The use of a hyperosmolar irrigation solution is safe in an equine stifle joint model but does not reduce joint swelling

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OBJECTIVE
To determine the following: (1) whether an irrigation solution that is hyperosmolar (HYPER) relative to synovial fluid decreases tissue extravasation during an arthroscopic protocol when compared to a relatively hypoosmolar solution, (2) the safety of a HYPER solution based on viability of joint tissues following joint irrigation, and (3) if the use of a HYPER solution decreases water content in stifle joint tissue.

ANIMALS
8 adult horses.

PROCEDURES
A prospective, blinded, randomized controlled trial was performed to compare lactated Ringer’s solution (LRS; 273 mOsm/L) and a HYPER (600 mOsm/L) irrigation solution for routine medial femorotibial joint (MFTJ) arthroscopy. Primary outcomes included quantification of periarticular fluid retention based on measured changes in defined stifle joint girth and ultrasonographic (US) criteria. Water content of tissue samples was assessed. The viability of articular cartilage was determined using a microscopic fluorescent cell viability staining system.

RESULTS
No significant difference in postprocedural joint swelling was observed between LRS and HYPER treatment groups. Percent increments in femorotibial joint dimensions (mean ± SD) 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 ≤ .05). Chondrocyte viability and tissue water content were maintained in both treatment groups, and differences were not statistically significant.

CLINICAL RELEVANCE
Doubling the osmolarity of an irrigation solution used routinely for arthroscopy does not result in detrimental effects on chondrocyte viability or tissue water content. However, use of a relatively HYPER irrigation solution did not attenuate procedural tissue swelling of the equine stifle joint.

Arthoscopic 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.1 Continuous irrigation is an essential component of arthroscopic surgery in order to assure adequate joint distention for visualization of intraarticular structures. However, constant irrigation causes localized joint inflammation and periarticular fluid extravasation.2–5 Inevitable intraoperative fluid extravasation results in compression of the synovial compartment interfering with optimal arthroscopic visualization, prolonging surgical time, and increasing pain following surgery.5,6 An irrigation solution that is hyperosmolar (HYPER) relative to plasma and the synovial environment may decrease fluid extravasation during arthroscopic procedures according to the principles of osmosis and has been reported in other species.7,8

Previous studies have indicated that arthroscopic irrigation of joints can lead to increased synovial fluid nucleated cell counts and total protein concentrations, indicating that joint inflammation may be an independent consequence of the procedure.3,4 Arthroscopic irrigation is typically performed using a isotonic electrolyte solution such as lactated Ringer’s solution (LRS) (273 mOsm/L), which is hypoosmolar relative to normal equine synovial fluid (350 to 450 mOsm/L).9–11 This hypoosmolar environment persists within the joint until the composition of synovial fluid is reestablished, restoring normal joint...
homeostasis. In human arthroscopy studies, chondroprotective effects have also been demonstrated with HYPER solutions (up to 600 mOsm/L).12

In a recently published study7 involving shoulder joint arthroscopy in research dogs, the mean percentage change in shoulder joint girth was higher in the control (isosmolar) group than in the HYPER group (600 mOsm/L), and there was no significant change in water content or chondrocyte viability. The results of this study were used to guide the development of a human clinical study that demonstrated that use of a hyperosmolar irrigation solution (593 mOsm/L) resulted in less mean weight gain, reduced increment in shoulder joint girth, and lowered visual analog scale pain scores in the immediate postoperative period.9 Therefore, the use of a HYPER irrigation solution has the potential to improve the welfare of horses undergoing common arthroscopic procedures through reduction of surgical time, less procedural inflammation, and reduced postsurgical pain.

Potential adverse effects associated with routine arthroscopic lavage (as currently performed) include postoperative synovitis, capsulitis, or articular cartilage damage/chondrocyte death, all of which highlight the need for safety studies prior to implementing new fluid lavage protocols in clinical practice.3,4,11,13 It is essential to demonstrate the safety of a HYPER irrigation solution with respect to chondrocyte viability and cartilage water content before being recommended and adopting them for 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 the following: (1) whether an irrigation solution that is HYPER relative to synovial fluid decreases fluid extravasation during an arthroscopic protocol when compared to a relatively hypoosmolar solution, (2) the safety of a HYPER solution by assessing the viability of joint tissues after joint irrigation, and (3) if the use of a HYPER solution decreases water content in stifle joint tissues.7 It was hypothesized that joint irrigation using a relatively HYPER irrigation solution would result in a significant decrease in fluid extravasation and periarticular joint swelling, without a significant decrease in the viability of cells in representatative joint tissues and water content of the joint tissues, compared to control joints receiving LRS as the irrigation solution.

Materials and Methods

Horses Results of a prestudy power analysis (α < 0.05; P ≥ .8) using data from previous studies7 indicated that a minimum of 6 individuals would be needed to ensure that power of significance. To ensure the power of significance was attained, 8 healthy, adult horses were enrolled. Basing our approach on the previous canine study,7 and with Animal Care and Use Committee approval (protocol no. 19306), 8 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 joint disease based on results of physical and lameness examinations as well as ultrasonographic examinations of both stifle joints undertaken prior to the study.

Surgical procedure Following sedation with xylazine hydrochloride (XylaMed: 1.1 mg/kg IV), general anesthesia was induced using ketamine hydrochloride (Ketamine: 2.2 mg/kg IV) and midazolam (0.05 mg/kg IV), and anesthesia was maintained using isoflurane (IsoSol) in 100% oxygen. All horses were then placed in dorsal recumbency with both hind limbs positioned for routine arthroscopy of the medial femorotibial joint (MFTJ).5 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. An approximately 8-mm incision was made through the skin and fascia with an 11 blade. Four millimeter 30-degree arthroscopes (Stryker) were introduced into the joints simultaneously through arthroscopic cannulas.5 An approach to each MFTJ was used to create instrument portals. Three-millimeter egress cannulas were placed into the instrument portals. All joints were explored arthroscopically to confirm appropriate portal placement and to inspect each MFTJ for evidence of disease.

Stifle joint irrigation Following establishment of both portals, irrigation was initiated with either LRS, (Lactated Ringer’s Irrigation (Baxter)) 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 (115 mL) (APP Pharmaceuticals) or 7.2% saline (373 mL) (HYPER Saline Solution 7.2%; Aspen Veterinary Resources) to a 3-liter bag of 0.9% saline (0.9% sodium chloride; Baxter). One MFTJ was irrigated using LRS and the other with the HYPER solution. The surgeons (LRH and MJM) were blinded to which solution was used to each stifle joint. Solution assignment was determined through a coin toss. Twelve liters of irrigation solution was delivered to each stifle joint at a constant rate (25 mL/min) through a double-headed irrigation pump (Cole-Parmer).

Stifle joint 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 (Appendix 1). Stifle joint caliper, tape measure and ultrasonographic measurements were obtained before instrumentation (time 0) and immediately after discontinuation of irrigation and portal removal (time completion).7 Caliper and tape measurements were used to measure

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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. Medial measurements were obtained to evaluate the swelling of the MFTJ, and lateral measurements were obtained as the lateral femorotibial joint (LFTJ) served as a control. 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) prior to and at the conclusion of the irrigation procedure. Three separate measurements were performed for each stifle joint at each time point; the mean of these measurements was used for statistical comparisons. Percentage of change in the stifle joint measurements (as listed above) was calculated using the following formula: [% (time 0 caliper distance - time completion caliper distance)/time 0 caliper distance] × 100.

**Sample collection**

After the postprocedural measurements had been obtained, horses were euthanatized under general anesthesia using a super saturated potassium chloride solution, and both stifle joints were disarticulated immediately postmortem. Control samples were obtained from the LFTJ as, under normal conditions, communication between the LFTJ and MFTJ joints does not occur.14 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 tibiotarsal joint (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 TTJs were obtained from the lateral trochlear ridge of the talus.

**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.7 Lyophilized tissues were subsequently digested in papain digestion (300 μg/mL dithiothreitol, 300 μg/mL papain, 20 mM sodium phosphate [pH 6.8], and 1 mM EDTA) overnight at 65°C. Digested tissues were tested for tissue GAG content using the dimethylmethylene blue (DMMB) assay, as previously described.15 The GAG content from 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 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°C to allow stain penetration and processing.7 After 30 minutes, the tissues were washed for 10 minutes with plain PBS, and images were obtained of the staining using a fluorescence microscope. The area of tissue section used for counting was measured using the MicoSuite Basic edition software program (Olympus American Inc).7 Viable cell density was determined using the formula: viable cell count/measured tissue area (mm²).

**Statistics**

Data for each group were compiled and mean and standard deviation were determined for each variable assessed. Data were compared for statistically significant ($P < .05$) 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 postirrigation measurements for the hypoosmolar and hyperosmolar treated groups using a paired $t$ test analysis with significance set at $P < .05$. 

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**Figure 1**—Measurements were performed as outlined. A and B—The origin to insertion of the medial and lateral collateral ligaments. C—Proximal aspect of the femorotibial joint.
Results

The breeds represented were American Quarter Horses (3/8 [37.5%]), American Quarter Horse crosses (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 6 to 23 years of age. All horses were free of evident stifle joint pathology based on palpation, ultrasonographic examination, and arthroscopic examination of the MFTJ, and overt lameness was not present.

Based on results of both external measurements (tape measure and caliper measure) and measurements obtained using ultrasound (US), postprocedural joint swelling developed in all treated joints (Figure 2). The measurement of medial to lateral FTJ had the highest percentage change from preprocedure to postprocedure compared to the origin to insertion lateral collateral ligament (LCL) and origin to insertion medial collateral ligament (MCL) measures. Therefore, this measure was selected to be indicative of changes in joint girth for this discussion. In general, the percent change in the measurement from the medial to lateral FTJ indicated that there was a percentage increase in joint dimensions for the majority of the joints postprocedure. There was agreement in the change in joint girth when the caliper (LRS: 5.5 ± 4.3%; HYPER: 7.5 ± 5.8%) or the tape measure (LRS: 5.7 ± 5.6%; HYPER: 4.2 ± 5.1%) was used to assess the medial to lateral FTJ, and there were no significant differences observed between groups for either measurement, or between measurements of the same group. However, for 2 of the joints with minimal change in joint swelling (<2% change), 1 of the measurements indicated a decrease in joint girth, while the other indicated an increase. This finding is indicative of the variability in measurements that can occur when there is minimal change in joint girth observed since the decrease in joint swelling was observed in both groups (LRS and HYPER) and in both measurement types (caliper and tape measure). 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%)

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 3).

Figure 2—Box and whisker plots for percent change of stifle girth measurements from presurgery to postsurgery. Ultrasonographic depth (A) and girth measurement (B) from medial to lateral femorotibial joint (FTJ), origin to insertion lateral collateral ligament (LCL) or origin to insertion medial collateral ligament (MCL) using either a tape measurer or a caliper. There were no significant differences between groups identified for any of the change in girth measures performed in this study. (X) indicates mean, bar indicates median, box indicates 25 to 75 quartiles, whiskers indicate lower and upper quartiles, and circles indicate outliers.

Figure 3—Chondrocyte viability (displayed in green) with calcein acetomethoxy and ethidium homodimer-1 staining. Articular cartilage samples from the lactated Ringer’s solution (LRS) and hyperosmolar solution (HYPER) groups from the medial femorotibial joint (MFTJ) (A and B, respectively) and the tibiotarsal joint (TTJ) control (C).
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. 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.

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 ($P > .05$) (Figure 4). Further, 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 ($P = .977$ for meniscal and $P = .505$ for articular cartilage) (Figure 5).16

**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 the nonoperated control group (TTJ). However, the results of this study did not support the hypothesis that swelling associated with intraprocedural fluid extravasation would be decreased in the hyperosmolar group compared to the standard of care solution in the femorotibial joint. Previous canine and human studies7,8,17,18 have not reported adverse effects of HYPER fluid accumulating within the periarticular tissue planes. The possible accumulation of this HYPER fluid within the periarticular tissue planes could account for the greater postprocedural ultrasound depth measurements that were observed in the HYPER group in this study. Further studies are needed to investigate

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**Figure 4**—Average percent water in the sampled tissues. The MFTJs were treated with either the HYPER or LRS. Control samples were obtained from the lateral femorotibial joint (LFTJ) (Control-HYPER and control-LRS refers to nonirrigated lateral stifle compartment for each treated MFTJ). The nonoperated samples were obtained from the TTJ. There were no identified differences between each of the columns.

**Figure 5**—Box and whisker plots of articular cartilage glycosaminoglycan content to dry weight ratios of the nonoperative group (tibiotarsal joints) (A), lateral femorotibial joint of an lactated ringers solution treated medial femorotibial joint (B), medial femorotibial joint of an hyperosmolar solution treated medial femorotibial joint) (C), lateral femorotibial joint of an hyperosmolar solution treated medial femorotibial joint(D), and lactated Ringer’s solution treated medial femorotibial joints (E). No significance was noted between groups.
greater time points following arthroscopy with HYPER irrigation fluids to evaluate postprocedural swelling in the immediate postoperative period.

High volume and pressure irrigation during arthroscopic procedures lead to fluid extravasation and 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 postoperative period. Extravasation can be limited through good anatomic placement of the portals, minimizing surgical time, and carefully monitoring the irrigation pump flow and pressure rates. Although intraarticular pressure was not recorded in this study, pump flow rates were set at the same rate to ensure that the delivery of fluid was held constant. Investigation into portal placement and instrument manipulation may be important when considering risks for extravasation.

As with most joints, there are various approaches described for the instrument portal for the MFTJ. In this study, a cranial instrument portal was utilized to prevent contamination of the LFTJ control joint. An approach using the standard instrument portal would potentially affect the periarticular swelling and warrants further investigation. Additionally, intraarticular swelling may be encountered secondary to repeat instrument manipulation, particularly large instruments or during the removal of large fragments as there is obstructed outflow and excessive perfusion pressure. It has also been shown that facial planes are weakened with repeat instrument entry or excessive instrument movement resulting in the extravasation of fluid. Three-millimeter egress cannulas were utilized for instruments in the current study as to prevent variability within instrument manipulation. Further investigation utilizing techniques to model removal of an osteochondral fragment where outflow is temporarily obstructed is warranted.

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. Although this observation was not supported by the findings from the present study, further research examining other joints (fetlock, carpus, or hock) would provide insights into the effect of portal location and how fluid extravasates differently in other joints. Additionally, extravasation commonly encountered during arthroscopy of the femoro-patellar joint and warrants investigation in the future. The femorotibial joints were chosen in this study as each horse had an unoperated control and meniscal tissue was present during arthroscopy of the femoro-patellar joint and 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 postoperative period. Extravasation can be limited through good anatomic placement of the portals, minimizing surgical time, and carefully monitoring the irrigation pump flow and pressure rates. Although intraarticular pressure was not recorded in this study, pump flow rates were set at the same rate to ensure that the delivery of fluid was held constant. Investigation into portal placement and instrument manipulation may be important when considering risks for extravasation.

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Lactated Ringer’s solution (273 mOsm/L) is the standard-of-care irrigation solution used for equine arthroscopic surgery. Normal saline (0.9% NaCl solution and 300 mOsm/L) has been shown to inhibit synthesis of GAG by chondrocytes and has therefore fallen out of favor in recent years. 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. 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. Therefore, further studies assessing the effect of hyperosmolar arthroscopic fluid on damaged cartilage and the other tissues of diseased equine joints are warranted.

Several limitations that must 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 clinically. 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.

With these limitations in mind, it can be concluded that the use of a hyperosmolar irrigation solution does not cause any immediate deleterious effects on synovial joint tissues for to use as an arthroscopic irrigation solution in equine patients. Assurance of this is supported by the facts that both chondrocyte viability and articular cartilage water content were not significantly different between irrigation treatments. Further long-term studies are needed to assure safety in the postoperative period with additional monitoring of postoperative comfort and periarticular swelling. 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 postsurgical 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.

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The authors declare that there were no conflicts of interest.

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Appendix 1

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

| 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) (cm) |
| 6                  | Femoral tibial joint width (medial to lateral joint capsule) tape measure (cm) |
| 7                  | Ultrasonographic depth (mm) |

LCL = Lateral collateral ligament. MCL = Medial collateral ligament.