

**The Effects of Perineural and Intrasynovial Anesthesia of the Equine
Foot on Subsequent Magnetic Resonance Images**

by

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ABSTRACT

THE EFFECTS OF PERINEURAL AND INTRASYNOVIAL ANESTHESIA OF THE EQUINE FOOT ON SUBSEQUENT MAGNETIC RESONANCE IMAGES

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Artifacts caused by regional anesthesia can influence image interpretation of ultrasound and nuclear scintigraphy. Perineural and intrasynovial anesthesia is commonly performed prior to magnetic resonance imaging (MRI); and the effects on MR images, if any, is unknown.

The objectives of this prospective, randomized, blinded experiment were to determine if perineural and intrasynovial anesthesia of structures in the equine foot cause iatrogenic changes detectable with MRI. A baseline MRI of both front feet was performed on 15 horses 2 to 6 days prior to mepivacaine injection adjacent to the lateral and medial palmar digital nerves (PDN), and into the podotrochlear bursa (PB), digital flexor tendon sheath (DFTS), and distal interphalangeal joint (DIPJ) of one randomly assigned forelimb. MRI was repeated at 24 and 72 hours post-injection; then qualitative and quantitative assessments of MRI findings were performed.

The results of this study showed MRI findings associated with the PDN, PB and DIPJ at 24 and 72 hours after mepivacaine injection did not alter significantly from those

at baseline. Compared to baseline, a significant increase in synovial fluid volume of the DFTS was detected with MRI at 24 and 72 hours post-injection.

Therefore, perineural anesthesia of the PDN and intrasynovial anesthesia of the PB or DIPJ did not interfere with the interpretation of MRI examinations performed at 24 or 72 hours after injection. However, intrasynovial anesthesia of the DFTS caused an iatrogenic increase in synovial fluid, which was detectable on MRI for at least 72 hours. Although a definite time frame for resolution of DFTS distension was not determined, we recommend waiting greater than 3 days between intrasynovial anesthesia of the DFTS and evaluation with MRI.

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DECLARATION OF WORK PERFORMED

I declare that all work reported in this thesis was performed by Belinda Black, with the exception of some design, programming and interpretation associated with statistical analysis, which was performed by Ms. Gabrielle Monteith. I would also like to acknowledge the contributions of Norm Konyer for organizing and implementing the FSL program and Alice Daw for MRI assistance.

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LIST OF ABBREVIATIONS

DIPJ Distal interphalangeal joint

DFTS Digital flexor tendon sheath

GRE Gradient echo

MRI Magnetic resonance imaging

PB Podotrochlear bursa (navicular bursa)

PDN Palmar digital nerves (lateral and medial)

STIR Short tau (TI) inversion recovery

SGRE Spoiled gradient echo

TI Inversion time

1.0 – INTRODUCTION

Magnetic resonance imaging (MRI) is currently the gold standard for diagnostic imaging of the equine digit due to its excellent visualization of both soft tissue and osseous structures within the hoof. The interpretation of any diagnostic imaging modality involves understanding how artifacts interfere with image acquisition or overall interpretation.

Diagnostic analgesia, which is commonly used during lameness examination in the horse, has been recognized to cause artifacts in other imaging modalities, such as radiography, scintigraphy and ultrasonography. Investigating the effects of diagnostic analgesia on scintigraphy led to the discovery that perineural and intra-articular anesthesia can interfere with the interpretation of soft-tissue phase scintigrams. Currently, the effects of diagnostic analgesia on MRI are unknown.

This investigation was conducted to determine if several diagnostic analgesic techniques used in the equine foot would cause iatrogenic changes detectable on subsequent MRI examinations. Commonly performed procedures, including intrasynovial injection of the podotrochlear bursa (PB) and digital flexor tendon sheath (DFTS), intra-articular injection of the distal interphalangeal joint (DIPJ), and perineural injection of the palmar digital nerves (PDN) were performed. Magnetic resonance images performed at 24 and 72 hours after injection were compared with a baseline pre-injection MRI, and qualitative and quantitative assessments of MRI findings were performed.

1.1 – GOALS AND HYPOTHESES

The study was designed to determine if injection of mepivacaine into perineural and synovial structures of the equine foot would cause iatrogenic changes detectable with MRI. We hypothesized that after injection of mepivacaine into the PB, DFTS and DIPJ, MRI would detect an increase in synovial fluid at 24 hours, but not at 72 hours post-injection. We also hypothesized that the injection of mepivacaine adjacent to the PDN and into the PB, DFTS and DIPJ would cause abnormalities at the needle entry sites, which would be detectable on these post-injection MRIs.

2.0 – LITERATURE REVIEW

2.1 – Introduction

Lameness is the most prevalent health problem among all horses, the most significant economic cost to the equine industry (USDA 2001), and the most important cause of wastage in racing horses (McIlwraith & Trotter 1996). Within the vast range of clinical signs that are associated with lameness, foot pain is one of the most common; and diagnostic methods for conditions within the foot are relatively limited (Dyson, Murray, Schramme, *et al* 2003).

Evaluation of the horse for general conformation, abnormalities in gait, and for abnormalities during palpation and manipulation of the limbs are routinely among the first steps in lameness assessment. However, diagnostic analgesia, which usually consists of perineural and/or intrasynovial anesthesia, frequently follows (Ross 2011). Diagnostic analgesia is arguably the most valuable tool in localizing lameness, and identifies a region of interest that becomes the focus for imaging modalities. The more specific the clinical localization of the source of lameness, the easier it is to differentiate clinically significant findings from non-significant findings during diagnostic imaging (Bassage & Ross 2011; Chaby, Coelho, Kinns 2012).

2.2 – Local Anesthetics

2.2.1 – Local anesthetic agents – The most common anesthetic agents used for diagnostic evaluations in the horse include lidocaine, mepivacaine and bupivacaine. These local anesthetics share the same mechanism of action, specifically preventing the increase in membrane permeability to sodium ions; thereby inhibiting the generation of

neuronal action potentials and subsequent nerve conduction (Butterworth & Strichartz 1990). Local anesthetics are made of 3 components, an aromatic ring, an intermediate linkage and a terminal amine. Each of these components contributes to the properties of the specific drug. The aromatic ring determines lipid solubility, which in turn, determines potency. With increased lipid solubility, a greater amount of the drug diffuses through neural coverings and cell membranes. The intermediate linkage between the aromatic ring and the terminal amine determines whether the local anesthetic is categorized as either an amide- or an ester-type agent. Lidocaine, mepivacaine and bupivacaine are classified within the amide group. These drugs tend to have a more rapid onset, greater potency and longer duration of action than ester-type agents, such as cocaine, procaine and benzocaine. The terminal amine component acts as an “on/off” switch to allow the drug to transform between a hydrophilic quaternary (amine with 4 bonds) weak-acid salt and a lipophilic tertiary (amine with 3 bonds) base (Skarda & Tranquilli 2007; Skarda, Muir, Hubbell 2009). The pH at which the local anesthetic exists in equilibrium between these 2 states is known as the dissociation coefficient (pKa). Local anesthetics with a lower pKa than others will have a greater percentage of the lipophilic form available; hence, more drug is able to penetrate the axonal membrane. The pKa values for mepivacaine, lidocaine and bupivacaine are 7.6, 7.9 and 8.1 respectively (Skarda & Tranquilli 2007; Skarda *et al* 2009).

Local anesthetics, such as lidocaine and mepivacaine, are commercially available as water-soluble quaternary salts in solution; hence, these will not penetrate the neuron. When injected into tissue with a normal pH of 7.4, a percentage of quaternary salts will convert to lipid-soluble tertiary bases; the proportion depending on the individual pKa of

the drug. When injected into an acidic environment, such as inflamed or infected tissue, a lower proportion of the lipid-soluble more-penetrable form of the drug will be present. In this situation, a drug with a lower pKa, such as mepivacaine, would be more likely to produce effective anesthesia (Skarda & Tranquilli 2007; Skarda *et al* 2009).

When infiltrated into normal perineural tissue, lidocaine has a rapid onset (5 minutes), intermediate potency, and an intermediate duration of action (1 - 3 hours). In horses, 2% lidocaine hydrochloride solution is commonly used for perineural, intrasynovial and caudal epidural anesthesia, for topical anesthesia during pharyngeal and laryngeal surgery, and systemically for treatment of ileus (Malone, Ensink, Turner, *et al* 2006). Lidocaine is metabolized principally by the liver. The central nervous system is sensitive to the toxic effects of lidocaine, which are comparable to those of mepivacaine. However, the severity of symptoms depends on the rapidity of administration and brain cell exposure, rather than the definite concentration in the blood. When used as an infiltrative anesthetic, horses can safely tolerate up to 10 mg/kg; but if administered as a rapid intravenous bolus, seizures may result at doses greater than 2 mg/kg (Skarda *et al* 2009).

Mepivacaine also has a relatively rapid onset (10 minutes) and an intermediate duration of action (2 - 3 hours). The decreased vasodilator activity of mepivacaine is partially responsible for this slightly extended duration of action, although increased protein binding also contributes. The intermediate potency of mepivacaine and its level of toxicity also resemble those of lidocaine (Skarda *et al* 2009; Park, Sutradhar, Hong, *et al* 2011). Mepivacaine is metabolized by the liver at a slower rate than lidocaine, but more rapidly than bupivacaine (Lamont 2006). In equine practice, 2% mepivacaine

hydrochloride solution is used for direct infiltration, perineural, intrasynovial and caudal epidural anesthesia (Skarda *et al* 2009).

Bupivacaine has an intermediate onset (20 minutes) and a long duration of action (3 - 6 hours). Bupivacaine hydrochloride (0.5%) solution is used in equine practice for both perineural and intrasynovial anesthesia, but the above characteristics sometimes make it better suited for therapeutic rather than diagnostic analgesia (Skarda & Tranquilli 2007; Bassage & Ross 2011).

2.2.2 – Application of local anesthetics for diagnostic analgesia – Local anesthetics can be used to perform diagnostic analgesia via direct infiltration, perineural anesthesia or intrasynovial anesthesia. Direct infiltration involves injecting local anesthetic into the tissues to be anesthetized, which may be subcutaneous, within muscle or between fascial planes. The anesthetic diffuses into the surrounding tissues and anesthetizes the nerve fibers and endings. Perineural anesthesia is the injection of local anesthetic solution into the immediate vicinity of a nerve. The nerve is anesthetized as the solution diffuses into it (Skarda & Tranquilli 2007; Skarda *et al* 2009). Intrasynovial anesthesia involves injecting local anesthetic into a synovial space (joint, tendon sheath), followed by diffusion of the anesthetic to nerve endings within the joint/tendon-sheath capsule and eventually into surrounding soft tissues. Despite the method of performing diagnostic analgesia, diffusion of local anesthetic continues over time; which must be taken into account when interpreting the results of diagnostic analgesia (Bassage & Ross 2011).

2.2.3 – Adverse effects of lidocaine and mepivacaine in relation to diagnostic analgesia – Systemic toxicity, affecting the central nervous system or cardiovascular

system, is extremely rare from diagnostic analgesic techniques in horses. Local reactions due to tissue damage are also extremely rare, but soft tissue swelling due to hematoma formation occurs occasionally. Although potential complications to diagnostic analgesia include cellulitis and septic arthritis or tenosynovitis, these sequelae are uncommon (Bassage & Ross 2011).

However, the irritating effects of local anesthetics on the synovial environment have been demonstrated. Injection of equine carpal joints with lidocaine or mepivacaine caused an increase in synovial-fluid neutrophil and mononuclear cell counts, increased total protein, and decreased hyaluronic acid; although clear differences between the responses of lidocaine and mepivacaine were not identified (White, Hodgson, Hancock, *et al* 1989). Alternatively, in another equine study, intercarpal joint injection with lidocaine caused significantly greater effects on leukocyte count and morphology, total protein, hyaluronic acid and mucin, when compared to mepivacaine. Some of these parameters remained altered beyond 7 days post-injection (Specht, Nixon, Meyer 1988).

In a more recent study, mepivacaine had no significant effect on synovial-fluid nucleated cell counts when injected into equine metacarpophalangeal joints with acute lipopolysaccharide-induced synovitis (Kay, Bolt, Ishihara, *et al* 2008). However, *in vitro*, mepivacaine was reported to cause chondrocyte necrosis (Park *et al* 2011). Another group demonstrated that intra-articular injection of either bupivacaine (equine intercarpal joints) or lidocaine (equine tarsocrural joints) induced a significant increase in synovial-fluid markers of cartilage matrix synthesis and a significant decrease in collagen degradation markers. Thus, there is now some evidence of an overall anabolic, rather than catabolic, effect of intra-articular local anesthetics on cartilage metabolism (Piat,

Richard, Beauchamp, *et al* 2011). Given these potential effects of local anesthetics on synovial structures and other soft tissues, they also have the potential to adversely affect diagnostic imaging, including MRI, as discussed later in this review.

2.2.4 – Methods, selection and limitations of perineural and intrasynovial local analgesia of the equine foot – The purpose of these techniques is to localize the lameness to a specific region, which can then be assessed with diagnostic imaging. The choice of imaging modality depends on the region identified and the type of suspected lesion.

Most commonly, a palmar/plantar digital nerve (PDN) block is the first perineural anesthesia to be performed. In the past, a properly performed PDN block was thought to anesthetize only the palmar/plantar third to half of the foot. However, it is now recognized that the area of desensitization includes the majority of the distal interphalangeal joint and the entire sole (Schumacher, Steiger, Schumacher, *et al* 2000; Schumacher J, Schumacher J, Schramme MC, *et al* 2004). It is also now known that performing the PDN block greater than 1 cm proximal to the proximal margin of the collateral cartilages may also anesthetize the proximal interphalangeal joint (Schumacher, Livesey, DeGraves, *et al* 2004). Improper performance or improper interpretation of the PDN block may explain why palmar digital analgesia has been associated with improvement of the lameness in horses with navicular disease and navicular bone fractures; solar margin, wing and extensor process fractures of the third phalanx; laminitis; second phalanx trauma; and mid-sagittal fractures of the first phalanx (Ross 1998). To properly perform the PDN block, a 25-gauge 1.6-cm (5/8-inch) needle is subcutaneously placed directly over the medial and lateral PDN, just proximal to the cartilages of the hoof, and approximately 2 mL of local anesthetic is injected around each

nerve (Bassage & Ross 2011).

Intrasynovial anesthesia of the distal interphalangeal joint (DIPJ) may also alleviate lameness originating from the podotrochlear bursa (Pleasant, Moll, Ley, *et al* 1997). Diffusion of mepivacaine between the DIPJ and the podotrochlear bursa, and vice versa, has also been demonstrated, proving that intrasynovial analgesic techniques are not necessarily specific to the synovial structure injected (Gough, Mayhew, Munroe 2002). Multiple approaches to the DIPJ joint exist, including the dorsal perpendicular (to the weight-bearing surface), dorsal inclined (to the weight-bearing surface, but perpendicular to the pastern skin surface) and dorsolateral techniques (Van Kruiningen 1963). Although the needle insertion sites for all of these methods are immediately proximal to the coronary band, the dorsal inclined technique has been shown to be the most successful. Inserting the needle perpendicular to the skin surface ensures that it is located within the DIPJ dorsal pouch, but not too close to the coronary band (Gandini 2007). To properly perform the dorsal inclined technique, a 20-gauge 2.5-cm (1-inch) needle is inserted perpendicular to the skin surface, on the sagittal plane, 1 cm proximal to the coronary band. Intrasynovial anesthesia of the DIPJ may improve the lameness of horses with disease involving the DIPJ, such as osteochondral fragments, synovitis, osteoarthritis and articular fractures of the third phalanx. As described above, diseases outside the DIPJ, such as navicular syndrome and collateral ligament desmitis, may also respond with varying results (Pleasant *et al* 1997; Turner & Sage 2002; Dyson, Murray, Schramme, *et al* 2004).

Intrasynovial anesthesia of the podotrochlear bursa is used to localize the source of lameness when navicular syndrome is suspected. It is still considered to be the most

specific diagnostic-analgesia technique for this disease, despite the demonstrated diffusion of local anesthetic into the DIPJ (Gough *et al* 2002). Multiple approaches to the podotrochlear bursa exist, including the distal palmar approach parallel with the coronary band (Scrutchfield 1977), the distal palmar approach parallel with the sole (Van Kruiningen 1963), the proximal palmar approach (Bishop 1960), the lateral approach (Van Kruiningen 1963), and the distal palmar approach to the “navicular position” (Verschooten, Desmet, Peremans, *et al* 1990, 1991). The “navicular position” is defined as a point 1 cm distal to the coronary band, on the lateral hoof wall, halfway between the most dorsal and palmar aspects of the coronary band (Verschooten *et al* 1990, 1991). Radiographic assessment should be used to confirm correct placement of the needle during any of the podotrochlear-bursa injection techniques. However, the distal palmar approach to the “navicular position” was between 2.3 and 5.7 times more likely to result in successful injection of the podotrochlear bursa than other techniques (Schramme, Boswell, Hamhougias, *et al* 2000). This technique also appeared to be successful regardless of variations in foot conformation. *In vivo*, the distal palmar approach to the “navicular position” again resulted in a significantly higher number of successful injections (14/17), when compared with the distal palmar approach parallel with the sole (6/16) (Piccot-Crezollet, Cauvin, Lepage 2005). To properly perform the distal palmar approach to the “navicular position”, an 18-gauge 8.9-cm (3.5-inch) spinal needle is inserted on midline between the heel bulbs, just proximal to the coronary band. Then, the needle is advanced in a sagittal plane toward the “navicular position” until resistance is met. The foot is generally rested in a 60-degree inclined wooden block for the procedure (Piccot-Crezollet *et al* 2005). Alternatively, an assistant may hold the limb.

Intrasynovial anesthesia of the digital flexor tendon sheath (DFTS) appears to produce analgesia specifically to the structures contained within it, without clinically significant diffusion of local anesthetic into adjacent tissue (Harper, Schumacher, DeGraves, *et al* 2007). The DFTS can be approached in several different ways, but when synovial fluid is limited, the palmar/plantar axial sesamoidean approach proves reliable (Hassel, Stover, Yarbrough, *et al* 2000). In this technique, the leg is lifted and the distal limb flexed (225-degree angle between the third metacarpal bone and first phalanx). The mid-body of the proximal sesamoid bone is palpated, and a 20-gauge 2.5-cm (1-inch) needle is inserted in a transverse plane, 3 mm axial to the sesamoid bone and through the palmar annular ligament. The needle is directed at a 45-degree angle to the sagittal plane, to a depth of approximately 2 cm, toward the intersesamoidean region (Hassel *et al* 2000).

2.3 – Advantages and Disadvantages of Different Imaging Modalities

The choice of imaging modality in equine lameness examination depends on the region and suspected nature of the lesion. For equine practitioners, radiography remains the most universal imaging modality. Radiographs are affordable, widely available, and reasonably easy to acquire when compared to more advanced imaging modalities. They usually allow excellent evaluation of osseous components. Radiographs are used not only to evaluate lame horses, but also to assess healthy horses, with thousands of radiographs being performed worldwide for yearling sales and prepurchase examinations (Butler, Colles, Dyson, *et al* 2000; Ballegeer & Nelson 2012).

Radiography provides an anatomical, not a physiological, assessment of tissue; and standard radiography does not have multiplanar or tomographic capabilities. The limitations of radiography include the minimal information available regarding soft tissue structures in the limbs (Butler *et al* 2000; Widmer, Buckwalter, Fessler, *et al* 2000; Ballegeer & Nelson 2012), with only relatively advanced changes visible radiographically. Early changes of osteoarthritis are also quite subtle. Within the equine foot, many lesions associated with navicular syndrome are ill defined or not observed radiographically (Biggi & Dyson 2010); and it is not unusual for horses with a clinical diagnosis of navicular syndrome to be radiographically normal (Widmer *et al* 2000).

Ultrasound is generally an excellent imaging modality for soft tissue evaluation (Bischofberger, Konar, Ohlerth, *et al* 2006); and has been shown to be more sensitive than radiography for detecting osteochondrosis lesions in the medial trochlear ridge of the equine distal femur (Bourzac, Alexander, Rossier, *et al* 2009). High-resolution ultrasonography is particularly useful for evaluating tendons and ligaments, but variations in normal ligament and tendon dimensions, altered suspensory-ligament echogenicity due to variable amounts of muscular tissue, and artifacts from vessels present limitations (Dyson, 2003). Unfortunately, ultrasonography of the foot is difficult and limited to midline sagittal evaluation, with frequent off-incidence artifacts (due to a non-perpendicular incident beam angle) that may confuse interpretation (Busoni & Denoix 2001).

Nuclear scintigraphy is a functional or physiologic image, which is highly sensitive compared to the anatomical image of radiography (Baum & Devous 1980; Ross & Stacy 2011). Scintigraphy is extremely useful in identifying active bone pathologies

and abnormalities in bone metabolism (Ueltschi 1977), which makes it valuable in the early detection of incomplete fractures and acute bone injuries. However, this sensitivity may decrease as the lameness becomes more chronic or when the horse is given significant rest before the scintigraphic exam. Specificity is lower than other imaging modalities because various conditions may similarly alter blood flow or metabolism in a region (Ross & Stacy 2011). Although the use of scintigraphy in diagnosing navicular syndrome has been well-documented (Ueltschi 1977; Trout, Hornof, O'Brien 1991), false positives in the navicular region have been reported in horses with lameness unrelated to the foot (Dyson 2002). Therefore, it is important to combine scintigraphy with diagnostic analgesia (e.g. intrasynovial anesthesia of the DIPJ or podotrochlear bursa) to further isolate the source of pain in horses with lameness related to the foot (Dyson 2002). Scintigraphy may also help determine the significance of radiographic or MRI lesions in the foot if more than one lesion is present (Dyson & Murray 2007b).

Computed tomography is most useful for complex bone abnormalities, including those in the equine distal limb (Peterson & Bowman 1988). Within the foot, high-detail tomographic images of the navicular bone can clearly define osseous lesions not seen on radiographs (Widmer *et al* 2000). The limitations of computed tomography lie in obtaining good resolution of soft tissue structures (Peterson & Bowman 1988), which is where MRI excels.

2.4 – Magnetic Resonance Imaging

2.4.1 – How MRI differs from other imaging modalities – The introduction of MRI for horses has revolutionized the diagnosis of foot lameness (Gavin 2011).

Clinically important lesions in the equine foot that have been diagnosed with MRI include: collateral ligament desmitis; cartilage and subchondral bone abnormalities of the DIPJ; periarticular osteophyte formation; distension of the DIPJ, navicular bursa or DFTS; surface and core defects of the deep digital flexor tendon; abnormal signal (“edema”) or cyst formation in the navicular bone; fragments in the distal sesamoidean impar ligament; laminitis; phalanx fractures; straight sesamoidean ligament desmitis; or a combination of lesions (Widmer *et al* 2000; Dyson *et al* 2003; Dyson *et al* 2004; Dyson, Murray, Schramme 2005; Dyson & Murray 2007a; Sampson, Schneider, Gavin, *et al* 2009).

Its multiplanar views allow excellent visualization of both osseous and soft tissue structures (Chaby *et al* 2012), which permits identification of changes that are not visible with computed tomography or radiography (Widmer *et al* 2000). Occult fractures, articular cartilage damage, and subchondral bone remodeling are examples of lesions better detected with MRI than with other imaging modalities (Whitton, Murray, Dyson 2003; Murray & Mair 2005). Compared to ultrasonography, MRI provides more detailed information regarding tendon and ligament fibers. For example, MR images of the origin of the suspensory ligament were more precise, less susceptible to artifacts, and correlated better with histologic morphology than ultrasound images (Bischofberger *et al* 2006). Specifically related to the equine foot, MRI is unmatched for recognizing changes in tendons, ligaments, cartilage, bursae and trabecular bone (Widmer *et al* 2000). Even measurements from acute laminitic horses that were obtained with MRI were more sensitive and specific predictors of histologic changes than those obtained with radiography (Arble, Mattoon, Drost, *et al* 2009).

The limitations of MRI are often associated with the field strength of the magnet. High-field (≥ 1.0 tesla [T]) MRI units use closed circular magnets, and have a high signal-to-noise image ratio, high image quality and resolution, and fast imaging times. However, these systems always require the horse to be under general anesthesia. Low-field MRI systems (≤ 0.3 T) have an open U-shaped magnet, in which examinations of equine distal limbs may be performed with the horse standing under sedation. However, the lower field strength results in a lower signal-to-noise ratio, lower image quality and resolution, and longer imaging times (Murray & Mair 2005; Werpy 2007). In general, acquisition times for MRI examinations are significantly longer than those for computed tomography and radiography; and although there is no radiation exposure, interaction with the magnetic field is potentially hazardous. The purchase and maintenance costs, particularly of a high-field system, are considerable; and specific room construction with temperature regulation and non-ferrous equipment is required for both high and low-field systems (Werpy 2007).

2.4.2 – Physics of MRI – The production of MR images is complex and involves both quantum and classical mechanics (McRobbie, Moore, Graves, *et al* 2006). The system comprises of a large magnet, transmitter and receiver coil, source of active nuclei (within the structure to be imaged), and computational facilities. Most of the MRI signal is derived from water and fat within tissues, as these are abundant sources of hydrogen, which has one proton as its nucleus (Whitton *et al* 2003). The spinning motion of atomic nuclei (i.e. resonance of atomic nuclei) forms the basis of MRI. Hydrogen-atom protons have their own “magnetic moment”, which makes each proton react with a magnetic field as if it were a tiny bar magnet. By placing a static magnetic field around the area to be

imaged, the hydrogen-atom protons either align with the direction of the field (longitudinal magnetization) or oppose it (Mitchell & Cohen 2004). In addition to their magnetic moment, the spinning protons also have “angular momentum”, which is the tendency for a spinning object to continue to spin about the same axis. When the main static magnetic field is applied, the axis of rotation changes to resemble a cone, similar to the wobbling of a spinning top (precession). The frequency of this precession is proportional to the strength of the magnetic field. The character of the radiofrequency pulse will also alter the characteristics of precession. (McRobbie *et al* 2006).

When energy (a radiofrequency pulse) is added to the system via the transmitter coil, it is absorbed by the hydrogen protons and causes them to change their energy state, their alignment (transverse magnetization) and their precession characteristics. After the energy input is discontinued, the protons will return (“relax”) to their original alignment (T1 relaxation) and precession (T2 relaxation) with the static magnetic field. Following the radiofrequency pulse, signals emitted during the relaxation phase (designated as T1, T2 or T2*, and to be described later) are collected to create the MR image (Mitchell & Cohen 2004). During this relaxation, the change in energy and net magnetization generates a signal, which can be measured in the receiver coil and subsequently used to construct an image. There are 2 common methods for generating MR images: spin echo and gradient echo sequences.

2.4.3 – Spin echo sequence – Spin echo was the main pulse sequence during the establishment of human and equine clinical MRI (Park, Nelson, Hoopes 1987; Mitchell & Cohen 2004). It is a robust sequence that is tolerant to magnetic field inhomogeneities and resistant to artifacts from ferrous materials, aberrant radiofrequency pulses, etc. Its

disadvantage is the prolonged acquisition time, which makes it less practical for equine imaging (Busoni *et al* 2004).

Fast spin echo has an intermediate acquisition time, but maintains a low susceptibility to the magnetic artifacts described above. Although fast spin-echo sequences have been used in clinical scanning of the equine foot (Busoni *et al* 2004; Bell *et al* 2009), for a similar acquisition time, gradient-echo sequences have shown better detail (Murray *et al* 2007).

2.4.4 – Gradient echo sequence – Gradient-echo sequences show a wide range of variations compared to the spin-echo and inversion-recovery sequences. A gradient echo is created by the application of a pair of magnetic gradient pulses in opposite directions, which alter the characteristics of the main static magnetic field (Mitchell & Cohen 2004). Gradient-echo sequences are commonly used in clinical scanning of the equine foot (Dyson *et al* 2003; Dyson & Murray 2007b; Murray *et al* 2007; Bell *et al* 2009) because their acquisition times are fast and they provide good anatomical detail (Werpy 2004). Gradient-echo sequences are capable of creating T1-weighted and T2*-weighted images (Murray *et al* 2007; Bell *et al* 2009). However, gradient-echo sequences are also more sensitive to magnetic field inhomogeneities and magnetic artifacts (Mitchell & Cohen 2004).

2.5 – Clinical Application: MRI Protocols for the Equine Digit

To highlight specific tissues, sequences with different image weighting can be created (McRobbie *et al* 2006). T1 and T2 relaxation occurs in all tissues with all imaging sequences. The differing T1 and T2 relaxation rates in various tissues are what

provide tissue contrast. Both spin echo and gradient echo sequences can be used to generate images of almost any weighting.

By altering the type and timing of the radiofrequency pulses or magnetic field gradients, specific T1 or T2 characteristics are generated (Mitchell & Cohen 2004). Two fundamental sequence parameters determine the image weighting. These are repetition time (TR) and time to echo (TE), both measured in milliseconds. Repetition time indicates the time interval between the radiofrequency pulses, while time to echo indicates the time interval between the introduction of the radiofrequency pulse and the time of signal collection. Table A demonstrates how these factors influence image weighting.

Table A – Influence of the MRI sequence parameters, repetition time and time to echo, on image weighting.

| Time to echo (TE) | Repetition time (TR) | |
|-------------------|----------------------|-------------------------|
| | Short | Long |
| Short | T1-weighted | Proton density-weighted |
| Long | ----- | T2-weighted |

2.5.1 – T1-weighted images – During T1 relaxation (spin-lattice relaxation/recovery), protons leave the transverse alignment and realign themselves in the direction of the main magnetic field; however, they do so at different rates for different tissues. T1-weighted images are characterized by bright (hyperintense) fat and dark (hypointense) fluid. Water-based tissues, such as muscle, are mid-gray. T1-weighted

sequences produce high-resolution anatomic images that are useful for bone and soft tissue evaluation (Mitchell & Cohen 2004; Murray & Mair 2005), such as the collateral ligaments of the distal interphalangeal joint (Dyson *et al* 2004). T1-weighted sequences are used in both low and high-field MRI of the equine foot (Sherlock, Mair, Blunden 2008; Bell, Howard, Taylor, *et al* 2009; Biggi & Dyson 2011). Contrast-enhanced (gadolinium) MRI studies, including arthrography or bursal injections, also use T1-weighted sequences (Werpy 2007).

2.5.2 – T2 & T2*-weighted images – Immediately after the radiofrequency-pulse input of energy, all individual protons realign to the transverse plane and precess simultaneously (in phase), creating a strong signal. Over time, differences in the precession rates cause the protons to move out of phase with one another; which causes the transverse magnetization, and hence the signal, to decay. This decay rate, known as T2 or spin-spin relaxation, is tissue-specific. Fluids generate the strongest signal intensity with a T2-weighted sequence, followed by water-based tissues being mid-gray, and fat-based tissue generating the lowest signal. Since most lesions are demonstrated by a change in the tissue water content, the fluid creates a bright signal against the normal tissue. This contrast makes T2-weighted scans useful for detecting pathology, but displays less anatomical detail than T1-weighted images (Murray, Dyson, Branch, *et al* 2007).

T2*-weighted images are acquired with the gradient-echo sequence, which is more susceptible to inhomogeneities in the magnetic field, such as imperfect uniformity of the main magnet or iron within venous blood or hemorrhage. In the gradient-echo sequence, these inhomogeneities can cause the precessing protons to move out of phase

more quickly, with a time constant $T2^*$. However, the advantages of $T2^*$ gradient-echo images are a short acquisition time and increased contrast for certain types of tissue, such as venous blood (Mitchell & Cohen 2004). Upon validating MRI use with equine limbs in 2007, Murray *et al* demonstrated $T2^*$ gradient-echo images to have the best detail for pathologic change in the laminae and osseous structures of the foot. This sequence is also commonly used for clinical imaging of the equine foot (Dyson *et al* 2003; Murray, Blunden, Schramme, *et al* 2006; Sherlock *et al* 2008).

2.5.3 – Proton density-weighted images – The contrast in a proton density-weighted image is intermediate between a T1-weighted and T2-weighted image. Proton density-weighted images are usually acquired with a spin-echo sequence, but a gradient-echo sequence is sometimes used. The number of hydrogen atoms in a particular volume of tissue typically determines proton density, and subsequently MRI signal intensity. Therefore, tissues with high water content, such as cerebrospinal fluid, blood and fat, have higher signal intensities than tendon and bone (Mitchell & Cohen 2004). Intermediate-weighted images have been clinically useful for the musculoskeletal system and equine foot (Busoni, Snaps, Trenteseaux, *et al* 2004; Werpy 2004; Tucker & Sampson 2007); where a low signal-intensity structure, such as a tendon, is contrasted against background higher signal-intensity soft tissue (Mitchell & Cohen 2004). However, laminar, osseous and ligamentous pathology in the equine foot was reported to be less clear with proton density-weighted images than with gradient echo sequences (Murray *et al* 2007).

2.5.4 – Short tau (TI) inversion recovery (STIR) sequence – By preceding the excitation radiofrequency pulse with an inverting 180-degree pulse, the initial

longitudinal magnetization is converted from positive to negative. After the excitation radiofrequency pulse ends, the realigned protons (transverse magnetization) relax to their initial positive equilibrium (longitudinal magnetization) similar to T1 relaxation. The inversion time (tau or TI) is the time taken between the inversion pulse and the excitation pulse. To create a fat-suppressed image, the excitation pulse occurs when the longitudinal magnetization of fat is zero (short tau or TI). Therefore, no signal is subsequently produced from fat tissue and it is said to be “nulled” (Mitchell & Cohen 2004). STIR sequences are particularly useful for imaging the navicular bone (Werpy 2004; Murray *et al* 2006; Tucker & Sampson 2007) and pathological change associated with the distal phalanx (Murray *et al* 2007). Use of STIR sequences for the equine foot has resulted in consistent fat suppression, with good contrast and clear definition of pathology, but it is time-consuming (Dyson *et al* 2003).

The best sequence protocols will not be the same for each system, and knowledge of tried and tested sequences for each specific unit is recommended. A minimum of 3 scanning planes, sagittal, dorsal and transverse, should be obtained; with the option of additional planes perpendicular to curved surfaces. A variety of sequences to obtain anatomical and pathological scans should be generated.

2.6 – Effects of Perineural and Intrasynovial Anesthesia on Diagnostic Imaging Modalities

Despite the increased use of MRI in equine lameness diagnosis, no published literature exists regarding the validation of MRI after perineural or intrasynovial anesthesia. It is unknown whether the inflammation caused by the injection of local

anesthetics during lameness examinations may alter the perineural tissue or synovial environment enough to cause changes detectable on subsequent MRI.

Lidocaine and mepivacaine are currently the 2 most commonly used local anesthetics for equine perineural and intrasynovial anesthesia (Bassage & Ross 2011). Both anesthetics cause varying degrees of synovial inflammation when injected into equine joints, with some synovial-fluid parameters remaining elevated beyond 7 days post-injection (Specht *et al* 1988; White *et al* 1989). Injection of local anesthetics into the soft tissues causes minimal side effects, although edema may develop if the injected site is in a distal or ventral area (Day & Skarda 1991).

While injection of local anesthetics causes minimal morbidity, their effect on ultrasonographic, radiographic and scintigraphic image interpretation can be significant (Zekas & Forrest 2003; Kirberger, Gottschalk, Guthrie 1996; Trout, Hornof, Fisher 1991a; Trout, Hornof, Liskey, *et al* 1991b). Air that is inadvertently injected during diagnostic anesthesia can cause ultrasound reverberation artifacts (Rantanen, Jorgensen, Genovese 2011). Zekas and Forrest (2003) investigated the effects of high and low palmar and palmar-metacarpal nerve blocks on ultrasound examination, identifying mild hypoechoic swelling of the surrounding soft tissue and gas in the regions of the injections. These findings were noted in all horses at one hour post-injection, but had essentially resolved by 24 hours. These artifacts partially obscured evaluation of the suspensory ligament and the accessory ligament of the deep digital flexor tendon in some horses, leading to recommendation of a repeat ultrasound at least 24 hours after injection.

Air introduced during intra-articular anesthesia of the metacarpophalangeal joint produced gas-capped radiolucencies on subsequent radiographs, evident as horizontal

fluid-gas interfaces in the proximal aspect of the joint. Air introduced during palmar-digital perineural anesthesia resulted in linear radiolucencies that dissected between tendinous structures and moved proximally with time. The majority of the gas was resorbed within 48 hours, and all gas was gone by 96 hours post-injection (Kirberger *et al* 1996).

Both perineural and intra-articular anesthesia cause focal accumulation of radiopharmaceutical activity during soft-tissue phase scintigraphy. During the soft-tissue phase, low and high palmar nerve blocks resulted in increased radiopharmaceutical uptake that was greatest at one day post-anesthesia, but persisted up to 17 days following perineural anesthesia (Trout *et al* 1991b). After intra-articular anesthesia of the antebrachiocarpal joint, focal accumulation of activity was evident on soft-tissue phase scintigrams. At 2 to 4 days post-anesthesia, this accumulation was most evident, however, this activity persisted for 14 days (Trout *et al* 1991a). No abnormal accumulation of activity was evident on bone phase scintigrams after perineural or intra-articular anesthesia.

The effect of palmar-digital and abaxial-sesamoid perineural anesthesia on infrared thermography has also been investigated. No significant changes were evident within 45 minutes of injection. Although only half of the horses were assessed at 24 hours post-injection, no changes were seen in any horses at that time (Holmes, Gaughan, Gorondy, *et al* 2003).

Important recommendations regarding appropriate times between diagnostic anesthesia and subsequent diagnostic imaging have resulted from these studies. Research investigating the effect of diagnostic anesthesia on MRI has not been performed to the

knowledge of the author. Therefore, no guidelines regarding the time between these procedures exist. Dyson *et al* (2003) documented at least a 2-week period between intrasynovial injections and MRI in their study, but an explanation regarding this time lapse was not provided, and perhaps this time interval is not required. Alternatively, an additional study by Dyson & Murray (2007a) of 264 horses with foot pain demonstrated that almost half had effusion of the distal interphalangeal joint and podotrochlear bursa on MRI. Of these horses, podotrochlear bursa effusion was likely to occur with other abnormalities of the navicular bone, but effusion of the distal interphalangeal joint was unexplained. All horses in this study received diagnostic anesthesia as part of their lameness evaluation.

**3.0 – THE EFFECTS OF PERINEURAL AND INTRASYNOVIAL
ANAESTHESIA OF THE EQUINE FOOT ON SUBSEQUENT MAGNETIC
RESONANCE IMAGES**

(In press, Equine Vet J)

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3.1 – Summary

Reasons for performing study: Artefacts caused by regional anaesthesia can influence image interpretation of ultrasonography and nuclear scintigraphy. Perineural and intrasynovial anaesthesia are commonly performed prior to magnetic resonance imaging (MRI); and the effects on MR images, if any, are unknown.

Objectives: To determine if perineural and intrasynovial anaesthesia of structures in the equine foot cause iatrogenic changes detectable with MRI.

Methods: A baseline MRI examination of both front feet was performed on 15 horses 2 to 6 days prior to mepivacaine injection adjacent to the lateral and medial palmar digital nerves (PDN), and into the podotrochlear bursa (PB), digital flexor tendon sheath (DFTS), and distal interphalangeal joint (DIPJ) of one randomly assigned forelimb. MRI was repeated at 24 and 72 hours post-injection; then qualitative and quantitative assessments of MRI findings were performed.

Results: MRI findings associated with the PDN, PB and DIPJ at 24 and 72 hours after mepivacaine injection did not alter significantly from those at baseline. Compared with baseline, a significant increase in synovial fluid volume of the DFTS was detected with MRI at 24 and 72 hours post-injection.

Conclusions: Perineural anaesthesia of the PDN and intrasynovial anaesthesia of the PB or DIPJ did not interfere with the interpretation of MR images acquired at 24 or 72 hours after injection. However, intrasynovial anaesthesia of the DFTS caused an iatrogenic increase in synovial fluid, detectable on MR images for at least 72 hours.

Potential relevance: Although a definite time frame for resolution of DFTS distension was not determined, we recommend waiting greater than 3 days between intrasynovial anaesthesia of the DFTS and evaluation with MRI.

3.2 – Abbreviations

DIPJ Distal interphalangeal joint

DFTS Digital flexor tendon sheath

GRE Gradient echo

MRI Magnetic resonance imaging

PB Podotrochlear bursa (navicular bursa)

PDN Palmar digital nerves (lateral and medial)

STIR Short tau inversion recovery

SGRE Spoiled gradient echo

3.3 – Introduction

Lameness in the horse is commonly associated with pain originating from the distal aspect of the limb, particularly the foot [1]. The equine foot is an anatomically complex structure, which is encased in a solid hoof capsule, and the specific causes of foot pain are multiple and varied [2]. Magnetic resonance imaging (MRI) is particularly suited to imaging of the equine foot due to the excellent detail of both soft tissue and osseous structures that it provides [3, 4]. The usefulness of imaging the equine foot with this modality is continuing to be proven and is gaining widespread use in both academic and private practices [5].

Magnetic resonance imaging often enables the determination of a precise diagnosis [6], but lesions that are clinically insignificant may also be identified. The clinical significance of a lesion is ultimately determined by the clinical examination findings and the response of the horse to regional anaesthesia. Perineural and intrasynovial anaesthesia are integral components of equine lameness evaluation [7] and are usually performed prior to MRI.

Lidocaine and mepivacaine are the 2 most commonly used local anaesthetic solutions for equine perineural and intrasynovial anaesthesia [7]. When injected into equine joints, both anaesthetic solutions cause alterations in the composition of synovial fluid consistent with inflammation, with some parameters remaining elevated after 7 days [8, 9]. Injection of local anaesthetic solutions into the soft tissues causes minimal side effects [10], although oedema may develop if the injected site is in a distal or ventral area. While injection of local anaesthetic solutions causes minimal morbidity, their effect on ultrasonographic and scintigraphic image interpretation can be significant [11-14]. Gas artefacts can prevent accurate ultrasonographic assessment after both perineural [11, 12] and intra-articular anaesthesia [12]. Radiopharmaceutical-uptake artefacts on soft-tissue phase scintigrams can persist up to 17 days following perineural anaesthesia [13] and up to 14 days following intra-articular anaesthesia [14].

The effect of perineural and intrasynovial anaesthesia on MRI interpretation is unknown. Some changes consistent with inflammation, such as synovial effusion, are common pathologic MRI findings in the distal interphalangeal joint (DIPJ) and podotrochlear bursa (PB) [15]. Therefore, it is important to know whether diagnostic anaesthesia within the digit alters the local environment enough to cause iatrogenic

changes on MRI of this region. The objective of this study was to determine if perineural or intrasynovial injection of mepivacaine in the equine foot would cause iatrogenic changes detectable with MRI. We hypothesized that: 1) after injection of mepivacaine into the PB, DFTS and DIPJ, MRI would detect an increase in synovial fluid at 24 hours, but not at 72 hours post-injection; and 2) perineural and intrasynovial injection of mepivacaine would cause abnormalities at the needle entry sites, which would be detectable with MRI.

3.4 – Materials and Methods

Fifteen mature healthy horses of several breeds and mixed gender were used in this study. Horses were excluded if they were lame on physical examination, or if they had received non-steroidal anti-inflammatory drugs or had perineural or intrasynovial anaesthesia performed within the previous 14 days. All procedures were in accordance with an Animal Utilization Protocol, approved by the Animal Care Committee, University of Guelph. Horses were confined to box stalls throughout the study.

For each horse, a baseline MRI study of both front digits was performed 2 to 6 days prior to mepivacaine injection adjacent to the lateral and medial palmar digital nerves (PDN), and into the PB, digital flexor tendon sheath (DFTS) and DIPJ of one randomly assigned forelimb. The contralateral limb, which did not receive any injections, served as an additional control.

Perineural and intrasynovial injections

All mepivacaine injections (PDN, PB, DFTS and DIPJ) were performed by one author (BB), using the techniques described below. Each horse was sedated with detomidine hydrochloride (Dormosedan)^a (0.01-0.02 mg/kg) and butorphanol tartrate (Torbugesic)^b (0.01-0.02 mg/kg).

Perineural injection of the PDN – A 25-gauge 1.6-cm needle was subcutaneously placed directly over the medial and lateral PDN, just proximal to the cartilages of the hoof, and 2 mL of 2% mepivacaine hydrochloride (Carbocaine)^c was injected around each nerve [7].

Intrasynovial injection of the PB – The distal palmar approach to the “navicular position” technique was used [16], with the modification that an assistant held the foot. After a lateromedial radiograph was obtained to determine correct positioning of the 18-gauge 8.9-cm spinal needle, 1.5 mL of iohexol contrast solution (Omnipaque)^d and 1.5 mL of 2% mepivacaine were injected simultaneously into the PB. An additional radiograph was immediately taken to confirm correct injection of the bursa.

Intrasynovial injection of the DFTS – The palmar axial sesamoidean approach was used [17]. The leg was lifted and the distal limb flexed, the mid-body of either the medial or lateral proximal sesamoid bone was palpated, and a 20-gauge 2.5-cm needle was inserted in a transverse plane, axial to the sesamoid bone and through the palmar annular ligament. The needle was directed at a 45-degree angle to the sagittal plane, to a depth of approximately 2 cm, toward the intersesamoidean region. Visual identification of synovial fluid was achieved prior to injection of 15 mL of 2% mepivacaine into the DFTS.

Intrasynovial injection of the DIPJ – The dorsal inclined technique was used [18]. A 20-gauge 2.5-cm needle was inserted perpendicular to the skin surface, in the sagittal plane, 1 cm proximal to the coronary band. Evidence of synovial fluid, or positive pressure refilling of the syringe, was used to confirm needle placement; then 6 mL of 2% mepivacaine was injected into the DIPJ. After completing all of the injections, both front limbs were bandaged from the proximal aspect of the metacarpus to the coronary band with stable wraps, which were removed immediately prior to induction of general anaesthesia for the MRI at 24 hours post-injection.

Magnetic resonance imaging protocol and assessment

Magnetic resonance images were acquired from the foot to the distal aspect of the metacarpus of both forelimbs (1.5-Tesla GE Signa Excite magnet, GE 8 Channel cardiac array coil)^e, with the horse under general anaesthesia in randomly assigned recumbency. A sagittal 2D short tau inversion recovery (STIR) sequence, a dorsal 3D T1-weighted fast spoiled gradient echo (SGRE) sequence, and sagittal and transverse T2*-weighted fast gradient echo (GRE) sequences were acquired of each limb. Slice thicknesses were 3 mm for T1-weighted and T2*-weighted sequences, and 4 mm for STIR sequences. The horse was recovered from general anaesthesia after completing the MRI. Identical MRI studies were repeated in each horse at 24 and 72 hours after the PDN, PB, DFTS and DIPJ injections.

Magnetic resonance images were assessed collectively at the completion of all MRI examinations. The MRI studies were randomised. Two authors (BB & SN)

independently assessed qualitative measures, while one author (BB) assessed quantitative measurements. Both authors were blinded to injection and timing.

Qualitative assessment of images was performed in a randomized fashion, using all sequences obtained. Prior to assessment, grading scales were produced for synovial fluid volume in the PB, DFTS and DIPJ, and for increased signal intensity in the distal sesamoid bone (navicular bone), evidence of needle tracts to the PB, and abnormalities at the PDN site (Table 1).

Quantitative assessment of synovial fluid volume in the PB, DFTS and DIPJ was obtained from the sagittal STIR images, using the FSL analysis tool^f. A threshold level, which was determined to be the minimum signal intensity of synovial fluid, was applied to the sequence. Then, a region of interest that contained the synovial structure being evaluated (PB, DFTS or DIPJ) was manually drawn on each slice. The total volume of fluid in each synovial structure (i.e. the volume above the threshold intensity level and within the region of interest for that structure) was calculated (mL) from all slices in the sequence. Intra-observer repeatability was determined by assessing 15 randomly selected limbs on 2 occasions for synovial fluid volume in the PB, DFTS and DIPJ.

Statistical analyses

To analyse the qualitative data grades for synovial fluid volume, navicular bone signal intensity, evidence of needle tracts to the PB, and abnormalities at the PDN site, a Wilcoxin Signed Rank test was paired by horse. Data from the control and injected limbs at the 24-hour and 72-hour post-injection MRI were analysed against the baseline MRI

study. Significance levels were set at $P \leq 0.05$. A weighted kappa value was calculated to determine agreement between the 2 investigators.

The quantitative data for synovial fluid volume in the PB, DFTS and DIPJ was analysed using an ANOVA (general linear mixed model fitted using ProcMIXED[®]) with fixed effects for injection and time. Both control and injected legs were included in the model. The repeated-measures analyses were made from the baseline MRI study to the subsequent post-injection MRI studies; thus, accounting for individual variation between legs. To assess the ANOVA assumptions, comprehensive residual analysis was employed, including the Shapiro-Wilk, Kolmogorov-Smirnov, Cramer-von Mises, and Anderson-Darling tests. Following significant f tests, multiple comparisons were conducted using protected least significant differences. An adjustment to the P-values, using a Dunnett's test, was performed for the quantitative DFTS data.

3.5 – Results

All 15 horses were healthy and free from lameness throughout the study period. The control limb STIR sequence at 24 hours post-injection was unable to be retrieved for one horse due to a technical error. All of the perineural and intrasynovial injections were performed successfully. During the PB injections, repositioning of the spinal needle (1-3 times) was required in 7 of the horses to obtain correct injection of the bursa.

Agreement analyses – The statistical outcome (significant or not significant) for qualitative assessment of synovial fluid volume in the PB, DFTS and DIPJ, navicular bone signal intensity, evidence of needle tracts to the PB, and abnormalities at the PDN site was consistent for both investigators. The weighted kappa agreement varied from

fair for the DIPJ fluid volume (0.31), to moderate for both the PB fluid volume (0.43) and navicular bone signal intensity (0.43), to substantial for the DFTS fluid volume (0.63), to “almost perfect” for the evidence of needle tracts to the PB (1.0) and PDN site abnormalities (1.0) [19]. For quantitative data regarding synovial fluid volume, the concordance (repeatability) was 0.91 (1.0 represents identical measurements each time).

Synovial fluid volume: PB and DIPJ – On qualitative and quantitative MRI assessment, synovial fluid volume in the PB and DIPJ did not significantly change from baseline in either the injected or control limbs at 24 or 72 hours post-injection (Figure 1A, Table 2). To make graphic comparisons of quantitative data more meaningful, values were normalized by expressing them as the mean percentage change from baseline. Repositioning of the spinal needle during the PB injection was not associated with any change in PB synovial fluid volume.

Synovial fluid volume: DFTS – On qualitative assessment, a significant increase in DFTS synovial fluid volume was present in injected limbs at both the 24-hour ($P < 0.01$) and 72-hour ($P < 0.05$) post-injection MRIs, when compared with baseline. No significant changes were identified in control limbs (Figure 2). On quantitative assessment, synovial fluid volume in injected legs was also significantly increased from baseline in MR images acquired at 24 hours (26%, $P < 0.01$) and 72 hours (36%, $P < 0.01$) post-injection. However, the increase between the 24- and 72-hour studies was not statistically significant. Synovial fluid volume in control limbs did not change significantly from baseline (Figure 1B, Table 2).

Repositioning the spinal needle during the PB injection may have contributed to some of the increases in DFTS fluid volume that were identified. In injected limbs, the 7

horses that had the PB needle repositioned showed a significant increase (41%, $P < 0.01$) in DFTS synovial fluid on the 24-hour MR images. Horses that did not have the PB needle repositioned did not have a significant change in DFTS fluid volume at 24 hours. However, by 72 hours all injected limbs had significant increases in DFTS synovial fluid volume, regardless of PB needle repositioning.

Abnormalities at injection (needle entry) sites – Neither investigator identified any abnormalities at the PDN, DFTS or DIPJ injection sites on any of the MR images. However, on MR images of injected limbs at 24 hours post-injection, 5 of the 15 horses had a clearly defined needle tract (Grade 2) to the PB; evidenced by an obvious linear area of low signal intensity extending from the heel-bulb region toward the navicular bone (Figure 3). Of these 5 horses, only 2 had the PB needle repositioned. Six horses had a poorly defined tract (Grade 1). At 72 hours post-injection, 4 of the 15 horses had a clearly defined needle tract to the PB, while 6 horses had a poorly defined tract. No evidence of needle tracts to the PB was present on baseline MR images of legs designated for injection or in control legs at any time.

Navicular bone signal intensity – A total of 7 navicular bones (6 horses) had increased signal intensity, with both control and injected limbs represented. However, no statistically significant change in the grade of signal intensity occurred during the study.

3.6 – Discussion

Injection of mepivacaine into the DFTS caused a statistically significant iatrogenic increase in DFTS synovial fluid volume, which was detectable both quantitatively and qualitatively on MR images at 24 and 72 hours post-injection (Figures 1B and 2, Table

2). The irritating effects of local anaesthetic solutions on the synovial environment have previously been demonstrated [8, 9]. Injection of equine carpal joints with mepivacaine caused increased neutrophil and mononuclear cell counts, increased total protein concentration, and decreased hyaluronic acid concentration [9]. *In vitro*, mepivacaine has also been reported to cause chondrocyte necrosis [20]. Therefore, in the current study, the observed increase in DFTS synovial fluid volume after mepivacaine injection was not unexpected. However, there were no significant MRI changes in the PB or DIPJ at either 24 or 72 hours following mepivacaine injection. Although a smaller volume of local anaesthetic solution was injected into the PB and DIPJ than into the DFTS, the injected volume was relatively proportional to the potential volume of the respective synovial structure.

It has been suggested that the DFTS is more sensitive to centesis and injection of medication than other synovial structures; possibly due to soft tissues associated with the injection site or from inflammation or proliferation of the synovial membrane at the injection site. The frequent lack of significant synovial fluid distension of the DFTS, despite possible pathology, may also make delineation of the injection site difficult and increase the risk of potential trauma during centesis [17, 21]. The palmar axial sesamoidean approach to the DFTS, which was used in our investigation, was previously developed to overcome these problems [17]. However, when using this same approach, Dykgraaf *et al* observed a transient non-infectious inflammatory response following injection of either amikacin or lactated Ringer's solution into the DFTS [21], which had alterations exceeding those previously reported for the antebrachiocarpal joint [22]. Two

of the 8 horses in Dykgraaf's study developed changes in DFTS synovial fluid that were suggestive of sepsis.

In our investigation, the DFTS may have been further inflamed by incorrect initial positioning of the spinal needle during the PB injection. Repositioning of the needle during injection of the PB is not uncommon, which is why radiographic confirmation is recommended [7]. Anatomically, the DFTS begins at the distal aspect of the metacarpus and terminates at the middle of the middle phalanx, proximal to the PB. The distal aspect of the DFTS, the proximal recess of the PB, and the proximopalmar recess of the DIPJ are adjacent to one another, although they do not naturally communicate [23]. It is possible that when initially positioning the spinal needle for injection of the PB, proximal deviation may have caused penetration of the DFTS; which may have contributed to the increase in DFTS synovial fluid volume at 24 hours post-injection. However, the need to reposition the PB needle did not appear to contribute to the increased DFTS synovial fluid at 72 hours post-injection. At that time, all injected limbs had significant increases in DFTS synovial fluid volume, regardless of PB needle repositioning. In a clinical situation, injections of local anaesthetic solution into multiple synovial structures may be performed on the same day to determine the location of lameness [7]. The injection protocol in this study was intended to mimic this scenario as best possible. Therefore, differentiation between the effects of needle placement alone and those of mepivacaine injection was beyond the objectives of this study.

As stated above, there were no significant changes in PB or DIPJ synovial fluid volume on MR images at either 24 or 72 hours after mepivacaine injection. Therefore, on MR images of clinical patients following detailed diagnostic anaesthesia of the foot,

distension in these structures is probably truly associated with pathology, not the injection of local anaesthetic solution. This information is of particular clinical importance, since DIPJ effusion had been reported as the most common finding on MR images of the equine foot [15]. A limitation of our study was that the mepivacaine injections were performed on horses free from lameness or pre-existing abnormalities detectable with MRI. In a recent report, mepivacaine had no significant effect on nucleated cell counts when injected into the metacarpophalangeal joints of horses with acute lipopolysaccharide-induced synovitis [24]. Still, it is currently unknown how equine synovial structures with other disease states respond to the injection of local anaesthetic solution, and if that response may be exaggerated. Also, we cannot speculate that identical results would be obtained if different injection techniques or significantly larger volumes of local anaesthetic solution were used. The addition of iohexol to mepivacaine in the PB injection also represents an uncontrolled variable. Positive contrast is frequently used when injecting the podotrochlear bursa in clinical practice. During this study, confirmation that the injectate was within the bursa was of paramount importance. Hence, our decision to include iohexol was warranted, despite adding another variable to the study. The techniques selected for this study were commonly used methods, which were previously critically evaluated.

A minor limitation of our study was the possible bias introduced by manually drawing regions of interest to accumulate quantitative data. Although most clinical MRI assessments of veterinary patients are performed qualitatively [25], we thought it important to also quantify synovial fluid volume for this study. For each synovial structure, this involved manually drawing a region of interest on each slice of the STIR

sequence; then using a computer algorithm^f to calculate the volume. Despite the decision on structural boundaries being consciously made by one author, the regions were drawn as anatomically correctly and as objectively as possible. A learning curve was thought to be present; therefore the regions of interest were repeated randomly at a later date. The excellent concordance (repeatability, 0.91) demonstrated that this method produced a consistent result. Similar FSL analysis programs have been used repeatedly in human MRI clinical studies [26, 27], and the authors do not currently know of a more accurate method.

Although both investigators reached the same statistical conclusions regarding the qualitative data results, the weighted kappa agreement was only “fair” (0.31) for the DIPJ synovial fluid volume. Review of the results from each investigator showed consistent assessment, however one investigator (BB) frequently assessed one grade higher than the other (SN). This was particularly evident between grades 1 and 2, where these differences were subtle. A grading system with improved repeatability could be achieved by decreasing the number of grades from 4 to 3; and is recommended by the authors for future studies of this nature.

Using an image sequence with a 4-mm slice thickness for quantitative assessment might give question to the absolute accuracy of synovial fluid volume calculation, due to the effect of partial volume averaging. The overall signal intensity of each voxel (volumetric or 3-dimensional pixel) in an MR image is an average of the different signals within that voxel [28]. As slice thickness of the specific MRI sequence increases, so does the thickness of each voxel and the associated signal averaging. Consequently, there would be a mildly altered representation of signal intensity, and therefore fluid volume.

In our study, selecting a thinner slice thickness would have decreased the degree of partial volume averaging [28], but it would have greatly increased the manual component of calculating synovial fluid volume. Obtaining a good representative volume to enable comparisons between baseline and subsequent MR images, rather than an absolute volume, was the rationale behind this method. Hence, the volumes presented are not expected to be the absolute volume of synovial fluid within each structure.

Injection of the PB caused abnormalities along the needle tract that were frequently detectable on subsequent MR images. No abnormalities were identified at the PDN, DFTS or DIPJ injection (needle entry) sites. Needle tracts were probably evident at the PB site due to the increased size and length of the PB needle, repositioning of this needle, and the large amount of soft tissue penetrated. At this institution, needle tracts are not typically seen in clinical cases, perhaps because our clinical MRI examinations are seldom performed within the week following PB injection. The easily identifiable PB needle tracts in the current study were not associated with any lameness and did not interfere with interpretation of the MRI sequences. Anecdotal concerns have been raised that the deep digital flexor tendon could be damaged by the injection method used in this study, since the needle was required to pass through the tendon to reach the PB. However, no abnormalities within the deep digital flexor tendon were identified on subsequent MR images, and to our knowledge, such an occurrence has not been reported.

No evidence of a significant change in navicular bone signal intensity in either control or injected limbs was identified during the study. Anaesthetic recoveries in horses can be traumatic. Therefore, navicular bone signal intensity could have been anticipated to increase throughout this study. It is possible that the injection of local

anaesthetic inhibited some inflammation of the surrounding tissues. Intravenously administered local anaesthetics, such as lidocaine, have been demonstrated to decrease granulocyte adherence in a dose-dependent manner; therefore, decreasing neutrophil accumulation at sites of inflammation [29]. Lidocaine has also been demonstrated to impair prostaglandin and leukotriene production and alter microvascular permeability, resulting in decreased albumin extravasation [30]. In the lungs of humans, lidocaine is a potent inhibitor of bronchial hyper-responsiveness, as well as having the capacity to decrease the production of toxic oxygen metabolites, reducing edema [31]. As no changes in navicular bone signal intensity were identified, the potential anti-inflammatory effects of the local anaesthetic on the navicular bone (or any tissues surrounding the injected regions) cannot be ruled out. However, it is unlikely that the inflammatory effects associated with the local injection of local anaesthetics are overshadowed by the systemic effects described above. Three sequences, in a total of 3 planes, were acquired for each MRI study: a sagittal 2D STIR sequence, a dorsal 3D T1-weighted SGRE sequence, and a sagittal and transverse T2*-weighted GRE sequence. Although this protocol is heavily weighted with GRE sequences, these were deemed sufficient for the objectives of this study and are commonly used for clinical imaging of the distal limb, at this, and other institutions [32, 33]. These sequences are also fast to acquire [28, 32, 33]; an important consideration when anaesthetising the equine patient. Although the resolution of STIR images may not be as high when compared with fat suppressed GRE images, the fat suppression produced in the STIR sequence is independent of magnetic field inhomogeneities and provides high contrast, making the STIR sequence good for pathology detection [32, 33].

In conclusion, our study showed that perineural anaesthesia of the PDN and intrasynovial anaesthesia of the PB or DIPJ in sound horses did not have any significant effect on the interpretation of subsequent MR images. However, intrasynovial anaesthesia of the DFTS caused a significant iatrogenic increase in synovial fluid, which was detectable on subsequent MR images for at least 72 hours. Determining the definitive time for the DFTS effusion to return to pre-injection values was beyond the scope of this study; and further investigation, particularly in lame horses, is warranted.

3.7 – Authors Declaration of Interests

No conflicts of interest have been declared.

3.8 – Source of Funding

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3.9 – Acknowledgements

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3.10 – Manufacturers’ Details

- a. Pfizer Animal Health, New York, New York, USA.
- b. Wyeth/Pfizer Animal Health, New York, New York, USA.
- c. Hospira Healthcare Corporation, Saint-Laurent, Québec, Canada.
- d. Amersham Health, Princeton, New Jersey, USA.
- e. GE Healthcare Technologies, Waukesha, Wisconsin, USA.
- f. FMRIB Software Library, Release 4.1 (c) 2008, The University of Oxford, Oxford, United Kingdom.
- g. SAS Institute Inc. 2007. SAS OnlineDoc® 9.2. Cary, North Carolina, USA.

3.11 – References

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3.12 – Tables

Table 1 – Grading system for qualitative assessment of synovial fluid volume in the podotrochlear bursa (PB), digital flexor tendon sheath (DFTS) and distal interphalangeal joint (DIPJ), increased signal intensity in the distal sesamoid bone (navicular bone), evidence of needle tracts to the PB, and abnormalities at the palmar digital nerve (PDN) site.

| Grade | Synovial fluid volume | | | Navicular bone signal intensity | Needle tracts to PB | Abnormal PDN site |
|----------|---|--|-----------------------------------|---------------------------------|------------------------------|----------------------------|
| | PB | DFTS | DIPJ | | | |
| 0 | Minimal fluid, proximal to NB | Minimal or no fluid | Minimal fluid, palmar | Not increased | None | Normal |
| 1 | Increased fluid, proximal and/or distal to NB | Mild fluid, distal plantar pouch and adjacent to flexor tendons | Mild fluid, dorsal and palmar | Increased in palmar region only | Poorly defined needle tract | Oedema, hematoma or seroma |
| 2 | Bulbous increase in fluid, proximal and distal to NB Fluid between NB and DDFT | Moderate fluid, distal plantar pouch, palmar to P2, and adjacent to flexor tendons | Moderate fluid, dorsal and palmar | Increased in 40-60% of the bone | Clearly defined needle tract | ----- |
| 3 | ----- | Marked fluid along entire sheath Bulbous increase in pouches | Marked fluid, dorsal and palmar | Increased in > 60% of the bone | ----- | ----- |

NB = Navicular bone.
DDFT = Deep digital flexor tendon.

Table 2 – Quantitative assessment of synovial fluid volume [mean (95% confidence interval)] in the podotrochlear bursa (PB), digital flexor tendon sheath (DFTS) and distal interphalangeal joint (DIPJ) at baseline and after intrasynovial injection of mepivacaine (injected limbs).

| Structure / Limb | Synovial fluid volume (mL) | | |
|------------------|----------------------------|-------------------------|-------------------------|
| | Baseline | 24 hours post-injection | 72 hours post-injection |
| PB Control | 1.9 (1.7 - 2.2) | 2.1 (1.8 - 2.4) | 2.2 (1.9 - 2.5) |
| | 2.0 (1.7 - 2.3) | 2.2 (1.9 - 2.5) | 2.1 (1.8 - 2.4) |
| DFTS Control | 8.0 (6.6 - 9.4) | 8.7 (6.5 - 10.0) | 7.0 (5.1 - 8.9) |
| | 8.7 (6.9 - 10.5) | *10.9 (8.9 - 12.9) | *11.8 (9.5 - 14.1) |
| DIPJ Control | 6.6 (5.7 - 7.6) | 6.9 (6.0 - 8.1) | 6.8 (5.9 - 7.9) |
| | 6.9 (6.0 - 8.1) | 7.2 (6.2 - 8.4) | 6.7 (5.8 - 7.8) |

*Statistically significant difference from baseline (P < 0.05).

3.13 – Figures

Figure 1 – Quantitative results for synovial fluid volume at baseline and after intrasynovial injection of mepivacaine (injected limbs), shown as the mean percentage change relative to baseline synovial fluid volume. *Statistically significant difference from baseline ($P < 0.05$). (A) Podotrochlear bursa (PB) and distal interphalangeal joint (DIPJ). (B) Digital flexor tendon sheath (DFTS).

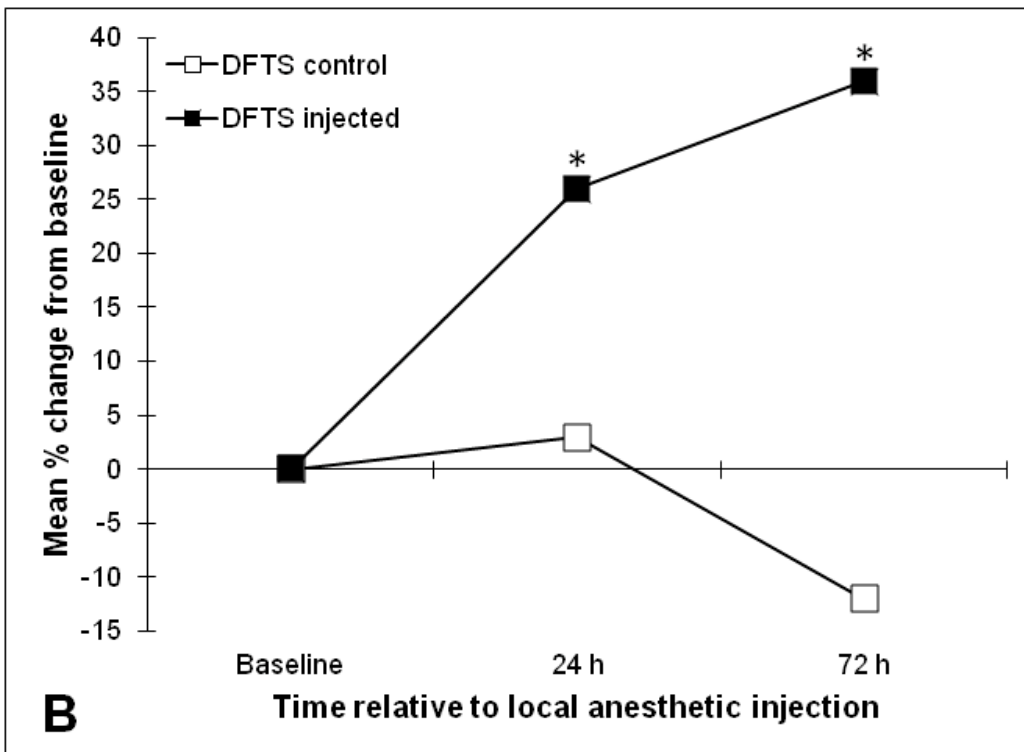
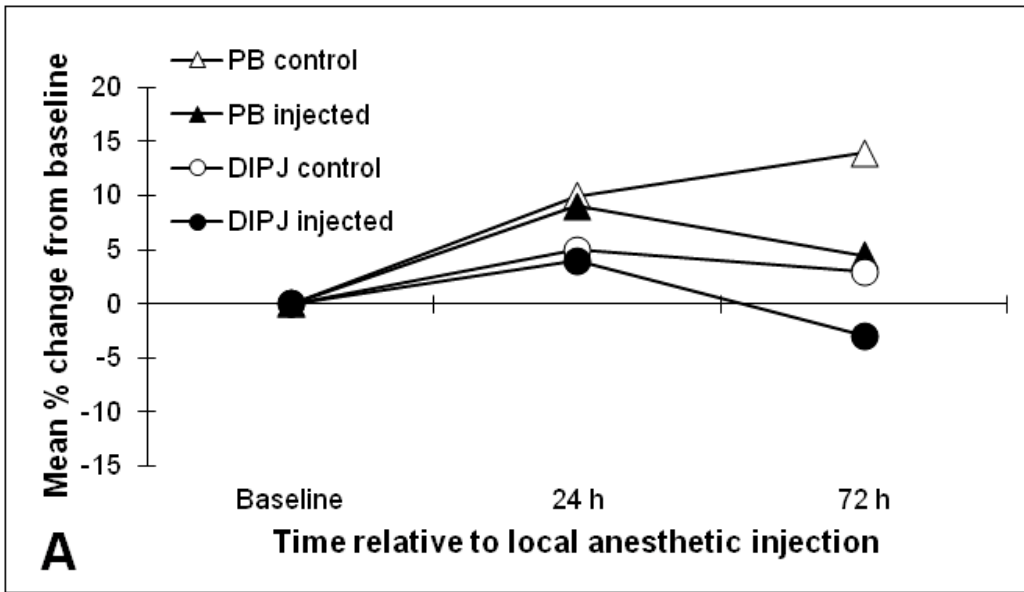


Figure 2 – Qualitative results for digital flexor tendon sheath (DFTS) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by BB. *Statistically significant difference from baseline ($P < 0.05$).

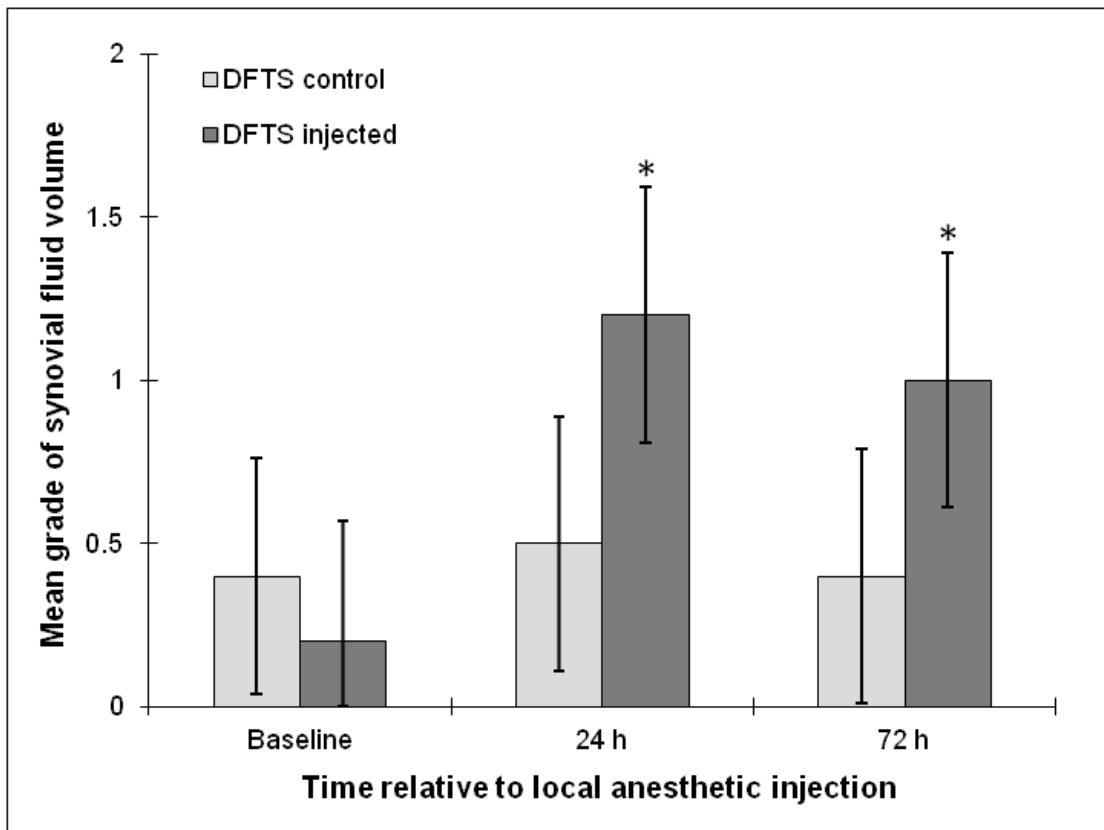


Figure 3 – T2*-weighted sagittal MR image of a foot, 24 hours after mepivacaine injection into the podotrochlear bursa (PB). The narrow linear area of low signal intensity (arrows), which extends from the heel-bulb region toward the navicular bone, is consistent with a clearly defined needle tract (Grade 2).



4.0 – LIMITATIONS AND FUTURE AREAS OF STUDY

The objective of this study was to determine if injection of mepivacaine into perineural and synovial structures of the equine foot would cause iatrogenic changes detectable with MRI. We hypothesized that: 1) after injection of mepivacaine into the PB, DFTS and DIPJ, MRI would detect an increase in synovial fluid at 24 hours, but not at 72 hours post-injection; and 2) injection of mepivacaine adjacent to the PDN and into the PB, DFTS and DIPJ would cause abnormalities at the needle entry sites, which would be detectable on the post-injection MRIs.

Injection of mepivacaine into the PB or DIPJ did not cause any significant MRI changes at either 24 or 72 hours post-injection. However, injection of the DFTS caused a significant increase in DFTS synovial fluid volume, which was detectable both quantitatively and qualitatively on both the 24- and 72-hour MRIs. The first hypothesis was supported by the increase in DFTS synovial fluid, but was opposed by the duration of the DFTS distension and by the lack of change in the PB and DIPJ.

On the post-injection MRIs, abnormalities were frequently noted along the tract of the spinal needle, which was used for the PB injection. Abnormalities were not evident at any of the other injection sites. Therefore, the second hypothesis was supported only by these visible PB needle tracts.

As previously discussed, the observed increase in DFTS synovial fluid volume after mepivacaine injection was not unexpected. However, at 24 hours post-injection, the increase in DFTS fluid volume was greater in horses that required repositioning of the spinal needle during the PB injection. It is possible that the distal aspect of the DFTS was penetrated during initial incorrect positioning of the spinal needle for injection of the

PB; which may have contributed to the increase in DFTS synovial fluid volume. Perhaps, just the close anatomical proximity of these 2 injected structures exacerbated the DFTS inflammation. However, the need to reposition the PB needle did not appear to contribute to the increased DFTS synovial fluid at 72 hours post-injection. At that time, all injected limbs had significant increases in DFTS synovial fluid volume, regardless of previous PB needle repositioning. Interestingly, horses which had the PB needle positioned only once showed the greatest increase in DFTS synovial fluid at 72 hours. Although this could represent a delayed inflammatory response, it makes no sense as to why it would be more pronounced in these horses.

In a clinical situation, injections of local anesthetic into multiple synovial structures may be performed on the same day. The injection protocol in this study was intended to mimic this scenario as best possible. To truly determine if PB injection does, or does not, have an effect on DFTS synovial fluid volume, the study would need to be repeated without injection into the DFTS. An argument could be made to reposition the PB needle multiple times to truly assess its effect. However, the cost benefit of such a study seems questionable.

There were no significant changes in PB or DIPJ synovial fluid volume on MRIs at either 24 or 72 hours after mepivacaine injection. Therefore, distension in these structures in clinical patients is probably truly associated with pathology, not the injection of local anesthetic. A limitation of our study was that the mepivacaine injections were performed on horses free from lameness or pre-existing abnormalities detectable with MRI. A similar study using lame horses would require a substantial number of animals to obtain adequate power for statistical testing of inherently more variable MRI data.

Lameness in the distal limb can originate from a multitude of sources, with more than one anatomical structure often affected.

Another limitation of the current study was the cessation of data collection at 72 hours post-injection, which prevent determining a definitive time for resolution of the DFTS effusion. Although this design was related to financial limitations, animal welfare issues associated with repeated general anesthetics were also considered. Subjectively, horses in the study tolerated the serial general anesthetics well, although no objective data was collected to confirm these observations. However, if the study were to be continued, the suggested protocol would be to inject the DFTS with mepivacaine and perform MRIs at 1, 3 and 5 days post-injection. This design would still allow assessment of early post-injection DFTS effusion at 24 hours (without any potential component from PB injection), and should also determine a definitive time for resolution of DFTS effusion.

A minor limitation of our study involves the accuracy of the manually drawn regions of interest to accumulate quantitative data. Although most clinical MRI assessments of veterinary patients are performed qualitatively, we thought it important to also quantify synovial fluid volume for this study. When drawing the regions of interest, the anatomical structure was localized as best possible, although at times this was difficult due to the close proximity of the distal aspect of the DFTS, the palmar pouch of the DIPJ, and the proximal pouch of the PB. Measuring an outcome measure quantitatively is encouraged in scientific experiments as this should allow repeatable measures between different people, however for some assessments qualitative assessment has been shown to be more accurate overall (Sullivan 2008).

4.1 – GENERAL CONCLUSIONS

In horses, MRI has proven to be a valuable tool in the diagnosis of lameness localized to the foot. Therefore, it is important to know if diagnostic analgesia of structures within the digit alters the local environment enough to cause changes on MRI of this region. The purpose of this study was to determine if perineural and intrasynovial anesthesia of structures in the equine foot cause iatrogenic changes detectable on subsequent MRI.

This study showed that perineural anesthesia of the PDN and intrasynovial anesthesia of the PB or DIPJ in sound horses did not have any significant effect on the interpretation of subsequent MRIs. However, intrasynovial anesthesia of the DFTS caused a significant iatrogenic increase in synovial fluid, which was detectable on subsequent MRIs for at least 72 hours. Determining the definitive time for the DFTS effusion to return to pre-injection values was beyond the scope of this study; and further investigation is warranted.

5.0 - MASTER REFERENCE LIST

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6.0 - APPENDIX

Figure A - Qualitative results for digital flexor tendon sheath (DFTS) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by BB. *Statistically significant difference from baseline ($P < 0.05$).

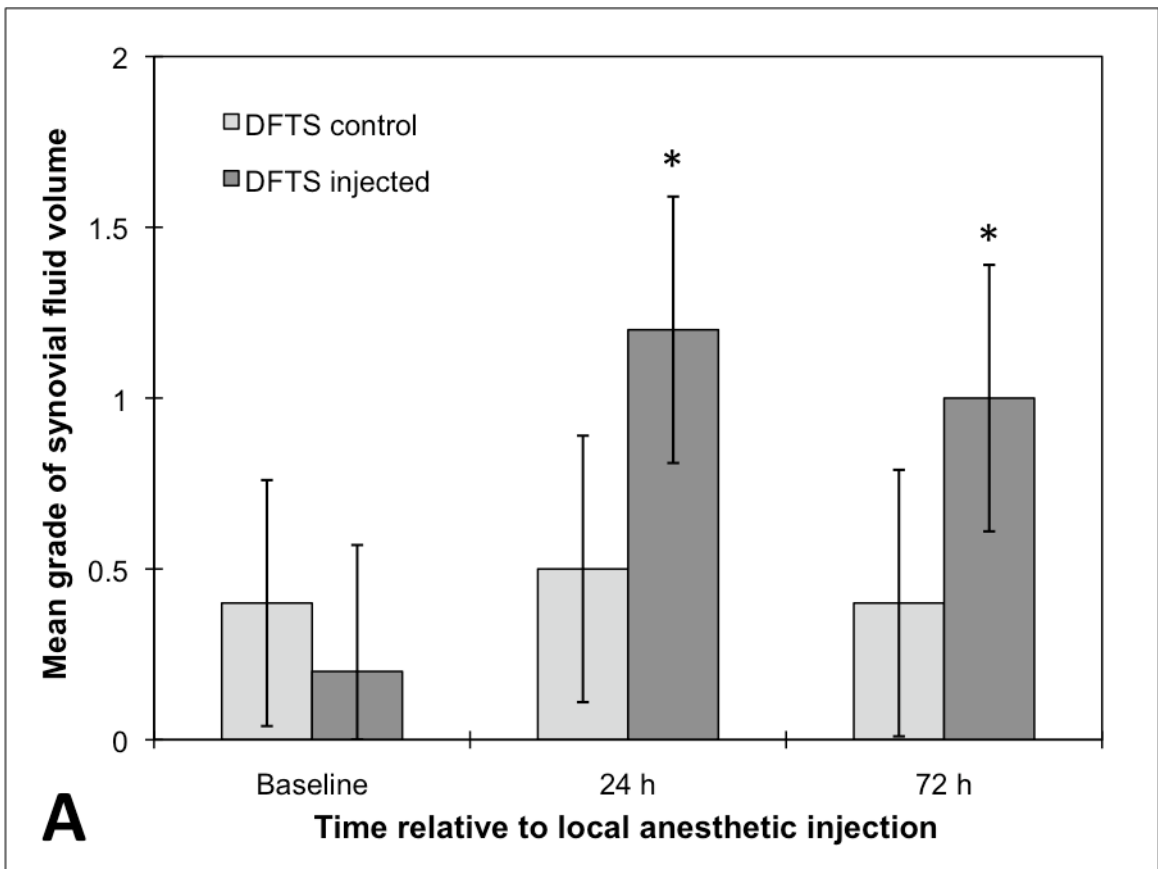


Figure B - Qualitative results for digital flexor tendon sheath (DFTS) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by SN. *Statistically significant difference from baseline ($P < 0.05$).

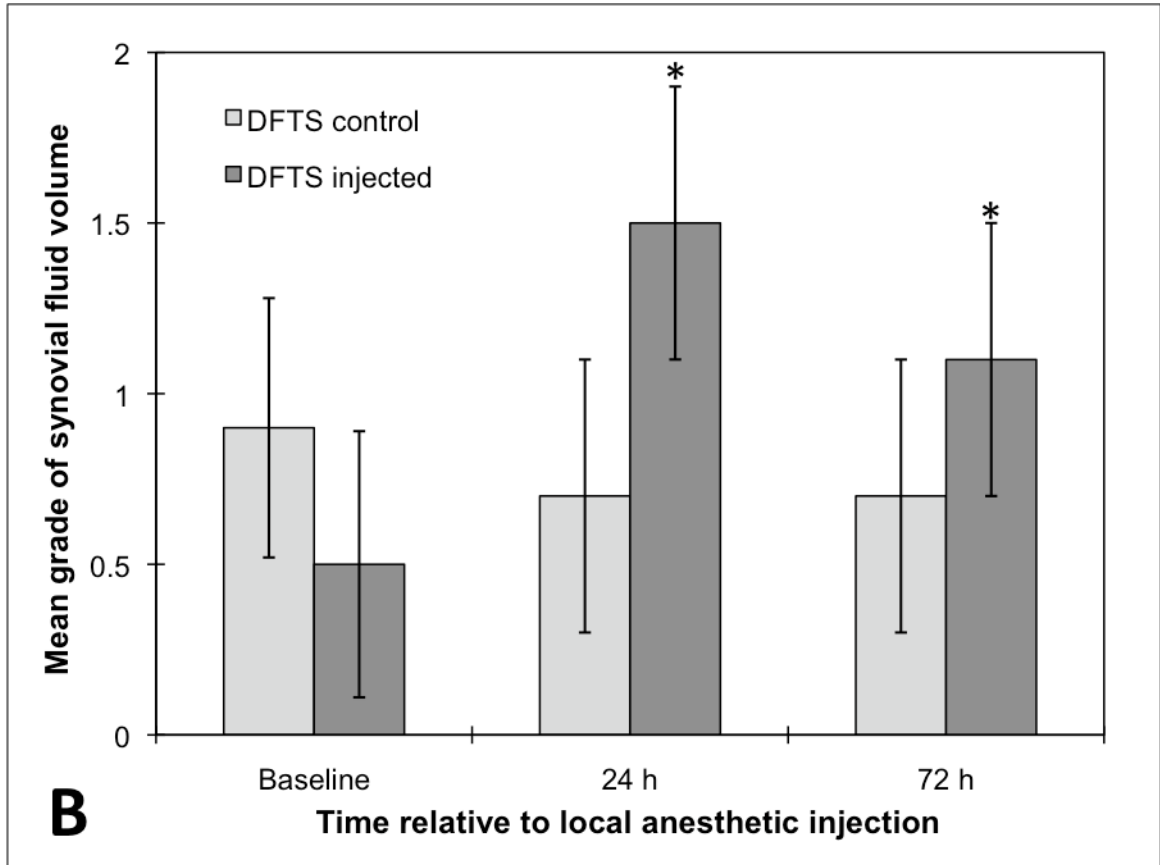


Figure C - Qualitative results for distal interphalangeal joint (DIPJ) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by BB. *Statistically significant difference from baseline ($P < 0.05$).

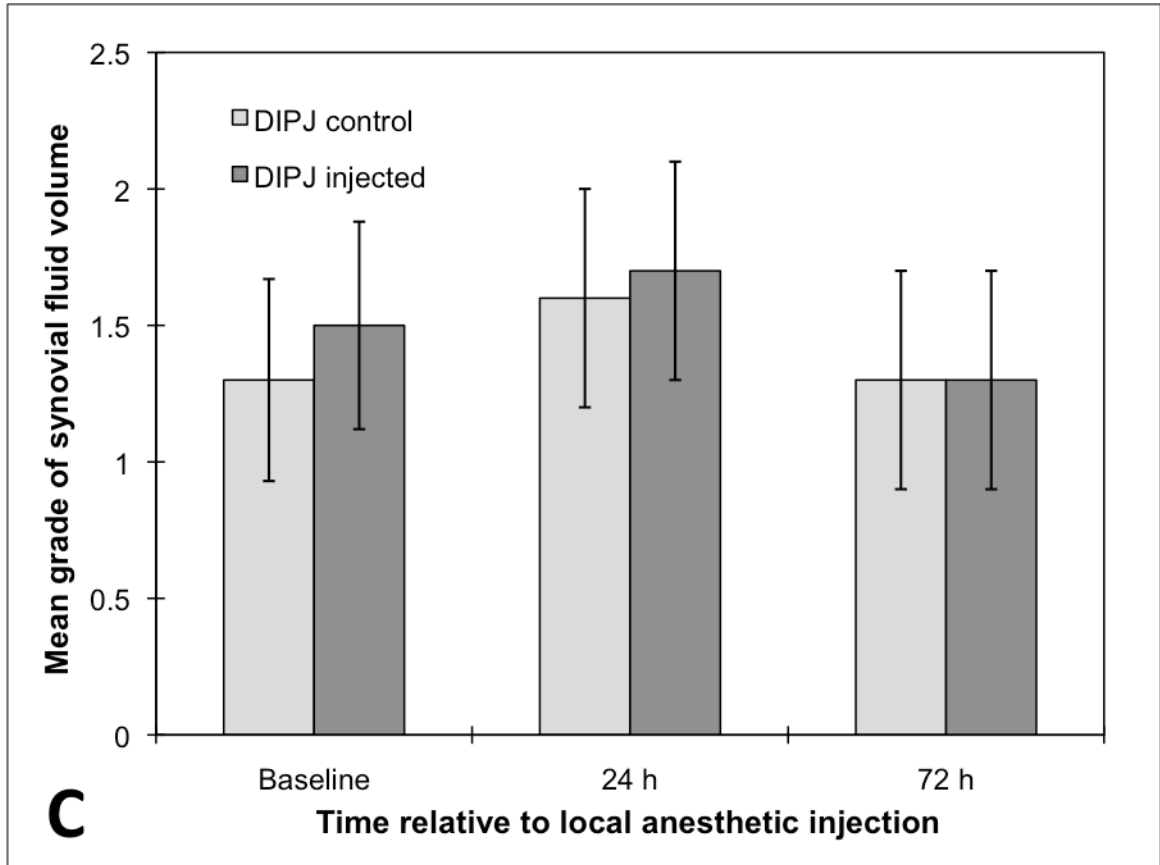


Figure D - Qualitative results for distal interphalangeal joint (DIPJ) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by SN. *Statistically significant difference from baseline ($P < 0.05$).

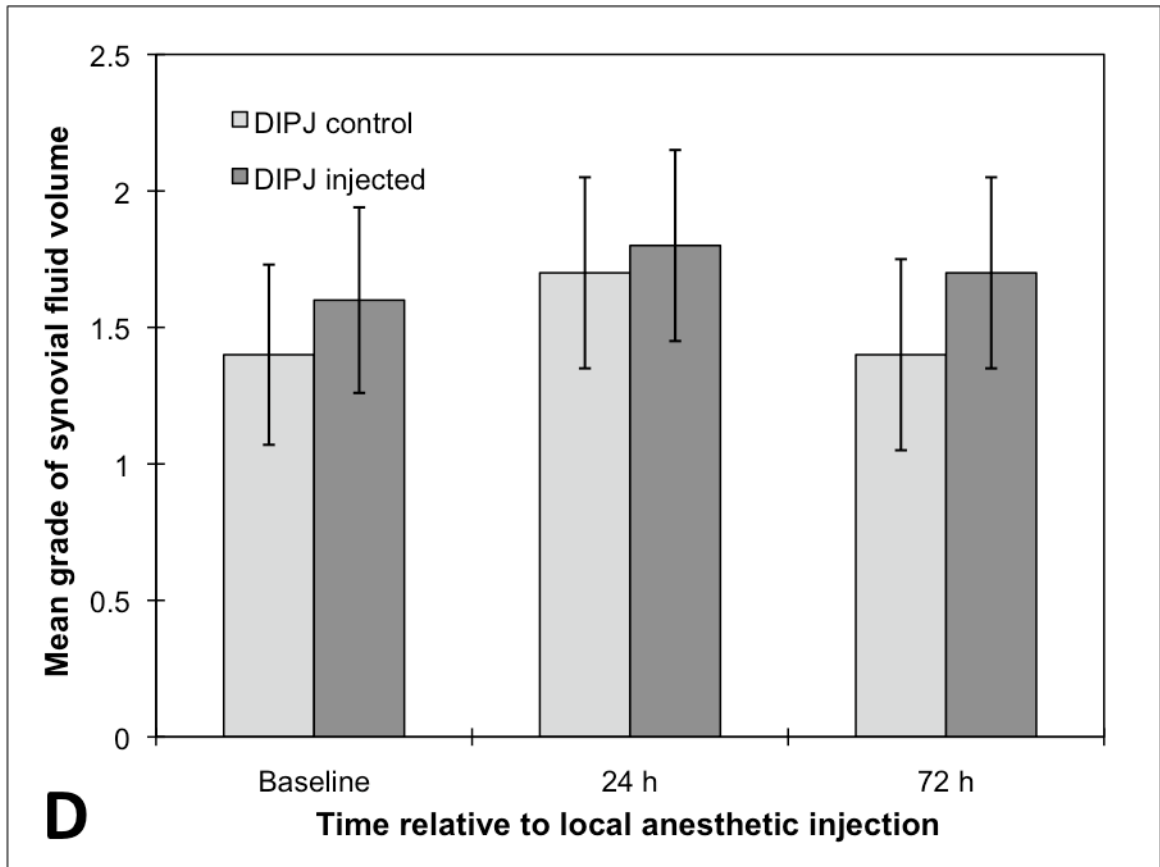


Figure E - Qualitative results for podotrochlear bursa (PB) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by BB. *Statistically significant difference from baseline ($P < 0.05$).

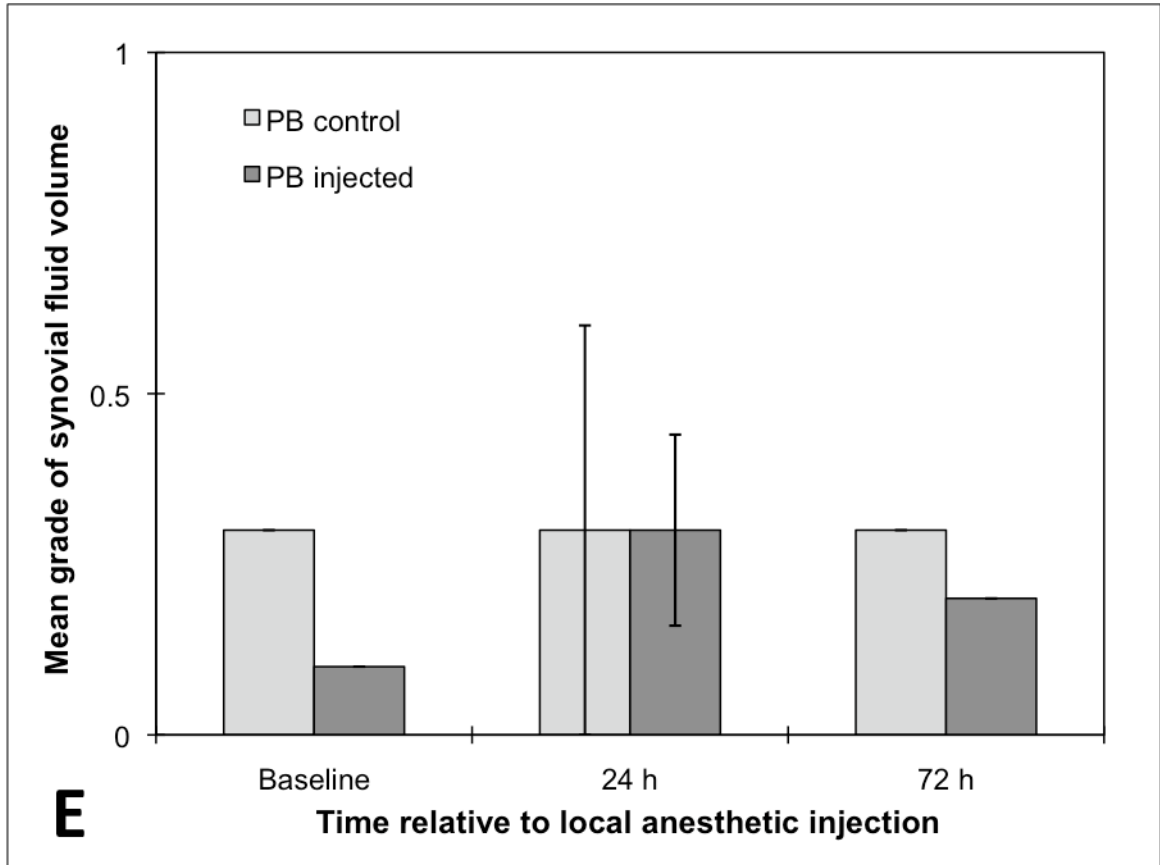


Figure F - Qualitative results for podotrochlear bursa (PB) synovial fluid volume (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by SN. *Statistically significant difference from baseline ($P < 0.05$).

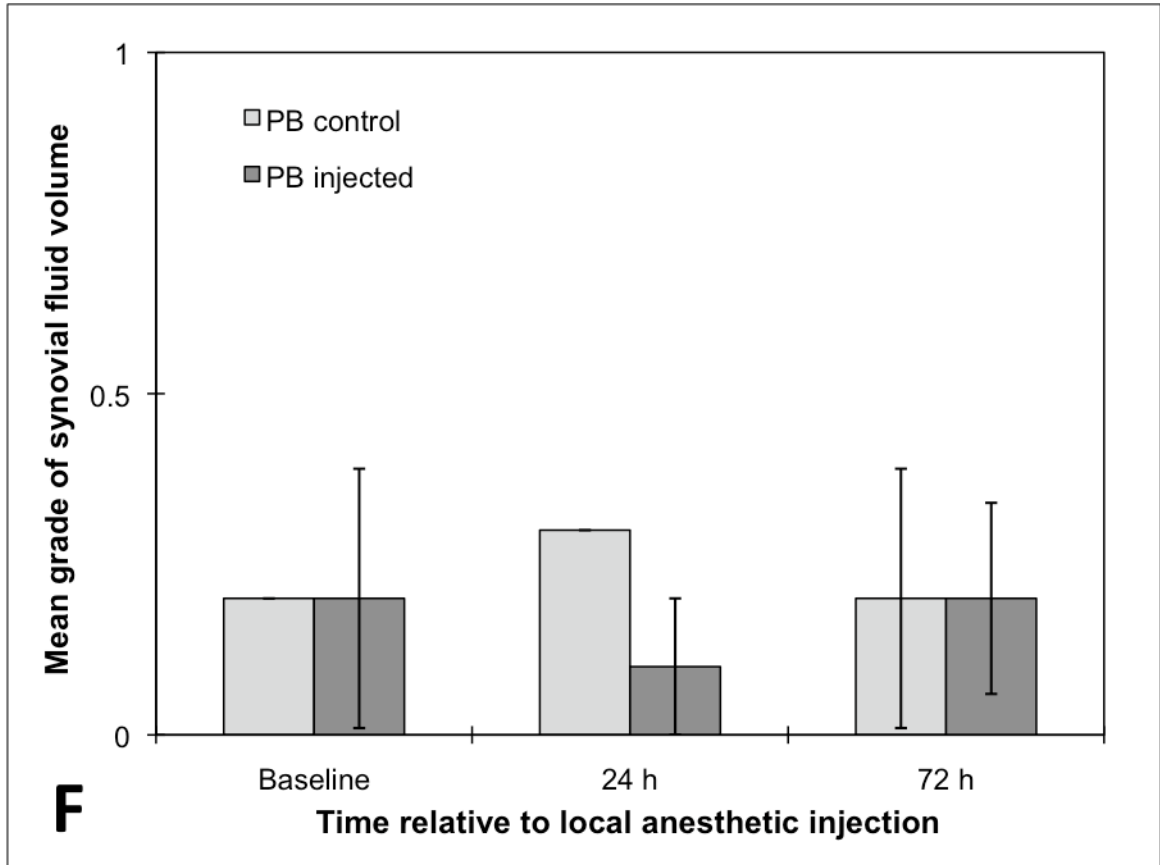


Figure G - Qualitative results for navicular bone (NB) signal intensity (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by BB. *Statistically significant difference from baseline ($P < 0.05$).

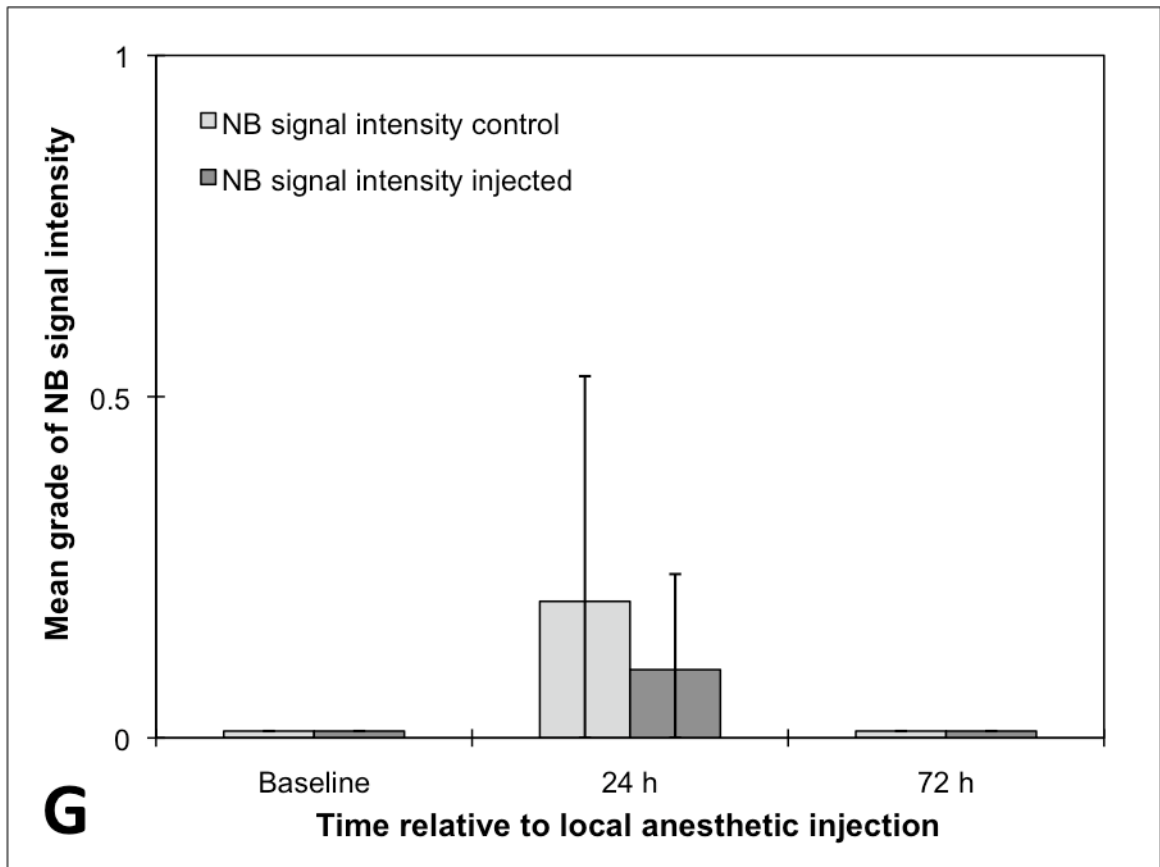


Figure H - Qualitative results for navicular bone (NB) signal intensity (mean grade, 95% confidence interval) at baseline and after intrasynovial injection of mepivacaine (injected limbs) as assessed by SN. *Statistically significant difference from baseline ($P < 0.05$).

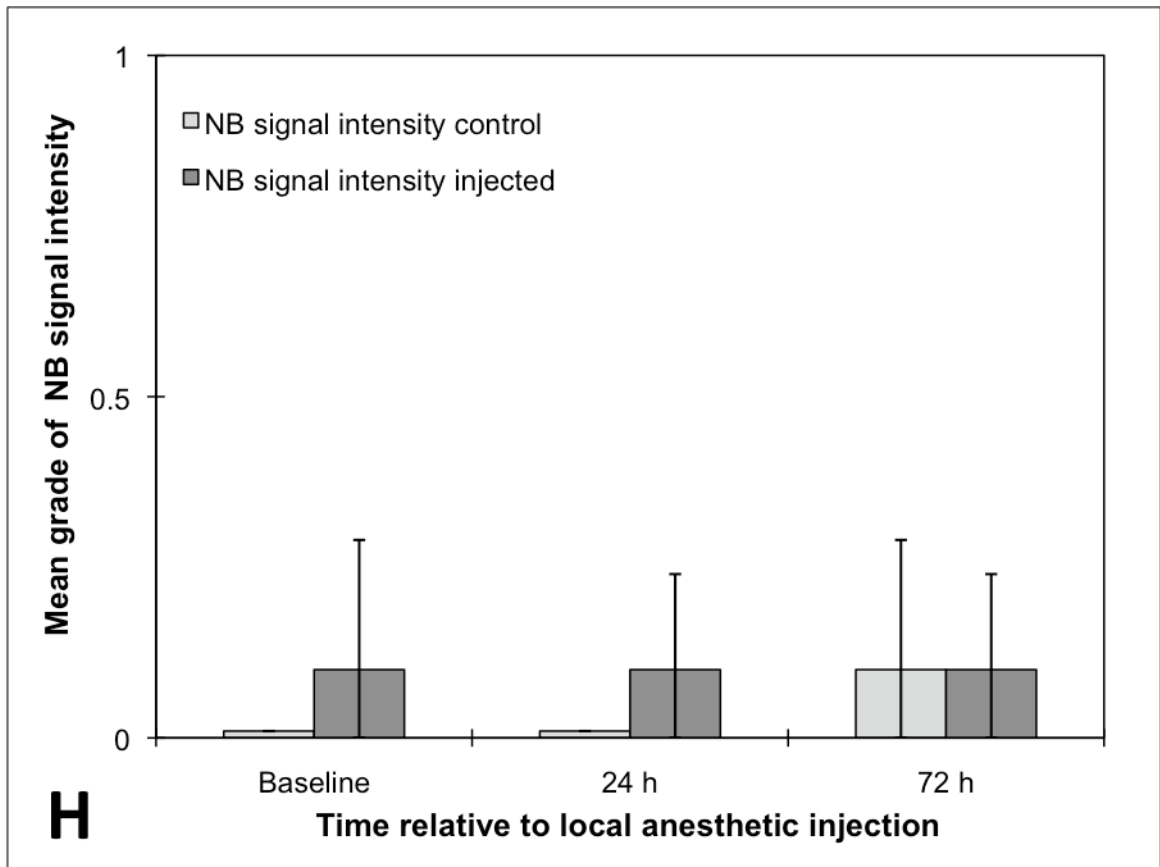


Figure I – Quantitative results for synovial fluid volume [mean volume (mL), 95% confidence interval] at baseline and after intrasynovial injection of mepivacaine (injected limbs). *Statistically significant difference from baseline ($P < 0.05$).

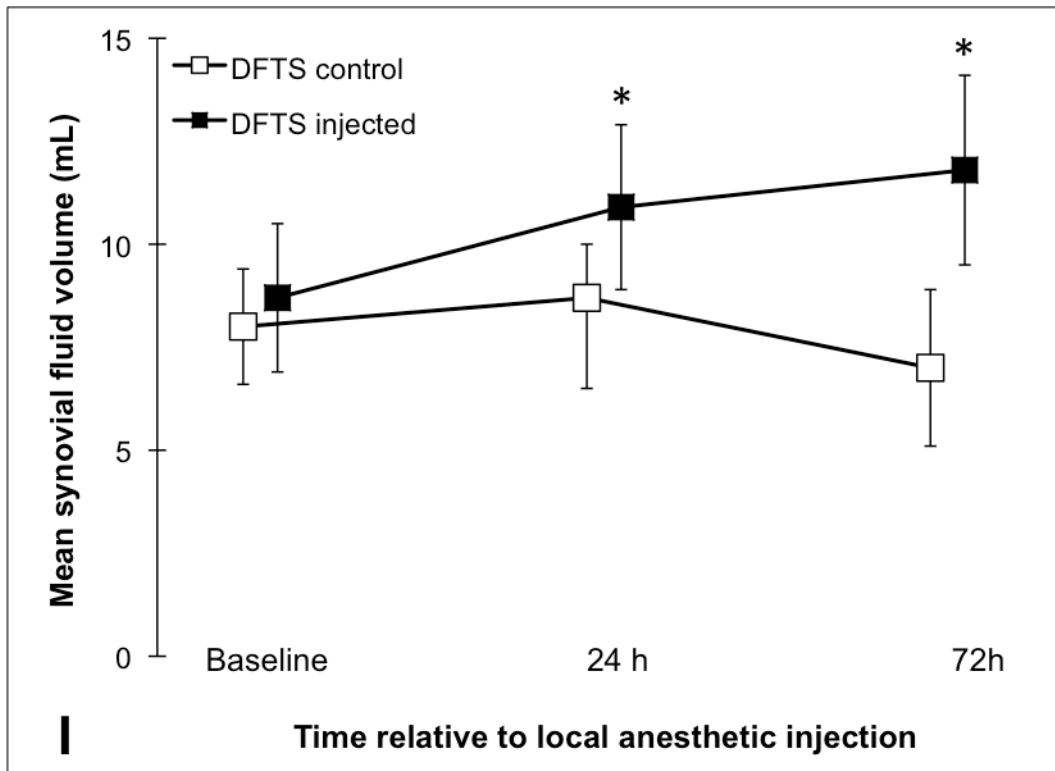


Figure J – Quantitative results for distal interphalangeal joint (DIPJ) synovial fluid volume [mean volume (mL), 95% confidence interval] at baseline and after intrasynovial injection of mepivacaine (injected limbs). *Statistically significant difference from baseline ($P < 0.05$).

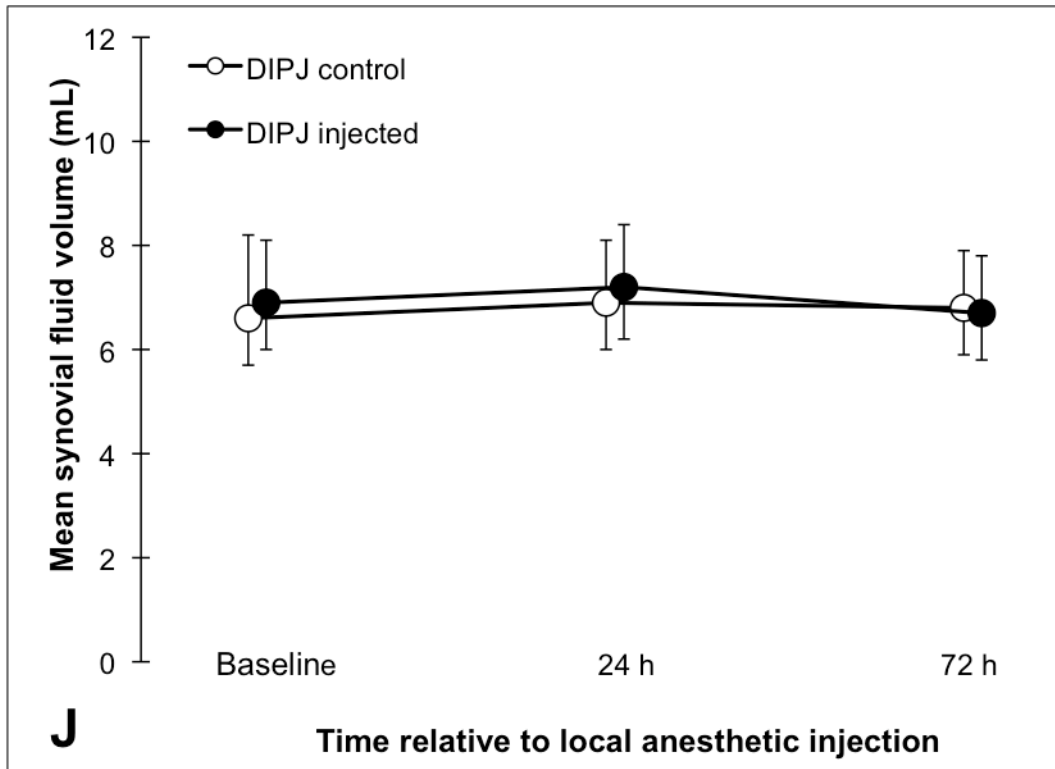


Figure K– Quantitative results for podotrochlear bursa (PB) synovial fluid volume [mean volume (mL), 95% confidence interval] at baseline and after intrasynovial injection of mepivacaine (injected limbs). *Statistically significant difference from baseline ($P < 0.05$).

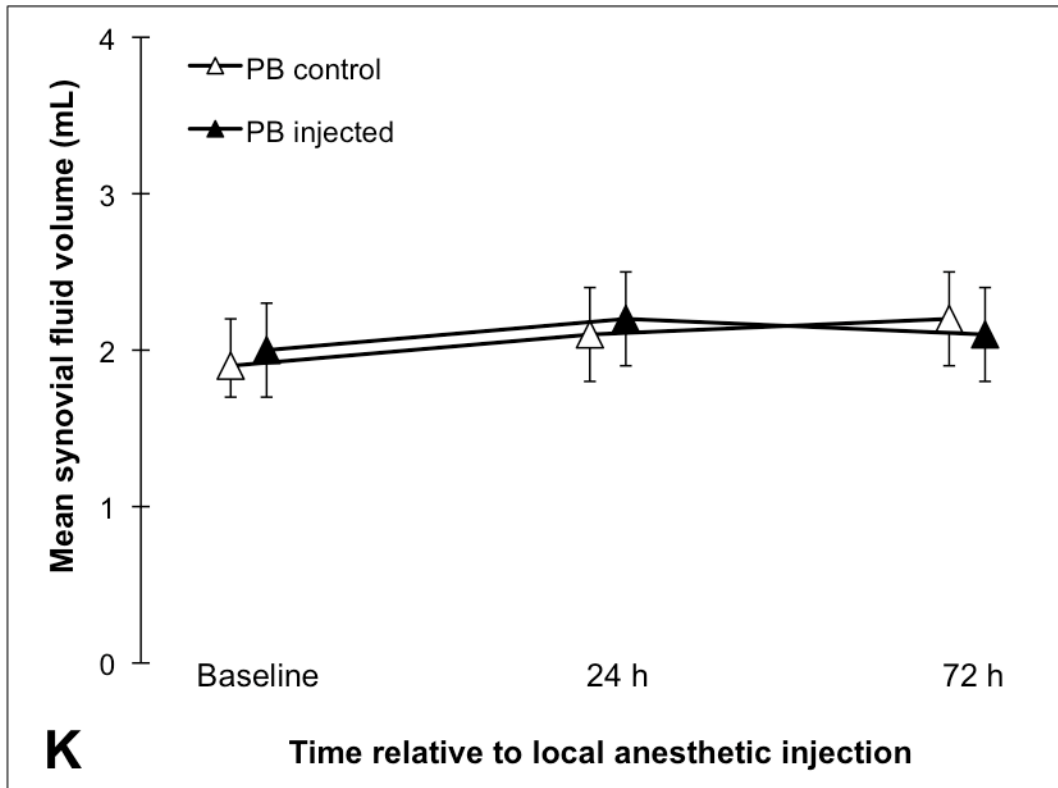


Table A – Qualitative assessment of needle tracts to the podotrochlear bursa (PB) observed within the foot (graded as per manuscript Table 1), at baseline and after intrasynovial injection of mepivacaine (injected limbs). Both investigators (BB & SN) obtained identical results; therefore, this table represents both investigators.

| Limb | Number of horses | | | | | | | | |
|-------------|--------------------------|----------|----------|---|----------|----------|---|----------|----------|
| | Grade at baseline | | | Grade at 24 hours post-injection | | | Grade at 72 hours post-injection | | |
| | 0 | 1 | 2 | 0 | 1 | 2 | 0 | 1 | 2 |
| Control | 15 | 0 | 0 | 15 | 0 | 0 | 15 | 0 | 0 |
| Injected | 15 | 0 | 0 | 4 | 6 | 5 | 5 | 6 | 4 |