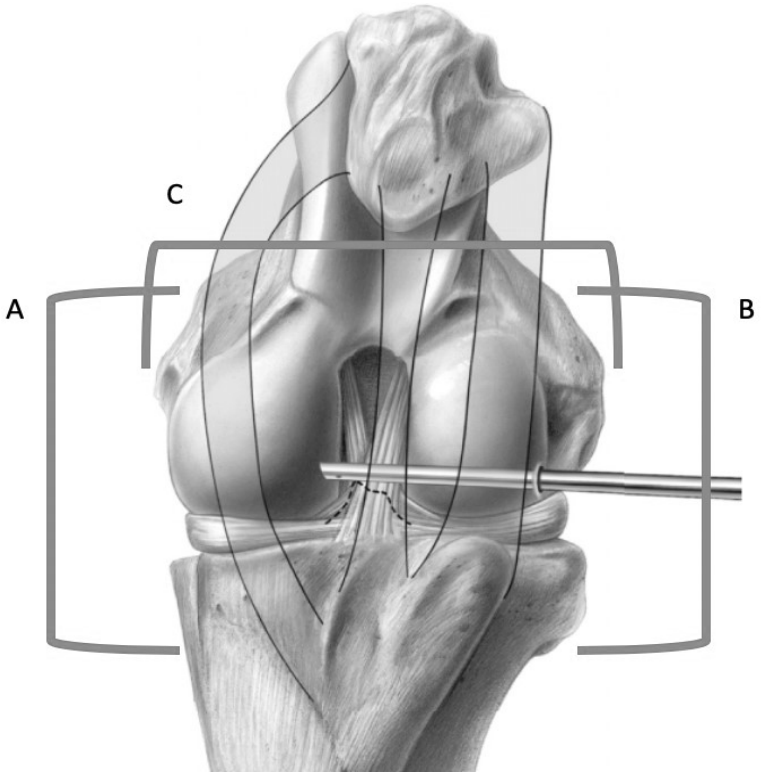

Figure 1. Measurements will be performed as outlined. A & B: the origin to insertion of the medial and lateral collateral ligaments. C: proximal aspect of the femorotibial joint. Adapted from Diagnostic and Surgical Arthroscopy in the Horse (4ed)[20] [Portal will not be utilized].

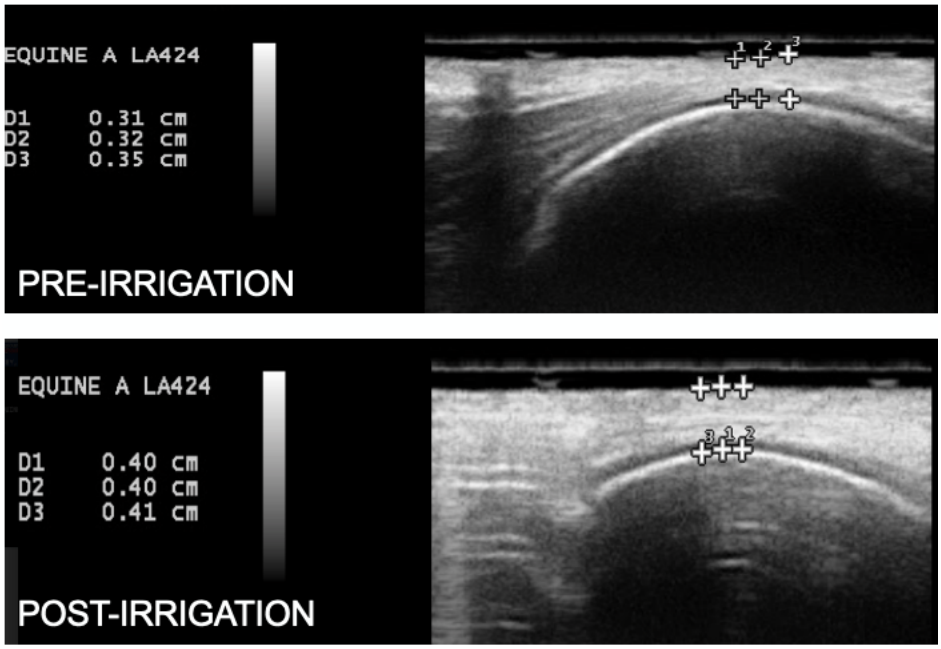

Figure 2. Ultrasound depth measurements were obtained with the ultrasound probe centered over the medial femoral condyle. Meauserements were obtained prior to and after arthroscopic irrigation.

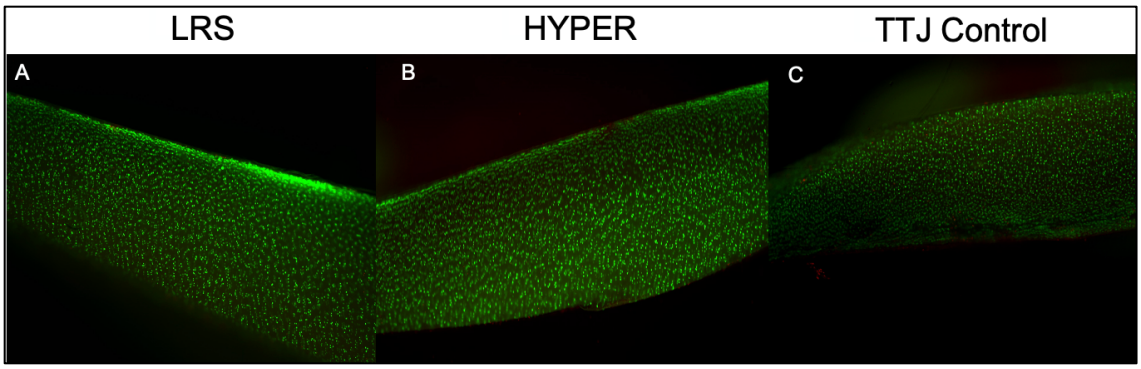

Figure 3. Chondrocyte viability (displayed in green) with calcein acetomethoxy and ethidium homodimer-1 staining. Articular cartilage samples from the LRS and Hyper groups from the MFTJ (A and B respectively) and the TTJ control (C).

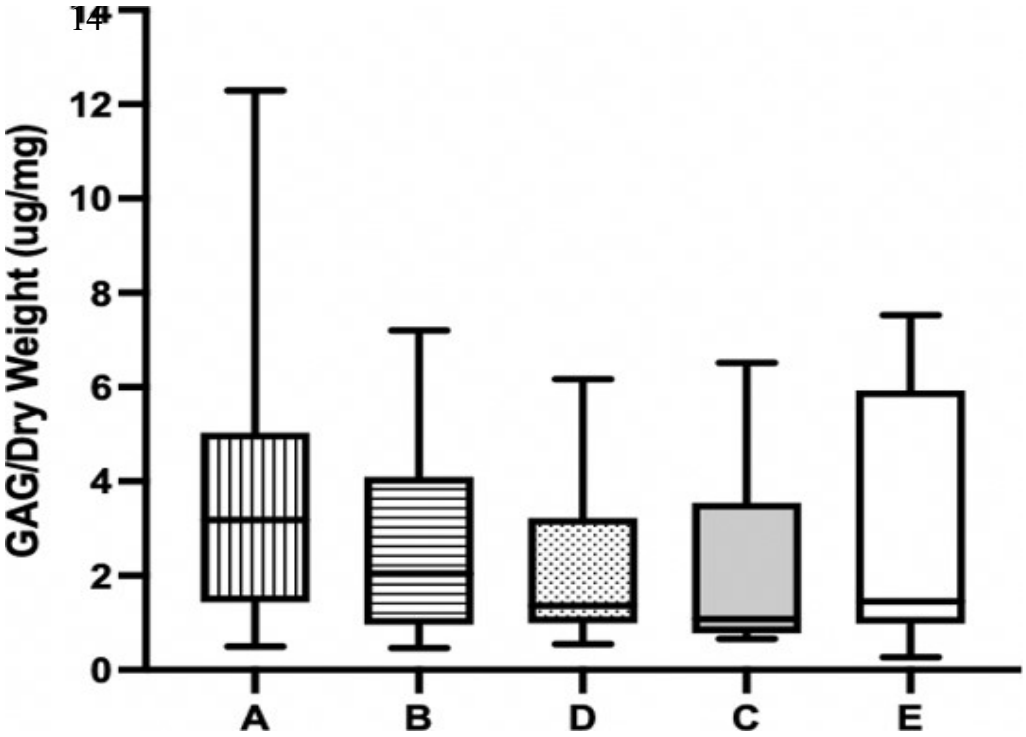

Figure 4. Box and whisker plots of articular cartilage GAG/Dry weight ratios of the (A) nonoperative group (tibiotarsal joints), (B) LRS controls (lateral FTJ), (C) HYPER controls (medial FTJ), (D) HYPER treated (MFTJ), and (E) LRS treated (MFTJ). No significance was noted between groups.

TABLES

Measurement Number	Measurement Description
1	Origin to Insertion of MCL Caliper (cm)
2	Origin to Insertion of MCL Tape Measure (cm)
3	Origin to Insertion of LCL Caliper (cm)
4	Origin to Insertion of LCL Tape Measure (cm)
5	Femoral Tibial Joint Width (medial to lateral joint capsule) Caliper (cm)
6	Femoral Tibial Joint Width (medial to lateral joint capsule) Tape Measure (cm)
7	Ultrasound Depth at medial femoral condyle (mm)

Table One: List of measurements obtained at time 0 and at the conclusion of the arthroscopic irrigation.

	LRS							HYPER						
MEASUREMENT	1	2	3	4	5	6	7	1	2	3	4	5	6	7
CHANGE MEAN	5.713	5.488	-0.9143	-0.625	-1.888	-1.129	83.91	4.213	7.525	0.4714	-1.313	-0.8	0.2857	131.2
STD. DEVIATION	5.598	4.287	2.234	3.462	2.534	6.028	84.58	5.049	5.482	1.5	1.28	3.395	2.249	144.9

Table Two: Precent change stifle girth measurements. Measurements: (1) medial to lateral FTJ (tape measure), (2) medial to lateral FTJ (caliper), (3) origin to insertion LCL (tape measure), (4) origin to insertion LCL (caliper), (5) origin to insertion MCL (caliper), (6) origin to insertion MCL (tape measure), (7) ultrasound depth

	HYPER			LRS			CONTROL-HYPER			CONTROL-LRS			TTJ	
	cartilage	meniscus	synovium	cartilage	meniscus	synovium	cartilage	meniscus	synovium	cartilage	meniscus	synovium	cartilage	synovium
MEAN	76.827	60.597	81.913	76.943	64.581	81.766	76.156	62.847	80.197	81.753	62.343	82.808	77.880	81.629
STD. DEVIATION	2.464	11.610	6.313	2.368	15.048	8.159	2.486	4.643	6.690	6.888	8.239	6.801	7.103	5.647

Table Three: Average percent water in the sampled tissues. Control samples were obtained from the lateral FTI, and the non-op samples were obtained from the tibiotarsal joints.

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