VASCULAR DISTRIBUTION OF CONTRAST MEDIUM DURING INTRAOSSEOUS REGIONAL PERFUSION IN THE EQUINE DISTAL LIMB

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ABSTRACT

VASCULAR DISTRIBUTION OF CONTRAST MEDIUM DURING INTRAOSSEOUS REGIONAL PERFUSION IN THE EQUINE DISTAL LIMB

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Intraosseous regional perfusion is a recent advance in the treatment of septic arthritis and osteomyelitis in the horse. The objective of this study is to describe the vascular distribution pattern of contrast medium during intraosseous regional perfusion (IORP) of the distal portion of the forelimb in horses. Serial lateromedial radiographs were taken of 10 heparinized cadaver distal forelimbs at 0, 1, 2, 6, 15, and 30 minutes during IORP of the third metacarpal bone (MCIII) using iodinated contrast medium and a tourniquet placed over the proximal MCIII. Vascular regions of interest (ROI) were created for each radiograph. Reviewers identified presence or absence of contrast in each ROI. This information was summarized to identify vessel-filling patterns over time. Vessel identification was verified using computed tomography angiography and latex perfusion studies on separate cadaver distal forelimbs. During IORP, contrast medium filled the medullary cavity of the MCIII, exited via trans-cortical vessels and diffused distally to the remaining arteries and veins of the limb, distal to the tourniquet. Maximum vessel and soft tissue opacification occurred in most specimens at 6 and 30 minutes, respectively. Serial radiography vessel patterns matched those of CT images and dissected specimens. It was concluded that intraosseous regional perfusion provides a repeatable pattern of vascular distribution in the distal portion of the equine forelimb. This is the first documentation of arterial perfusion using this technique.

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LIST OF ABBREVIATIONS

AIPMMA	Antibiotic-Impregnated Polymethylmethacrylate
CTA	Computed Tomography Angiography
GJK	Graham J. Keys
IORP	Intraosseous Regional Perfusion
IVRP	Intravenous Regional Perfusion
JCJ	Jeryl C. Jones
LEF	Larry E. Freeman
MCIII	Third Metacarpal bone
MIC	Mean Inhibitory Concentration
PMMA	Polymethylmethacrylate
RBC	Red Blood Cell count
WBC	White Blood Cell count
ROI	Region of Interest
SR	Serial Radiography
Tc 99m	Technetium isotope

1.0 INTRODUCTION

Horses commonly injure their lower limbs from external traumatic events. Because of the sparse soft tissue protection on these limbs, such injuries can easily introduce infection into vital structures. [1] Examples include joint spaces, tendon sheaths and bone. Two additional routes for bacterial inoculation and subsequent infection are hematogenous spread from the bloodstream, or complications of a surgical or medical procedure, iatrogenic introduction. [2] Infections of the lower limbs can one of the most challenging diseases to treat in the horse. [3] Until recently, established infections carried a grave prognosis. However, with the advent of perfusion techniques to establish high concentrations of antibiotics and modern veterinary care, many more horses are surviving and returning to their athletic careers. [4] [5]

Regional perfusion techniques have gained widespread acceptance as an effective method of delivering high concentrations of antibiotics to the tissues of the equine distal limb. [4, 6] [3] Intraosseous regional perfusion is a variation of this technique that has been used successfully in the treatment of septic arthritis and osteomyelitis. [7] [8] But many of the details of this technique are based on empirical information only. [6] Futhermore, there is conflicting evidence that intraosseous techniques may be inferior to intravenous regional perfusion. [9-11]

In order to understand these differences and use intraosseous regional perfusion, more understanding is required about the method by which medications distribute from the intraosseous space to the local tissues.

The aim of this study is to use established techniques such as angiography and computed tomography to detail the distribution characteristics of aqueous solutions or

medications infused into the medullary cavity (intraosseous or IO) of the third metacarpal bone of an equine distal limb. This information will provide clinicians with evidence-based information to guide their decisions about application of regional perfusion in the horse.

2.0 OBJECTIVES AND HYPOTHESIS

2.1 Objectives of the Study

To describe the distribution pattern of radio-opaque contrast medium during intraosseous (IORP), using serial radiographs of cadaveric forelimbs perfused with iodinated contrast medium. From this information, future accurate dosing requirements can be determined.

2.2 Hypothesis

It is our hypothesis that following injection into the medullary cavity of the MCIII, contrast medium will follow the normal distribution of blood flow out of the bone and into the superficial venous vessels. Contrast medium will travel from the medullary cavity, into the open canaliculi within the endosteum through transcortical vessels, and into the periosteal venous vessels. From there, contrast medium will distribute to the larger venous vessels and distribute to equilibrate the pressure gradient to the remaining veins of the distal limb, perfusing the vessels of the pastern and hoof. With time and increasing volume, this contrast medium will diffuse from the vessels and into the surrounding soft tissues distal to the tourniquet.

3.0 LITERATURE REVIEW

3.1 Orthopedic Infections in Horses

Orthopedic infections, such as septic arthritis and osteomyelitis, can be a devastating and life-threatening condition in the horse. [2, 3, 6, 12, 13] Minimal soft tissue surrounds the bones and synovial structures of the equine distal limb, including the coffin joint, navicular bursa, digital flexor tendon sheath, pastern joint and the fetlock joint. This region is prone to traumatic infection and can be particularly challenging to treat. [1] Infection may also spread to the bones and joints of neonates via hematogenous routes, and iatrogenic infection may result from surgical or medical procedures.[2] Treatment must be prompt and complete to increase the chance for a successful outcome. Even so, synovial infections of the equine distal limb carry a poor prognosis for return to function. [5] Infections of the bone, particularly after the surgical repair of a fracture, is one of the most difficult diseases to successfully resolve in the horse. [14]

3.2 Surgical Therapy for Orthopedic Infections

When available, surgical methods are advocated to debride infected bone and lavage infected synovial structures. [6, 15] Physical removal of bacteria, fibrin, diseased tissue, and inflammatory mediators is imperative for successful treatment. Yet surgical methods are limited by the extent of tissue that can be safely removed. Foreign materials, including orthopedic implants used to repair fractured bones, will potentiate bacterial

colonization and hinder attempts to treat the infection. [2] The foreign materials must often be removed before the infection can be fully resolved. Even though infected orthopedic implants delay osseous healing, they must remain in place until the osseous structures have healed. Until then, the infection must be managed with open drainage and antibiotics. [3, 12] Established orthopedic infections once carried a hopeless prognosis. But with early recognition, improved surgical techniques, and improved methods of delivering high concentrations antibiotics to the site of infection, many complicated orthopedic infections can be treated successfully.[3]

3.3 Systemic Antimicrobial Therapy for Orthopedic Infections

Systemic antimicrobials are the cornerstone of treatment for orthopedic bacterial infections. [2] The focus is the ubiquitous *Enterobacteriacae* family of bacteria commonly found in the animal's environment, opportunistic dermal Gram-positive flora and pathogenic anaerobic organisms. Broad-spectrum, intravenous antibiotic therapy must be administered promptly, before results from bacterial culture and antibiotic sensitivites are known. [3] Once the causative organism(s) is identified, more tailored antimicrobial therapy can be initiated.

3.3.1 Limitations of Systemic Antibiotics

In many cases, systemic antimicrobial therapy alone is not enough to resolve an orthopedic infection. [14] Systemic antibiotics have limited penetration to areas that are

isolated by ischemia, fibrosis or abscessation. [15, 16] Antibiotic penetration is further limited by the formation of a bacterial biofilm; a combination of bacteria and a glycoproteinaceous material formed by bacterial extracellular exopolysaccharides, which encapsulate the bacterial aggregate, protecting them from host defenses and environmental stresses such as antimicrobials. [17] The shielding effect of a biofilm may require a dramatic increase in the concentration of antibiotic to be effective. Minimum inhibitory concentrations (MIC) are laboratory-derived values that describe the minimum concentration of antibiotic required to kill a planktonic (free-floating) susceptible microorganism. It may require a 1000 fold increase in this concentration to kill the same organism protected by a biofilm.

3.4 Increased-Dose Antimicrobial Therapy

In dogs, it is advocated to increase the dose of certain antibiotics in order to achieve therapeutic concentrations in osteomyelitic bone with reduced local blood flow. [16] But toxicity and cost limit the dose of antibiotics that can be systemically administered to an equine patient. [4] For example, aminoglycosides are an effective, concentration-dependent group of antibiotics commonly used in the treatment equine orthopedic infections. [18] Although they are more effective at higher tissue concentrations, they carry the risk of renal toxicity. [19] Toxicity is also of concern to other organs, including the liver and the gastrointestinal tract. [4] It is warranted, then, to increase the concentration of an antibiotic at the site of infection, without increasing the systemic dose of the antibiotic.

When administered locally, very high concentrations of antibiotics can be achieved in tissues. The systemic toxic side effects as well as the cost of therapy can be minimized. Recently, innovative techniques have been developed to accomplish this goal. [6] Each of these therapies has unique advantages and disadvantages.

3.5. Intra-Articular Antimicrobial Therapies

3.5.1 Direct Injection of Antibiotic

Direct injection of gentamicin into equine joints has been shown to establish very high concentrations of antibiotics in the synovial fluid as well as the adjacent subchondral bone. [20, 21] These concentrations far exceed those achieved with systemic intravenous therapy. [20] Following direct articular injection, synovial fluid gentamicin concentrations remained above reported MIC for susceptible pathogens for greater than 24 hours. [21] Adjacent subchondral bone concentrations remained above reported MIC for 8 hours. Intrasynovial therapy had no significant effect on serum gentamicin concentrations, implying that it could be used safely in combination with systemic therapy. However, repeated intra-articular synoviocentesis can lead to synovial hyperplasia, capsular fibrosis, and subsynovial and pericapsular hemorrhage. [22] Intrasynovial injection of gentamicin into a joint causes a mild but transient chemical synovitis. [23] These findings may deter a clinician from repeated daily intra-articular injections.

3.5.2 Continuous Infusion Device

Lescun et al [24] describe a method to deliver a continuous infusion of gentamicin into the tarsocrural joint of horses using modified, commercially available balloon infuser and flow-control tubing. This device was reported to deliver 16.4 (+/- 2.5) ml/day of 100mg/ml gentamicin into the tarsocrural joint of 12 adult healthy horses. Peak synovial fluid gentamicin concentrations were comparable to those reported in other studies following intra-articular injection. [20] Yet the concentrations achieved using the continuous infusion device were sustained for the duration of the study (5 days). Synovitis was present in both the treated and control joints; control joints had an intrasynovial catheter placed, and balanced electrolyte solution was infused into the joint. Although there was no significant difference in the WBC or RBC between the treatment groups, synovial fluid pH was significantly lower and total protein concentration was significantly higher in treated joints compared to control joints. A complication rate of 29% was observed when using this device. Complications consisted of catheter kinking and failure of the balloons. To the author's knowledge, no studies have been performed using this device on clinical cases.

3.6 Antibiotic-Releasing Materials

Several antibiotic-releasing materials are available to deliver high local concentrations of antibiotics to infected tissues. Antibiotic-impregnated polymethyl methacrylate (AIPMMA) has been used in the successful treatment of cases of equine

septic osteomyelitis and arthritis. [25] Polymethyl methacrylate (PMMA) is an acrylic used in orthopedic surgery. Antibiotics can readily be combined with the polymers at the time of surgery or prepared and sterilized for future use. [4] Elution of antibiotics from PMMA is dependent on the type of PMMA, its porosity, surface area, the concentration of the antibiotic, the choice and state of antibiotic (powder or liquid), and the rate of local fluid turnover. [22, 26-28] Bactericidal concentrations of aminoglycosides are eluted from PMMA for at least 30 days. [27] Antibiotic-impregnated PMMA implanted in the site of infection will release sustained higher concentrations of antibiotics than can be achieved with systemic medications. [26] When used intrasynovially, this concentration is considerably less than that reported in other studies following intrasynovial injection. [20] [24] It may also lead to synovitis, and if the horse is not stall confined, bead migration and cartilage damage. Therefore it is recommended to remove intrasynovial AIPMMA beads from synovial compartments 9 days after implantation. [22] Infection has been observed to persist following implantation of antibiotic impregnated PMMA into wounds. [15] Because PMMA is not absorbed, it can act as a foreign body and may need to be removed to fully resolve an infection. Fibrous and granulation tissue formation around implanted AIPMMA beads may make removal complicated.

Bioabsorbale materials have also been evaluated as antibiotic-releasing devices. Antibiotic-impregnated hydroxyapatite is a water-based osteoconductive material that has superior antibiotic elution properties to PMMA [27]. Plaster of paris is also an osteoconductive material that will elute antibiotics effectively in vitro. However, 80% of the elution occurs within 48 hours, and subsequent elution remains below reported MIC values. [29] Collagen sponges impregnated with gentamicin have been used successfully

to treat septic arthritis in horses and cattle. [15] Bioabsorbable materials have the tremendous advantage of not requiring a second surgical procedure to remove them. Presently the cost and availability of bioabsorbable devices limits their clinical use. [15, 27]

3.7 Regional Perfusion Techniques

Regional perfusion techniques have gained widespread acceptance as an effective method to deliver high antibiotic concentrations to the tissues of the distal limb of horses. [3, 4, 6] Regional perfusion is the administration of medications into the vessels of an extremity, using a tourniquet to prevent the systemic distribution of the medication, [11] and isolate the distribution of that medication to the site of infection. [30] The medication is administered under pressure, distending the vasculature of the limb, and encouraging its diffusion from the vessels to the local tissues. [31] In so doing, the medication will distribute from healthy to diseased tissues. [32]Thus regional perfusion has particular value in delivering medications to poorly perfused tissues (ie tissues affected by ischemia, abscessation, fibrosis, or sequestration). [4]

3.7.1 Intravenous Regional Perfusion

Intravenous regional perfusion (IVRP) is the preferred method of regional perfusion used in the horse. [3] It can be performed on horses under general anesthesia, or on standing, sedated patients. [32] [33] There are many variations in the technique reported.

Typically, a catheter is placed in a local vein with a tourniquet applied proximally on the limb. Antibiotics are diluted to a higher volume using physiologic saline, and infused using hand pressure. The volume of perfusate is based on empirical information only, and should depend on the volume of the extremity being treated. Reported volumes range from 20 – 60 ml. [6] It has been reported to leave the tourniquet in place from 20-30 minutes. This is to allow time for the medication to diffuse from the vascular space to the surrounding tissue. The location of catheter placement depends on the site of infection. There is no consensus on the appropriate dose of antibiotic to be used. Often the systemic dose, or a portion thereof, is used in conjuction with systemic antimicrobial treatment. [4, 9, 10, 31, 34]

Intravenous regional perfusion has been shown to establish very high concentrations of antibiotics in the bone, synovial fluid, and soft tissues of the equine limb. [9, 10, 21, 30] Peak synovial fluid gentamicin concentrations following IVRP are surpassed only by intra-articular administration. [21] Reported peak subchondral bone gentamicin concentrations following IVRP are greater than 18 times the reported MIC for susceptible pathogens. These concentrations remained above MIC for over 8 hours. Synovial fluid concentrations exceed reported MIC values by 25-50 fold, and remained above MIC for greater than 24 hours. [31]

3.7.2 Limitations of Intravenous Regional Perfusion

Clinical situations exist that may limit venous access for IVRP. [4, 9, 35]Severe trauma may damage the vessels needed for catheter placement in the affected limb.

Swelling may make identification of vessels impossible, and infection of surrounding soft tissues may make catheterization of a local vessel unwise. In dehydrated or shocky patients, the venous vessels may be collapsed and unavailable for catheterization. Preservation of the local vascular supply to an infected wound or a repaired fracture is of paramount importance. Therefore the smallest catheters should be used and every effort must be made to avoid trauma to the vessel. [4] Even so, repeated catheterizations of the same vessel may lead to thrombosis, hematoma or abscess formation. [31] [9] [36] In these situations, intraosseous regional perfusion (IORP) may be a suitable alternative.

3.7.3 Intraosseous Regional Perfusion

Intraosseous regional perfusion uses the medullary cavity of a long bone to gain access to the local vasculature. [31] Because the medullary cavity is protected by a strong diaphyseal cortex, it provides a reliable, non-collapsing portal through which medications can be safely and repeatedly administered. [35] The portal is established through one cortex of the diaphysis of the long bone. Either a specific adapter or the male insertion of an intravenous extension set can be secured into this portal and medications are administered as they would for IVRP. A tourniquet is applied proximal to the portal and the site of infection and remains in place for 30 minutes during the perfusion. Although there is considerable variation in recommended techniques, experiments have consistently demonstrated that IORP in the equine distal limb establishes very high concentrations of aminoglycosides in the bones and synovial fluid distal to the tourniquet. [9, 10, 30, 31, 34]

3.7.4 Comparison Between Intraosseous and Intravenous Regional Perfusion

Several studies have compared IVRP to IORP. Most recently, Mattson et al [11] compared each technique using nuclear scintigraphy. An intraosseous cannula was placed in the dorsal cortex of the third metacarpus and (MCIII) a tourniquet was applied over the proximal MCIII. Technetium Tc 99m was perfused into the distal limb and a gamma camera was used to quantify the amount of the radionuclide uptake into the local tissues. The same volume was applied to the equine distal limb using IVRP. There was no significant difference in radionuclide uptake using either method. This study indicated that IVRP and IORP are similarly effective for the perfusion of the equine distal limb.

Butt et al [9] compared amikacin concentrations in the synovial fluid of the distal interphalangeal joint, digital flexor sheath and metacarpophalangeal joint of horses following IORP and IVRP. Although there was no significant concentration difference in the digital flexor sheath and metacarpophalangeal joint using either method, there was a significantly higher concentration achieved in the distal interphalangeal joint following IVRP. In a similar study, IORP was compared to IVRP by measuring the synovial fluid concentration of amikacin following perfusion of the equine tarsocrural joint. [10] In this study, IVRP established significantly higher synovial fluid concentrations compared to IORP.

It remains unclear why IVRP establishes higher synovial fluid antibiotic concentrations than IORP. There may be a different method of distribution of a

substance from the medullary cavity to surrounding tissues following IORP versus IVRP. But until more information exists about the circulation of the equine distal limb during regional perfusion, this question cannot be answered. Our intent is to contribute to the understanding of intraosseous perfusion to better elucidate rational for observed antibiotic tissue concentrations.

3.8 Blood Flow of Bone

3.8.1 Normal Blood Flow in Bone

The normal blood supply of the bone has been well described, [37]. Blood enters the medullary cavity through nutrient arteries via the nutrient foramen. It is supplemented by arterial inflow from the metaphyseal arteries at each end of the bone. Blood radiates in a centrifugal direction to the periosteum by way of transcortical vessels such as Volkmann's canals. During this transit across the cortex, the blood supplies nutrients to the lacunae in the cortical matrix. The remaining blood collects in the venules of the periosteum, and joins the systemic venous circulation by way of the venous drainage of the limb.

3.8.2 Blood Flow During Regional Perfusion

Both arterial inflow and venous outflow can be obstructed by the placement of a tourniquet. During IVRP, injection of a volume of medications into the venous system

distends the venous vessels distal to the tourniquet. Palmer et al describe this distribution pattern during IVRP in the standing horse. [33] They infused contrast medium into the palmar digital vein, and radiographs were taken immediately after. The authors describe the complete vascular distribution of the contrast medium distal to the tourniquet. Although specific vessels were not identified, contrast medium was observed in the venous as well as the arterial vessels distal to the tourniquet. By 15 minutes, the contrast medium was observed to diffuse from the vessels to the local tissues. Redden also reported the arterial distribution of contrast medium during digital venography in cadaveric horse limbs. [38] One might speculate that because the injection is performed under pressure, the contrast passes from veins, through local capillary beds in a retrograde fashion and subsequently fills the arteries. But to the author's knowledge, no further research has been performed on this subject. Other investigators have made contradictory claims, stating that IVRP distends only the superficial venous system. [10]

A similar confusion exists with IORP. It is not clear how a medication distributes from the medullary cavity to the local vessels during IORP. Scheuch et al [10] used contrast angiography to identify the vascular filling pattern of contrast medium following IORP of the equine tarsus. They reported that the superficial venous vessels were filled with contrast medium. These results are similar to those reported by Whitehair et al. [30] In this study, contrast medium was observed in the superficial venous vessels around the carpus following IORP.

With the limited knowledge about distribution patterns during IORP, it is not possible to determine why arterial perfusion has been observed following IVRP, but not IORP. This difference may be related to the differences reported earlier in synovial fluid

antibiotic concentrations following IORP compared to IVRP. Until more is known about the flow of medications during IORP, these questions will remain unanswered. Because many of the recommended IORP techniques are based on empirical evidence only, this technique may not be utilized to its full effect. To ensure that IORP is used appropriately in a clinical setting, an improved understanding about the circulation of the equine distal limb during IORP is necessary.

Therefore it is the object of this study to describe, in detail, the distribution pattern of contrast medium during IORP. Because the equine distal limb is commonly injured, [1, 39] and more regional perfusions appear to be performed in this region than elsewhere in the horse, [9, 11, 21, 31, 32] it is chosen as the subject of the present study. To the author's knowledge, no angiographic studies have been performed during IORP of the distal portion of the equine forelimb (below the carpus). It is hoped that this information will shed light on the discrepancies between IORP and IVRP observed by previous researchers. [9, 10] Furthermore, it will provide the clinician with scientific evidence to make educated decisions about IORP, rather than drawing on empirical information. To accomplish this goal, serial radiographs will be taken of cadaveric equine forelimbs during IORP using a contrast medium. Vascular distribution patterns will be described. These vascular distribution patterns will be confirmed using computed tomography and dissection studies of separate cadaver limbs perfused with blue latex.

It is our hypothesis that following injection into the medullary cavity of the MCIII, contrast medium will follow the normal distribution of blood flow out of the bone and into the superficial venous vessels. More specifically, contrast medium will travel from the medullary cavity, through transcortical vessels, and into the periosteal venous

vessels. From there, contrast medium will distribute to the larger venous vessels. Because the tourniquet will obstruct venous outflow, contrast medium will distribute in a retrograde direction to the remaining veins of the distal limb, perfusing the vessels of the pastern and hoof. With time and increasing volume, this contrast medium will diffuse from the vessels and into the surrounding soft tissues. This hypothesis is based on the description of normal equine blood flow, [37] and the observations made by previous researchers. [30] [10]

3.9 Radiographic Imaging Techniques

Previous investigators have used radiographic imaging techniques to evaluate the vasculature of the equine distal limb. Such techniques include radiography angiography and computed tomography angiography. Radiography is the use of X-ray beams emitted from a an X-ray tube, which pass through an object and photoemulsify an X-ray film. [40] An image is created on the X-ray film depending on the number of X-rays that pass through the object and contact the film. Because objects such as biologic organs have different physical densities throughout the tissues, different amounts of photons are absorbed by these tissues, and therefore different numbers of photons reach the X-ray film. This differential absorption is responsible for the image contrast that enables a viewer to evaluate internal structures of the object being radiographed.[41]

Contrast medium can be injected into the vessels of a biologic organ to evaluate the vascular pattern of that organ. This contrast medium is a liquid that contains atoms of high anatomic number (eg. iodine). High anatomic number correlates with increased

physical density, which absorbs increased amounts of X-ray photons. It is therefore considered radiopaque. A radiograph using a liquid contrast medium injected into the vessels of an organ is called an angiogram. This technique has been used to describe the vessel-filling pattern of a normal equine distal forelimb [42] as well as the vessel-filling pattern during intravenous regional perfusion.[33, 38]

Computed tomography is the use of a rotating X-ray tube and receiver (third generation computed tomography) to create a 3-dimenshional attenuation map of the object being imaged. This device allows the construction of serial cross-sectional radiographic images of an object. [43] It has been used to describe the 3 dimensional vascular anatomy of the equine distal limb following the arterial infusion of an iodinated contrast medium. [39]

Because radiographic imaging techniques have been used to accurately assess the vasculature of the equine distal limb, such techniques may be used to also depict the vascular distribution of a contrast medium during IORP of the equine distal limb.

4.0 Material and Methods

4.1 Specimen Collection

Thirteen forelimbs were collected from 12 heparinized equine cadavers. Horses were euthanized for reasons unrelated to the study. Based on history and a routine physical examination, horses did not suffer from vascular disease or distal forelimb orthopedic disease. Horses that suffered from colic or endotoxemia were similarly excluded from the study. Horses comprised 8 geldings and 4 sexually intact females. There were 8 Quarter Horses, 2 Warmbloods, and 2 American Saddlebreds. Mean age was 13.8 years (range 2-25 years). Mean weight was 440.4 kg (range 400 – 641 kg). Table 1 depicts the parameters evaluated and volumes used in each horse.

4.2 Specimen Preparation

Ten minutes prior to euthanasia, each horse was administered 120,000U heparin sodium[a] through the left jugular vein. Horses were euthanized using an overdose of intravenous pentobarbital.[b] Immediately after euthanasia, both forelimbs were transected in the middle of the radius. One limb was randomly assigned to the treatment group. A 1 cm incision was made through the skin, subcutaneous tissue, and periosteum, 2cm medial to the common digital extensor tendon in the middle of the MCIII (Figure 1). A 4.0mm

unicortical portal was drilled through the dorsomedial cortex of the MCIII. Threads for a 5.5 mm orthopedic screw were tapped into the portal using standard AO technique for the placement of a single cortical screw, [44] with the exception that only one cortex was drilled and tapped. A custom-made 5.5mm X 24 mm cannulated intraosseous bone screw was inserted into this portal (Figure 2). This cannula was designed to accept the male insert end of a standard intravenous extension line. Heparinized lactated Ringer's solution (3,000U of heparin sodium[c] / 100mls lactated Ringer's) was infused through this cannula using a syringe and an intravenous extension set attached to the cannula. This was continued until the fluid emerging from the cut surface of the limb was clear, requiring between 120 – 160mls.

4.3 IORP and Serial Radiography (SR)

To each prepared specimen, a pneumatic tourniquet[d] was placed distal to the carpus and proximal to the cannula (Figure 3). It was inflated to a pressure of 550mmHg. The specimen was secured to a custom-made table that accommodated a 7 X 17" radiograph cassette[e] (Figure 4). This table was secured to a portable radiograph stand. Radiographs were taken using a portable radiograph tube[f] affixed to this stand. Images were taken using high detail, rare earth film.[g] A standardized technique was employed for each film: using a distance of 90 cm, 80 kVp, and 15mA. An angiographic injector[h] was used to infuse iodinated contrast medium[i] under pressure into the medullary cavity of the MCIII (Figure 5). The injector syringe was loaded with a previously calculated volume of contrast medium and connected to the intraosseous cannula via an extension

set. The volume of infused contrast medium was calculated using the dose of 0.15ml/kg body weight of the horse. Infusion was performed at a set rate of 10ml/min, not exceeding a pressure of 450psi. Time to complete the infusion (injection of the contrast medium into the medullary cavity) was variable depending on the volume calculated for each specimen. Perfusion (distribution of the perfusate to local tissues following infusion) of the distal limb was allowed to take place passively while the tourniquet remained in place for the remainder of the 30 minutes. Lateromedial radiographs were obtained before infusion (time 0) and 1, 2, 6, 15, and 30 minutes after infusion was initiated. On two specimens, a dorsopalmar radiograph was taken before removal of the tourniquet to aid in the identification of vessels perfused with contrast medium. Opacified vessels were identified and named by a consensus reached between 2 reviewers (LEF and GJK).

4.4 IORP and CTA

One prepared specimen was placed within the gantry of a helical computed tomography (CT) scanner.[j] The dorsal surface of the specimen was oriented toward the top of the gantry. Contiguous transverse images (thickness of 4mm) were acquired in helical acquisition mode from the toe to the proximal MCIII. Settings for the scanner were: 120 kV, 250mA, FOV 200mm. Intraosseous regional perfusion was then performed on this specimen using the same technique as for the SR study. With the tourniquet in place, and immediately following the infusion of contrast medium, the same CT scan was repeated. Images were viewed using a soft tissue window. Opacified vessels were identified and named based on a consensus opinion between 2 reviewers (LEF and GJK).

4.5 IORP with Latex and Dissection

Intraosseous regional perfusion was performed on 2 prepared specimens using the same technique as the SR study, except 0.15ml/kg body weight of blue latex[k] was used as the contrast medium. The latex was allowed to set up according to manufacturer's recommendations. The specimen was frozen at –18ºC until used. While frozen, one limb was sectioned in a transverse plane in 2.4 cm increments using a band saw. The other limb was thawed and dissected to identify perfused vessels. This limb was then sectioned in a sagittal plane. Latex-filled vessels were identified and named by a consensus reached between 2 reviewers (LEF and GJK).

4.6 SR Analysis

Based on the vessels identified in the SR, CTA and latex-dissected specimen studies, thirteen vascular regions of interest (ROI) were selected for the lateromedial SR films. A vascular template was prepared, highlighting these 13 ROI's (Figure 6). They included the: medullary cavity of MCIII (1), palmar metacarpal vessels (2), dorsal MCIII periosteal vessels (3), proper palmar digital veins (4), proper palmar digital arteries (5), dorsal vessels of the proximal phalanx (6), coronary vein and dorsal branch (vein) of the middle phalanx (7), bulbar branch artery and vein (8), dorsal vascular plexus of laminar corium (9), terminal arch vessels (10), solar marginal vessels (11), bulbar plexus (12), parietal plexus (13).

A questionnaire was prepared by one investigator (GJK). It asked reviewers (JCJ and LEF) to identify the ROI's in every film. It then asked the reviewer to assign a "present" or "absent" score if the vessels of that ROI were opacified with contrast medium (Figure 8). The total number of "present" scores was counted for each region of interest for every specimen at every time interval. This total was divided by the number of specimens to provide the percentage of ROI's opacified by the contrast medium.

All radiographs for one specimen were organized in a series. Radiographs in each series were arranged in chronological order, corresponding to time 0, 1, 2, 6, 15, and 30 minutes of perfusion. One questionnaire was completed for each series. Reviewers were then asked to identify which radiograph in each series demonstrated maximum vessel opacification. They were also asked to identify which radiograph in each series demonstrated maximum soft tissue opacification. Reviewers were not blinded to treatment. Reviewers assigned each ROI score and maximum opacification score by consensus.

5.0 Results

5.1 Anatomic Analysis

5.1.1 Dissected Specimens Analysis

Blue latex was identified in all the vessels identified on CTA and SR. Latex filled the medullary cavity of MCIII (Figure 8c). Blue latex was evident in the cortex of MCIII in all areas except directly underneath the tourniquet and at the articular margins, where it was sparse (Figure 10). Large vessels under the tourniquet were not filled with latex. Minimal latex was present in the vessels of the periosteum under the tourniquet. Latex was observed in the lumen of the medullary cavity underneath the tourniquet. Latex was also observed in the cortex and periosteal vessels of MCIII proximal to the tourniquet. In each specimen, the tourniquet was situated over the level of the nutrient foramen of MCIII. In this location only, the nutrient vein was filled with blue latex, but the artery was not. Vessels draining the limb proximal to the tourniquet and connected to these proximal periosteal vessels and nutrient vein were filled with latex. The periosteal vessels of MCIII distal to the tourniquet were uniformly filled with latex, as were the large distal metaphyseal vessels and palmar metacarpal vessels distal to the tourniquet. These latex-filled vessels communicated with the major vessels of the distal limb, including the proper palmar digital arteries and veins (Figure 11). All grossly visible arteries and veins communicating with these major vessels distal to the tourniquet were filled with latex. Latex was observed in the coronary vessels, the plexus of the laminar

corium, and the vessels of the frog (Figure 12). Latex was also identified in the vessels of the digital flexor tendons and suspensory ligament, the collateral ligaments of each joint, and the ligaments of the proximal and distal sesamoid bones. No latex was observed in the medullary cavity of the proximal or middle phalanx or within the bone of the distal phalanx or the sesamoid bones. No latex was observed free in any synovial compartment, but was visible within the vessels of every synovial capsule distal to the tourniquet (Figure 13). The latex-perfused specimen cut in transverse slices demonstrated identical filling patterns to the corresponding CTA images (Figure 8).

5.2 SR and CTA Analysis

A total of 59 films were used in the SR analysis. One film was excluded due to experimental error. The SR analysis questionnaire was completed successfully for all available films. The ROIs tended to fill in order from proximal to distal with an average infusion volume was 72.3 ml (range 59.3 – 96.2ml).

No ROIs were opacified at Time 0 and ROIs were completely opacified by 15 minutes. Figure 14 summarizes the order of opacification for time periods 1, 2, and 6 minutes. ROIs 1-3 were opacified 100% by time period 1 minute. ROIs 4, 5, and 6 were opacified in 60, 80 and 70 % of specimens (respectively) by time period 1 minute. They were 100%, 89%, and 100 % opacified by time period 2 minutes. ROIs 7, 8, 9 were opacified in 78%, 67%, and 44% of specimens, respectively, by time period 2 minutes. Meanwhile ROIs 10, 11, 12, and 13 were 78%, 67%, 22%, and 0 % opacified,

respectively, by time period 2 minutes. All vessels in the study were opacified by time period 6 minutes, with the exception of ROI 13, which was opacified in 90% specimens by time period 6 minutes. In 8/10 specimens (80%), all vessels appeared maximally opacified at 6 minutes. The vessels in the remaining 2 specimens appeared to be maximally opacified at 15 minutes. In 8/10 specimens (80%), it appeared that maximum soft tissue opacification occurred at 30 minutes. In the remaining two specimens, maximum soft tissue opacification appeared to occur at 15 minutes.

5.2.1 SR Analysis

The vascular patterns identified on SR (Figure 6) were consistent and repeatable with every specimen following IORP, regardless of horse, left or right limb. These patterns were consistent with the CTA images and dissected specimens. All named vessels were identified according to established anatomical terms, when available. [42, 45-47] When discrepancies existed in the literature for the name of an anatomical structure, the most descriptive term was chosen. Although minor variations existed in vessel appearance and branch location amongst specimens, this did not preclude vessel identification. Smaller, unnamed vessels demonstrated more variation, but these vessels were not included in the ROI, and this did not affect the SR analysis.

Overall, every named vessel in the equine limb distal to the tourniquet was opacified following IORP. This includes veins and arteries. Serial radiography was the least detailed method of identifying these vessels compared to CTA and latex dissected

specimens, but it provided a consistent and repeatable method of evaluating these structures over time. Identification of superimposed major vessels was challenging with SR alone. However, once these vessels were confirmed using dorsoventral films, CTA, and latex-dissected specimens, identification of named vessels was not problematic.

5.2.2 CTA Analysis

CTA provided detailed images of the vascular anatomy of the distal portion of the equine limb (Figure 8). No vessels were opacified prior to perfusion. Following perfusion, contrast medium was identified in the medullary cavity of MCIII and the transcortical channels of the MCIII distal to the tourniquet (Figure 9). All named vessels and many unnamed vessels were opacified. This included all vessels used in the ROIs for the SR analysis. Contrast medium was not identified in the medullary cavity of the proximal or middle phalanx. Contrast medium was identified in the vessels of the solar foramen, solar marginal vessels, and the vascular plexus of the laminar corium of the distal phalanx.

5.3 Experimental Error

One radiograph during the SR was double exposed. The double exposure occurred for specimen number 3, during the 6-minute radiograph. This radiograph was superimposed over the 2-minute radiograph. Because the two films were taken identically, and there was no difference in positioning, the information was determined to

be valid for the 6-minute radiograph. Consequently, the information for the 2-minute radiograph was lost. During radiography of specimen # 1 at time 0, a small amount of contrast had diffused into the medullary cavity prior to the initiation of perfusion, giving an aberrant positive score for that ROI.

5.3.1 Leakage of Contrast

Leakage occurred from two sources in every specimen. One source was from around the intraosseous cannula. This leakage was considered minor (less than 5 ml). It was determined that the leakage predominantly came from the incised soft tissues around the intraosseous portal (ie. skin, subcutaneous tissue, periosteum). The other leakage source was from the transected vessels at the cut surface of the limb. This amount of leakage was considered major (approximately 15 – 20 ml) despite the presence of a tourniquet. Leakage was consistent amongst specimens, although the exact volume of leakage was not recorded for each specimen.

6.0 Discussion

6.1 Perfusion Pattern And Vasculature

In the present study, serial radiography angiography, computed tomography angiography, and latex-perfused dissected specimens were used to describe the vascular distribution of contrast medium during intraosseous regional perfusion of the distal portion of the forelimb of the horse. Previous investigators have evaluated the angiography of the distal limb of the horse using IVRP [33, 38]. Others have studied the distribution of contrast following IORP of the equine tarsus [10] and carpus. [30] The results of these studies indicated that IORP distributes contrast to the superficial venous system isolated by a tourniquet. To the author's knowledge, this is the first study to evaluate the angiography of the equine distal limb, below the carpus, following IORP. This is also the first study to document the presence of contrast medium in the arteries of the distal limb using this technique. We conclude the intravenous pressure gradient proceeds toward equilibrium and arterial perfusion is possible.

The results of this study demonstrate that there is a consistent pattern of distribution of contrast medium from the medullary cavity to the local tissues following IORP. Contrast medium enters the medullary cavity of the MCIII through the intraosseous cannula, and distributes through the cortical bone proximal and distal to the tourniquet. The periosteal and metaphyseal vessels collect this medium and deliver it to the major veins and arteries of the limb. With time and increasing volume, this medium diffuses to the remaining

vessels distal to the tourniquet. Of particular interest, both arteries and veins fill simultaneously in a proximal to distal direction.The hypothesis of this study was that contrast medium would distribute from the medullary cavity to the superficial venous vessels distal to the tourniquet. The null hypothesis is that contrast medium would have no discernable distribution pattern. This null hypothesis was refuted. Indeed, venous vessel distribution was observed in the predicted pattern. In addition, arterial opacification was observed. This was an unexpected finding.

The presence of contrast medium in the arteries suggests that a unique pressure difference exists in the equine distal limb during IORP. This pressure difference encourages diffusion of a substance out of the medullary cavity in a manner different that observed during normal blood flow. Once in the superficial vasculature, contrast medium was observed to distribute normograde via arterial vessels to distal capillary beds. It also traveled retrograde via venous vessels to the same capillary beds. The result was a complete perfusion of the vessels distal to the tourniquet. It is compelling, then, to investigate the relationship between intramedullary and vascular pressures, to explain how IORP creates this unique vascular distribution.

6.2 Intraosseous Pressures and Physiologic Implications

Intraosseous, venous and arterial pressures of the mature pony metatarsus while under general anesthesia are reported by Stolk et al. [48]. The mean intramedullary pressure of the mature pony metatarsus was 49.7 +/- 7.2 mmHg. The mean metatarsal

artery pressure of the plantar metarsal artery was 69.6 +/- 15.9 mmHg. The mean saphenous venous pressure was 12.3 +/- 1.7 mmHg. From this information it is conceivable that if intramedullary pressures exceed the arterial pressures (approximately 70 – 80mmHg), then retrograde filling of the arterial vessels would occur.

In our investigation, maximum infusion pressure was set at 450 p.s.i. based on the recommendations of previous researchers. **[30]** There was no difficulty encountered during any of the contrast medium perfusion studies. However, when maximum infusion pressure was set at 100psi using the same angiographic injector during pilot investigations in this study, infusion was not possible. This finding correlates with the author's clinical impression that intra-osseous perfusion performed by hand can be difficult and requires significant hand pressure.

Of interest is the lack of contrast medium and blue latex observed in the cortex and medullary cavity of the bones distal to the MCIII (including the proximal, middle and distal phalanx, as well as the proximal and distal sesamoid bones). The normal intramedullary pressure of the distal sesamoid bone is reported to be 19.13+/- 2.8 mmHg, which is less than in the medial palmar artery 80.25 +/-17.9 mmHg. [49]. Intuitively, if enough pressure was exerted to overcome arterial pressures and escape the medullary cavity of the MCIII via arterial cannels, one might expect this pressure would translate distally via the vessels, and thereby enter the cortex and medullary cavity of more distal bones. Why this did not occur is not clear from the study. It may be that the cortex of the MCIII is a pressure-limiting step. Outside of the medullary cavity, the pressures in the

peripheral vasculature is established only by the volume of the perfusate in this circulation. If this is the case, then higher volumes of perfusate may be required to increase the penetration into enclosed spaces with increasing pressures found in the distal osseous structures such as the proximal sesamoid bones or first and second phalanx.

Intraosseous pressures were not recorded in this study. But based on this information, it is likely that the infusion pressures exceeded the mean arterial pressures in the cadaveric forelimbs, allowing retrograde diffusion of the contrast medium out of the medullary cavity via arterial channels. Because venous pressures are considerably less, diffusion into these vessels likely occurs simultaneously. Indeed, both veins and arteries filled simultaneously during IORP. This is demonstrated by the proximal to distal distribution of contrast medium distal to the tourniquet in both veins and arteries.

6.3 Transcortical Migration of Medium

With the technique used in this study, it was impossible to observe the transcortical migration of the contrast medium. However, CTA images taken after 6 minutes of perfusion demonstrated transcortical channel opacification (Figure 9), as well as massive periosteal uptake of contrast medium throughout the MCIII distal to the tourniquet. The latex-perfused, dissected specimens cut in the transverse and sagittal plane also demonstrated intracortical latex filling (Figure 8,10). Based on this observation, it is reasonable to speculate that a substance exits the medullary cavity via transcortical vessels and enters both venous and arterial periosteal vessels. From there, a

substance will continue to distribute down a pressure gradient to the larger vessels that communicate with the periosteum – both arteries and veins.

An alternative explanation is that venous vessels fill with contrast medium first, and then diffuse to distal capillary beds. The contrast medium then traverses the capillary beds to the terminal arterioles and subsequently fills the larger arterial vessels in a retrograde fashion. This explanation is unlikely because during the serial radiograph study, contrast medium was immediately present in both veins and arteries at the distal aspect of the metacarpus. Contrast then diffused distally in both arterial and venous networks simultaneously. If this explanation were true, one would expect to see contrast medium in venous vessels first, distribute distally, then to distal arteries and distribute proximally.

According to Rhinelander, [37] the transcortical vessels of adult long bones act as an analagous capillary bed. Afferent (arterial) blood enters the medullary cavity via the nutrient artery and metaphyseal artery. Efferent (venous) blood exits via the periosteum over all surfaces of the long bone. The afferent and efferent vascular systems are connected through the cortex by the intermediate vascular system, the transcortical vessels. This includes channels such as Volkmann's canals and Haversian canals. Within these channels are capillary-sized vessels. These vessels are not true capillaries, however. They are non-expansile, and do not provide nutrients to surrounding tissues. Instead, small cannaliculi radiate from these channels and communicate with the lacunae, providing nutrients to the osteocytes. Flow in these transcortical channels is not

direction-specific, and in cases of disease, blood flow has been observed in a centripetal direction (outer cortex to medullary cavity). Normally, blood flows in a centrifugal direction, from the medullary cavity to the capillaries of the periosteum. Yet at facial attachments, and along the palmar surface of the long bone, the periosteum normally supplies arterial blood to the outer 1/3 of the cortex. These periosteal arterioles anastomose with the medullary arterioles in the transcortical channels.

It is the author's belief that during IORP, contrast medium travels from the medullary cavity to the periosteal vessels via these transcortical vessels: both venous, as in normal blood flow, as well as arterial. The arterial diffusion occurs at the level of periosteal anastomosis with the medullary arterioles within transcortical channels. Injection of a substance into the medullary cavity will increase the intramedullary pressure, above that of the peripheral arterial pressure. At this point, the substance enters both arterial and venous transcortical pathways, and diffuse toward their accompanying venous and arterial peripheral networks. The presence of the tourniquet ensures the retention of this substance as it collects in the peripheral vasculature. With increasing volume, and since the substance cannot exit via normal venous drainage because of the tourniquet, a new pressure gradient develops. This pressure gradient ecnourages flow of the substance distally, away from the tourniquet, to less-filled vessels of the distal limb.

6.4 Contrast Medium and Latex Perfusion

It is important to note that other researchers have determined that IORP establishes high concentrations of antibiotics in these distal bones. [31] These researchers used less volume compared to that used in the present study - 0.1 ml/kg versus the 0.15 ml/kg used in the present study. Thus it is likely that the design of this study does not fully appreciate the distribution of a medication during IORP. Latex and iodinated contrast medium are two dissimilar substances that have different diffusion patterns compared with antibiotics diluted in physiologic solution.

The latex is a commercial, radio-opaque product, intended for use in microvascular perfusion studies. [50] In liquid form, the latex has a viscosity of 25 centipoise. No latex was observed into the surrounding soft tissues. According to visual assessment, it remained confined to the intravascular spaces. Because the latex does not extravasate, the exact pattern of diffusion of the latex product may not be identical to that of the contrast medium. However, the latex product was felt to be a suitable choice for the dissected specimens in this study, because before set-up, it is a liquid that distributes throughout the vessels to the level of capillaries. Once it sets up, it forms a solid, highly visible contrast medium that is easily identified during dissection. It was decided that for the purpose of identifying the vessels perfused during IORP, the two substances would be comparable. The degree of radio-opacity provided by this product was not adequate for the angiography portions of the present study. Therefore contrast medium was chosen instead for the radiographic and computed tomography studies.

The iodinated contrast medium used in this study has a viscosity of 7 centipoise at 25ºC. The iodinated contrast medium diffuses rapidly from the vascular to the extra-vascular space in non-neural tissue. [51] Further studies are necessary to determine what affect the viscosity and molecular size of medications used in IORP has on their distribution to local tissues.

6.5 Effect of Tourniquet

The influence of the tourniquet during IORP has been clearly demonstrated by previous investigators. [11] During IORP and IVRP of the distal portion of the forelimb of horses using ^{99m} Tc pertechnate, it was found that a tourniquet is essential to prevent rapid systemic redistribution of the radionuclide. Placement of a tourniquet for 30 minutes was essential to maintain high radionuclide counts in the distal limb tissues, even after tourniquet removal. These authors concluded that ^{99m}Tc pertechnate diffused into the extracellular space during the 30 minute perfusion. These conclusions are consistent with the results of the present study, where soft tissue opacification was observed in the later stages of perfusion. Maximum soft tissue opacification occurred in most specimens at 30 minutes. No radiographs were taken beyond 30 minutes. So further studies are necessary to determine what effect longer durations of tourniquet application have on this distribution pattern.

Time for diffusion to occur was also evaluated in this study. At six minutes, nearly all vessels distal to the tourniquet were opacified. Gradually, this contrast medium

exited the vessels and distributed to the surrounding soft tissues. In this study, maximum soft tissue opacification by contrast medium occurred at 30 minutes.

It was unexpected that contrast medium would drain from the transected surface of each specimen. No leakage was observed through vessels underneath the tourniquet. However, the dissected specimens clearly demonstrated that contrast medium travels proximal to the tourniquet via the medullary cavity, then exits via transcortical vessels near the proximal MCIII. During pilot experiments for this study, variations in tourniquet pressures (450mmHg – 600mmHg), and type of tourniquet (Esmarch versus pneumatic tourniquet), had no appreciable influence on the amount of this leakage. A pneumatic tourniquet was used in the present study in order to minimize variability between specimens. The tourniquet was applied in the same manner by the same investigator (GJK), and inflated to the same pressure in each specimen.

6.6 Relevant Contributing Literature

In the present study, only grossly and radiographically visible vessels were identified on dissected specimens, SR images and CTA images. Previous investigators have determined that, following IORP, bone, synovial fluid and synovial membrane concentrations of aminoglycosides distal to the tourniquet far exceeded recommended MIC's for susceptible bacteria commonly implicated in equine orthopedic infections. [9, 30, 31] Whitehair et al [30] used histological methods to identify India ink within the venous system of the joint capsule, extensor carpi radialis tendon and venules of the

synovial villi of the equine carpus following IORP of the proximal metacarpus. Using a similar technique, but perfusing with iodinated contrast medium, these same investigators also demonstrated that contrast medium was present in the carpal bones following IORP of the carpus. These perfusions were all performed on living horses, which were later euthanized and their tissues analyzed.

A difference may exist in the vascular distribution between cadaver limbs and living subjects. The use of cadaver limbs is a major limitation of the present study. Cadaver limbs were chosen to minimize the variability between specimens, allowing identical serial radiographic films to be taken and later compared. Although the pattern of distribution of contrast medium was consistent amongst specimens, it remains to be seen if this pattern is repeatable in living horses and is as consistent between living individuals. The influence of injury and disease of the distal limb on this distribution pattern is also an important area for future investigation.

Numerous variations in technique of IORP have been reported. [9, 10, 31, 52] [7, 8, 30, 34] Published regional perfusion volumes vary considerably and are based on empirical data only. [2, 6] In order to reduce the amount of variation between individuals, Mattson et al [31] [11] report a perfusate volume based on body weight (0.1ml/kg). This principle was adopted in the present study. A volume of 0.15ml/kg was used in this study. This higher volume was used to approximate the upper level of the reported volume range. [2] Further studies are required to determine what effect volume has on the

distribution of contrast medium following IORP, as well as what volume is optimal for therapeutic effect.

The effect of other variables on IORP distribution pattern have not been scientifically studied. Such variables include the age of the horse, the concentration of medications administered, the rate of delivery, pressure of infusion, the location of the tourniquet placement and the location of intraosseous cannula placement. There was a wide range in ages of horses used in this study. No obvious difference in distribution pattern was observed between the ages of 2 and 25 years of age, but further investigation into this subject is warranted. The concentration of contrast medium and latex used in this study was based on manufacturer's recommendations for vascular perfusion studies. [50, 51] Rate of delivery in this study was set at 10ml/min. This was to approximate clinical conditions used by the author. Maximum infusion pressure was based on previous studies. [30] Location of tourniquet was based on recommendations from previous studies [9, 11, 31] and on the available room between the carpus and the intraosseous cannula.

Location of intraosseous cannula placement was unique in this study. Previous investigators have reported placing the catheter in the dorso-lateral cortex of MCIII, at the junction of the middle and distal 1/3 of this bone. [9, 11, 31] Pilot experiments for the present study raised the concern that in some horses, there may be metaphyseal trabecular bone in this region, impeding the distribution of contrast medium and reducing the ease of infusion. To ensure that the infusion went directly into the medullary cavity, the

catheter was placed in the middle of the MCIII. In this study the intraosseous cannula was placed medial to the common digital extensor tendon, to avoid soft tissue complications with the lateral digital extensor tendon. The dorsal cortex of the MCIII is thicker than in other areas, especially in exercised animals. [53, 54] Mean dorsal cortex thickness of racing thoroughbreds is 14.4 mm. [55] therefore the cannula was placed as dorsal as possible to maximize contact between the bone and cannula, ensuring a secure fit of the intraosseous cannula. It was hoped that this would minimize leakage from the bone-catheter interface. However, leakage occurred in every specimen, and location of catheter placement did not appear to have an effect on the amount of leakage during pilot studies.

Leakage from the intraosseous perfusion site is a commonly described, albeit minor complication during IORP. [9, 10, 30, 31] Poor catheter fit has been attributed to this complication. Cyanoacrylate ester [30] and the use of an intraosseous cannula [11, 31] have been suggested to minimize this leakage. A custom-made intraosseous cannula was used in this study for this reason. However, during pilot experiments for this study, it was determined that the intraosseous cannula did not influence the amount of leakage, especially when compared to using the male adapter of an intravenous extension set. The majority of leakage occurred not from the catheter-bone interface, but from the incised soft tissues around the infusion portal (ie. skin, subcutaneous tissue and periosteum). It is the author's opinion that the use of a custom-made stainless steel intraosseous cannula is not necessary in clinical cases.

It remains to be determined why arterial perfusion was observed during IORP in this study and not in others. [10, 30] As mentioned, it is likely that the tourniquet plays an important role in establishing the pressure differential that facilitates arterial perfusion in the IORP described in the present study. It may be that different locations of tourniquet placement create different perfusion patterns. For example, in this study, the tourniquet was placed over the location of the nutrient foramen. Latex was observed in the venous vessels proximal to this tourniquet, but not in the arteries. This pattern may be different if the tourniquet was located distal or proximal to the nutrient foramen. Furthermore, the aforementioned studies performed IORP on the equine tarsus and carpus, using intraosseous portals in the proximal third metacarpus, proximal metatarsus, and distal tibia. Further studies are necessary to determine the influence of anatomic location and tourniquet placement on the vascular distribution patterns during IORP.

6.7 Our Contribution to Literature

Based on the information in the present study, IORP can be advocated in the treatment of septic arthritis and osteomyelitis of the equine distal limb. This study demonstrates that a substance infused into the medullary cavity of the MCIII will distribute rapidly throughout the arteries and veins of the limb distal to the tourniquet. Because both the venous and arterial vessels are perfused, it is likely that the capillary beds of the tissues of the distal limb will be similarly perfused. This includes the soft tissues surrounding the bones and joints of the limb, the laminar corium and solar tissue. The work of previous investigators indicates that this perfusion will also access the bones

and synovial fluid of these distal limb structures. The vascular perfusion is rapid, but it is important to leave the tourniquet in place for at least 30 minutes to allow adequate distribution of the substance from the vascular space to the local tissues.

Drawbacks to this therapy have not been thoroughly investigated. Other than mild objection by the standing patient to the procedure and leakage from the cannula site,[9] no significant clinical complications have been reported following IORP. The effect of a unicortical defect on the integrity of the MCIII should be recognized. The presence of a fresh-drilled hole on the tension side of a bone will reduce the breaking strength of that bone by 30 +/- 10% during single cycle to failure testing. [56] Therefore it must be recommended that training not be resumed at least until radiographic resolution of the defect in the MCIII following IORP.

Intraosseous regional perfusion may have applications other than antimicrobial therapy. Intravenous regional perfusion with contrast medium has been described for the diagnosis, prognosis determination, and treatment of laminitis in the horse. [38] In this application, it is recommended to take several radiographic projections within 30-45 seconds of perfusion. Based on the author's personal experience, this can be a challenging task. Because the intraosseous perfusion of digital vessels appears to take 6 minutes and establishes a similar vascular pattern, it may be a favorable alternative - especially when preservation of the vascular supply to the digit is of paramount importance during laminitis. [38]

7.0 Conclusion

In conclusion, IORP using contrast medium in the distal limb of horses produces a consistent and repeatable pattern of distribution. This distribution is in a centrifugal direction from the medullary cavity to peripheral vessels distal to the tourniquet, including veins and arteries. Although studies using living horses and clinical cases are necessary to further validate this technique, the results of this study support the use of IORP to deliver medications to the tissues of the distal portion of the forelimb in horses.

[a]Heparin sodium, 10,000 USP units/ml, Elkin-Sinn Inc, Cherry Hill, NJ.

[b]Fatal Plus, pentobarbital sodium 390mg/ml, Vortech Pharmaceuticals, Dearborn, MI.

[c]Heparin sodium, 1,000 USP units/ml, Baxter Healthcare Corporation, Deerfield, IL.

[d]VBM tourniquet 2500 ELC, Noblesville, IN.

[e]X-Omatic lanex: regular screen cassette, Eastman Kodak Company, Rochester, NY

[f]Kramex DX – 30N-LBC, Saddlebrook NJ.

[g]IP-1 Insight film, Eastman Kodak Compant, Rochester, NY

[h]Angiographic injector, Medrad, Pittsburgh, PA.

[i]Conray 400, iothalamate sodium 66.8% injection U.S.P, Mallinckrodt Inc, St. Louis, MO.

[J]Picker PQ 5000 helical CT scanner, Philips Medical Systems, Bothell, WA.

[k]Microfil MV-120 injection compound, Flow Tech, Inc, Carver, MA

Figure 1. Cadaveric equine forelimb demonstrating correct position to place intraosseous portal: 2cm medial to the common digital extensor tendon, in the dorsal cortex of MCIII.

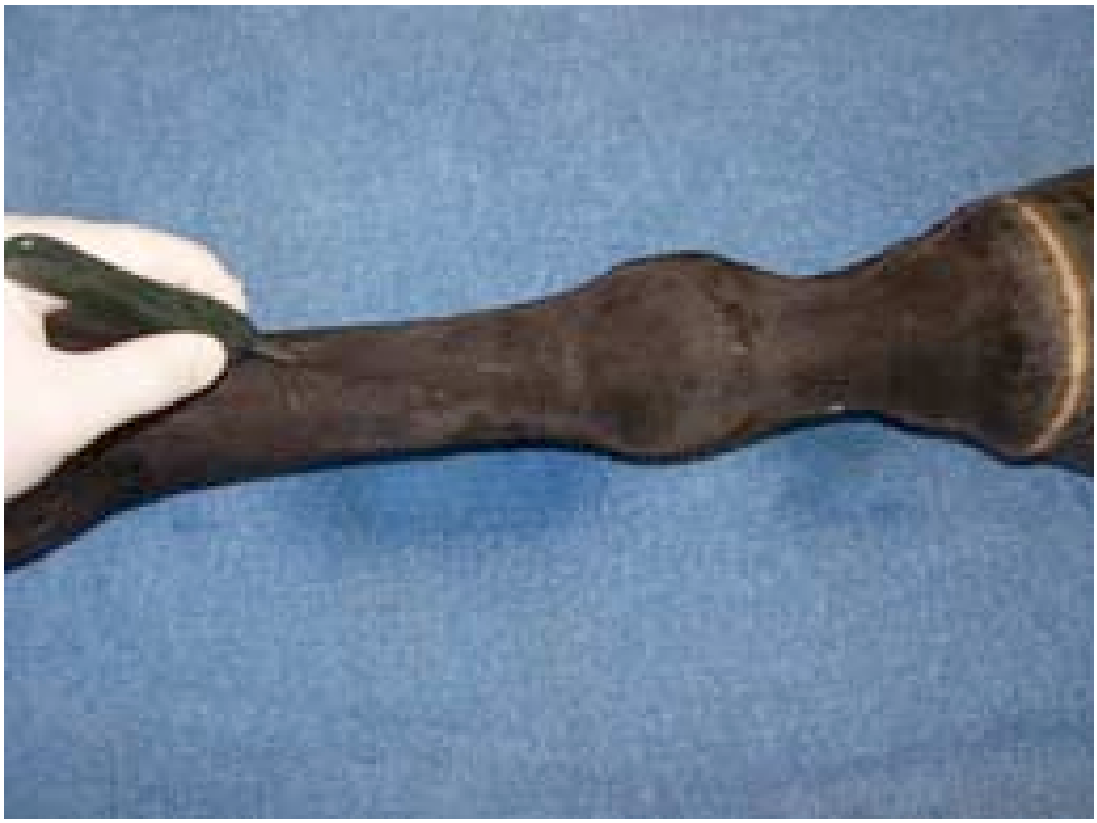


Figure 2. Custom designed 5.5 mm X 24mm cannulated screw with Luer adapter for intraosseous perfusion of the MCIII.

Figure 3. Placement of pneumatic tourniquet: distal to the carpus and proximal to the cannula.

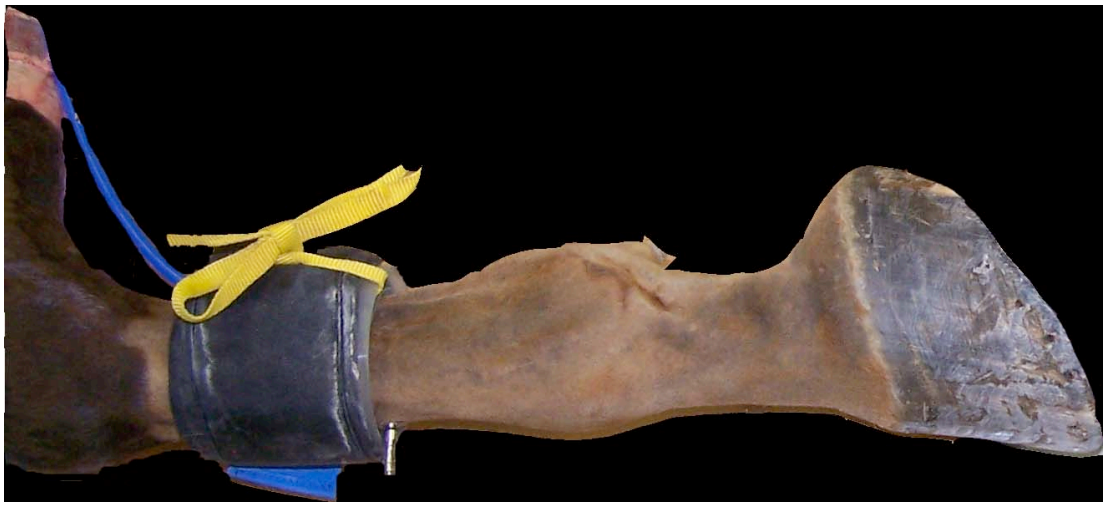


Figure 4. Custom designed table to accommodate a 7 X 17” radiographic cassette. Specimens were secured to the surface of the table in the position depicted.

Figure 5. (i). Equipment for acquiring serial radiographs of perfused specimens, including the specimen (A), radiograph cassette table (B), radiograph stand and tube (C), pneumatic tourniquet (D) and angiographic injector (E). (ii) Close up of pneumatic tourniquet (D) and angiographic injector (E).

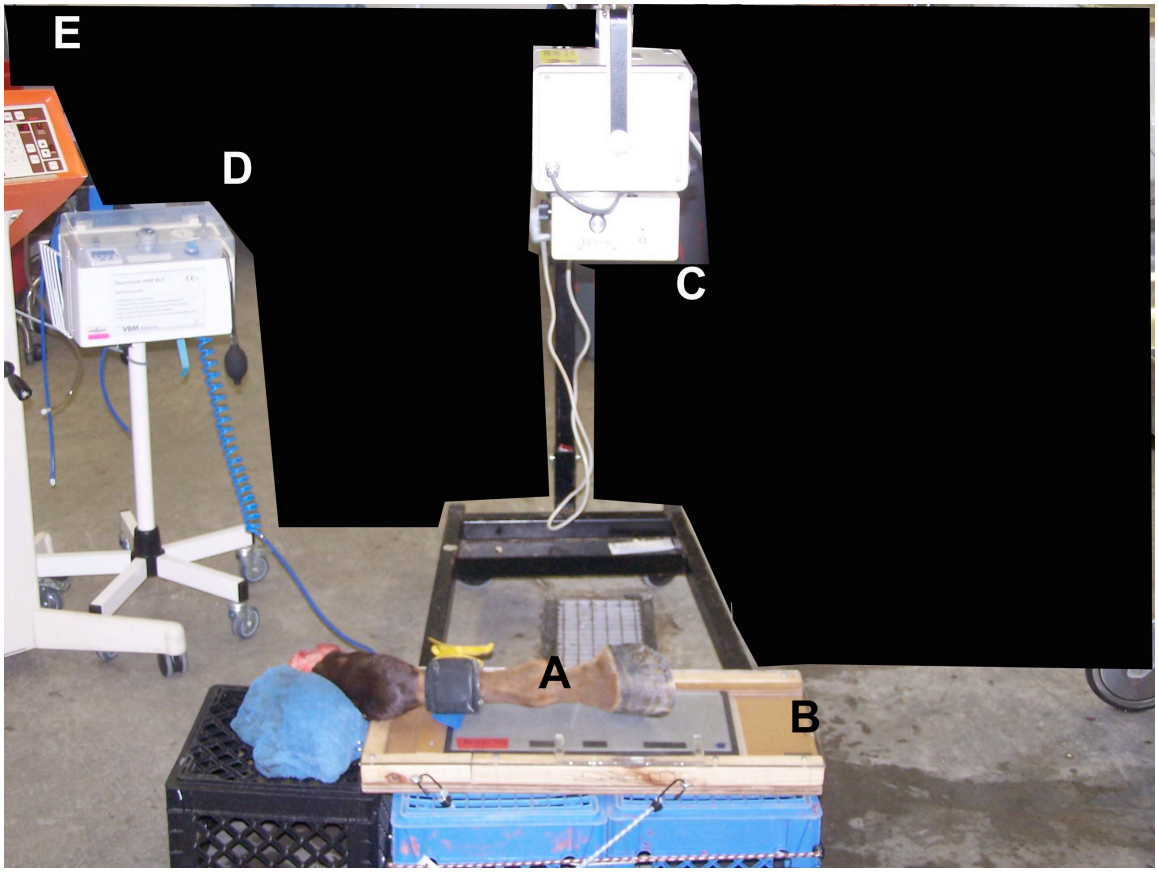
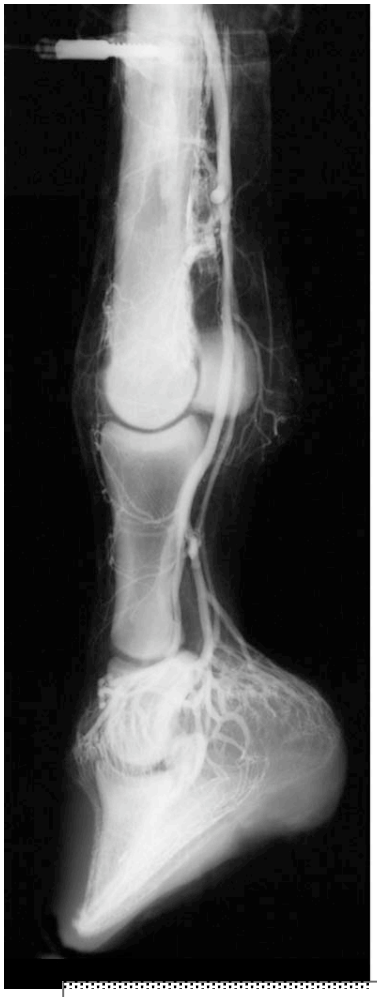
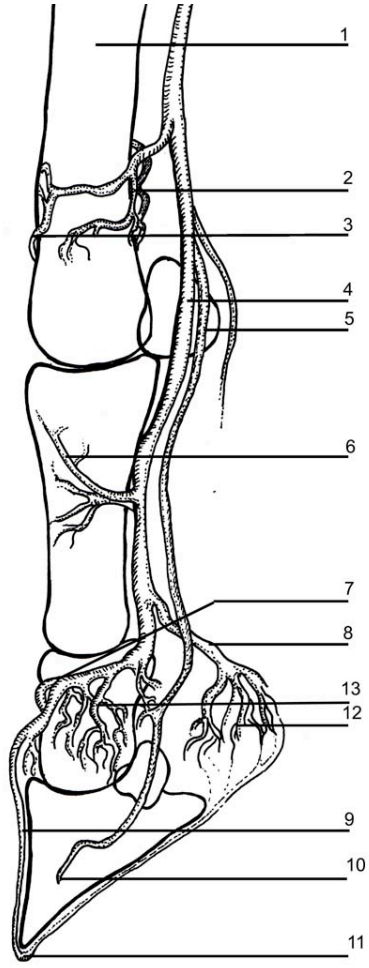


Figure 6. A) Lateromedial radiograph of the distal equine forelimb 6 minutes after initiation of IORP using contrast medium. B) Drawing identifying the vascular distribution pattern of contrast medium opacified in A.





A **B**

LEGEND

1. Medullary cavity of MCIII.
2. Palmar metacarpal vessels
3. Dorsal MCIII periosteal vessels
4. Proper palmar digital veins
5. Proper palmar digital arteries.
6. Dorsal vessels of the proximal phalanx
7. Coronary vein and dorsal branch (vein) of the middle phalanx
8. Bulbar branch artery and vein.
9. Dorsal vascular plexus of laminar corium
10. Terminal arch vessels
11. Solar marginal vessels
12. Bulbar plexus
13. Parietal plexus.

Figure 7. Questionnaire to identify presence or absence of contrast medium in the regions of interest depicted in Figure 6. One questionnaire was completed for the series of radiographs taken for each specimen.

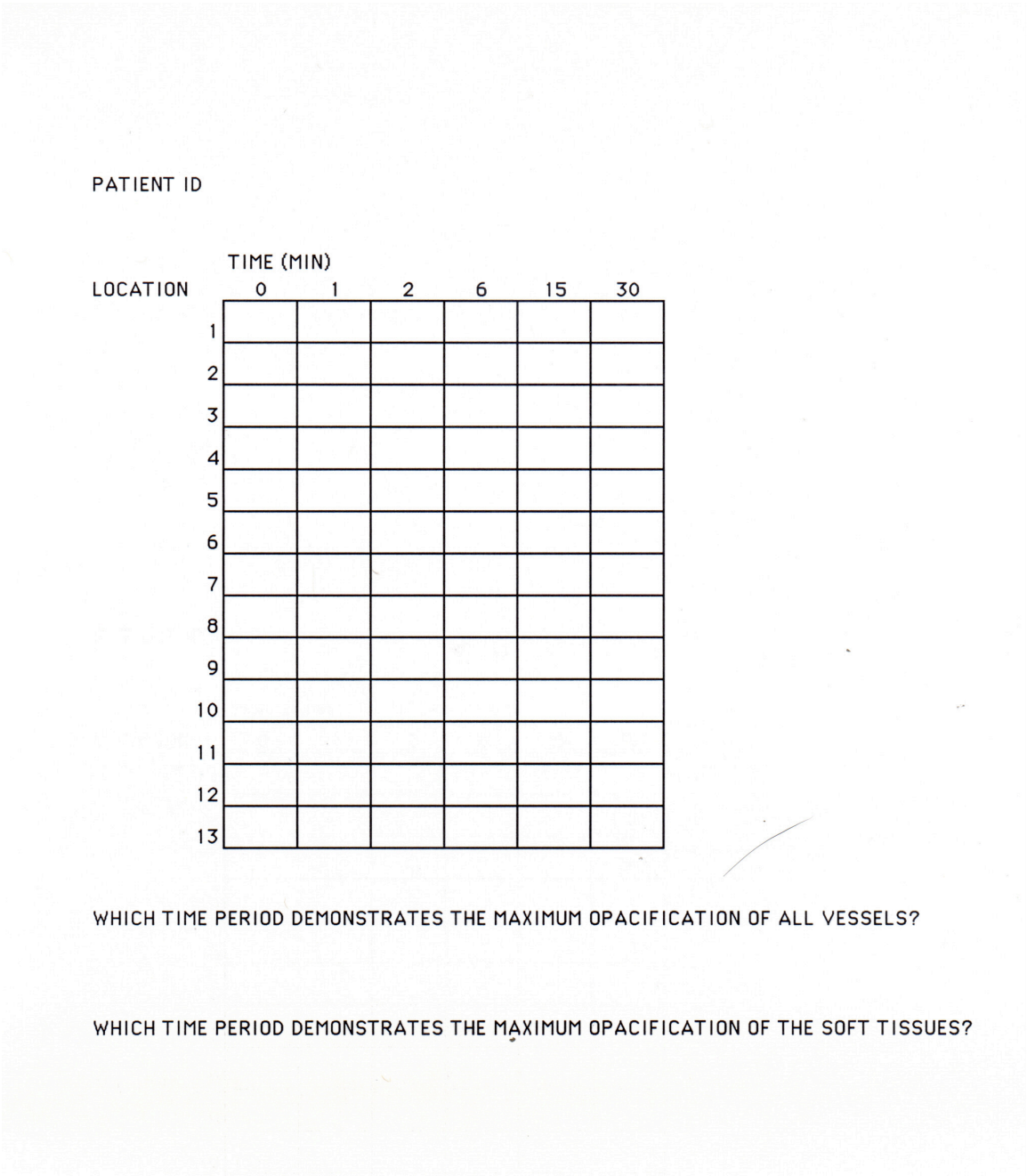
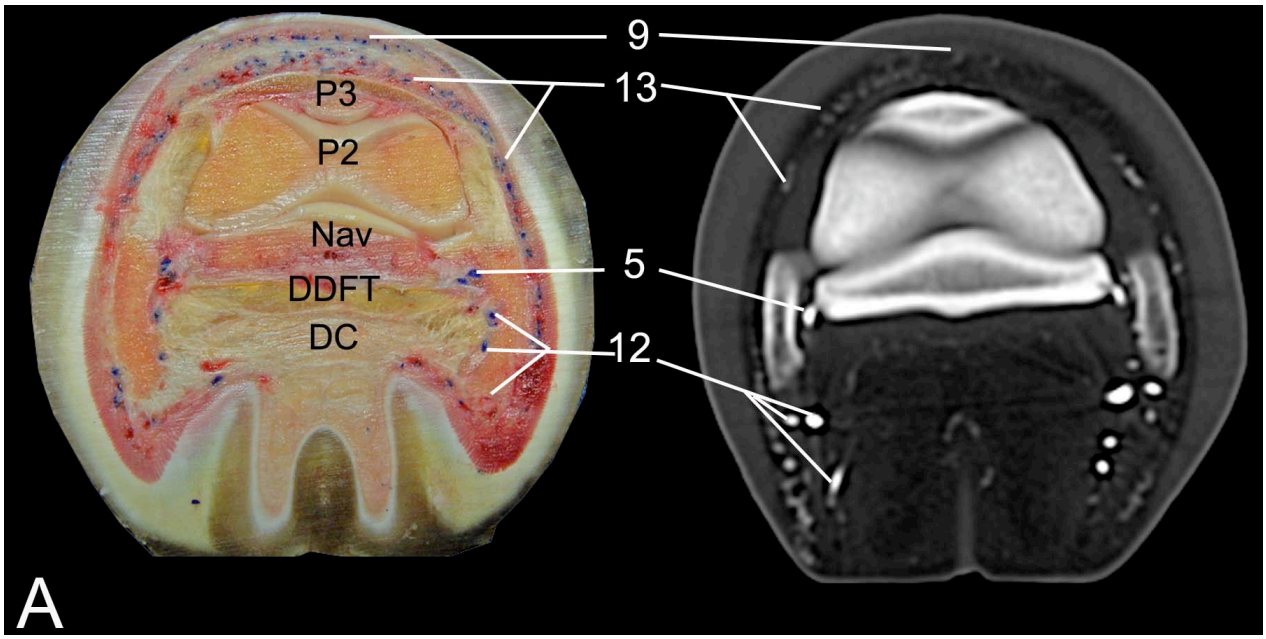
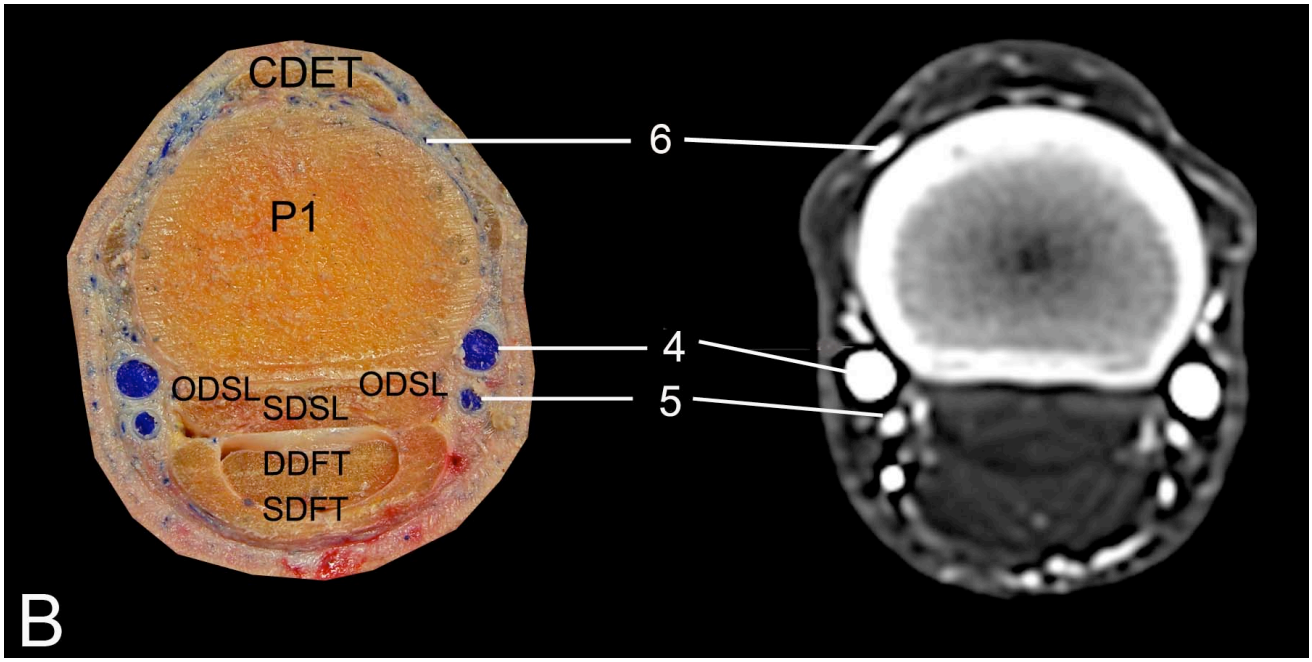


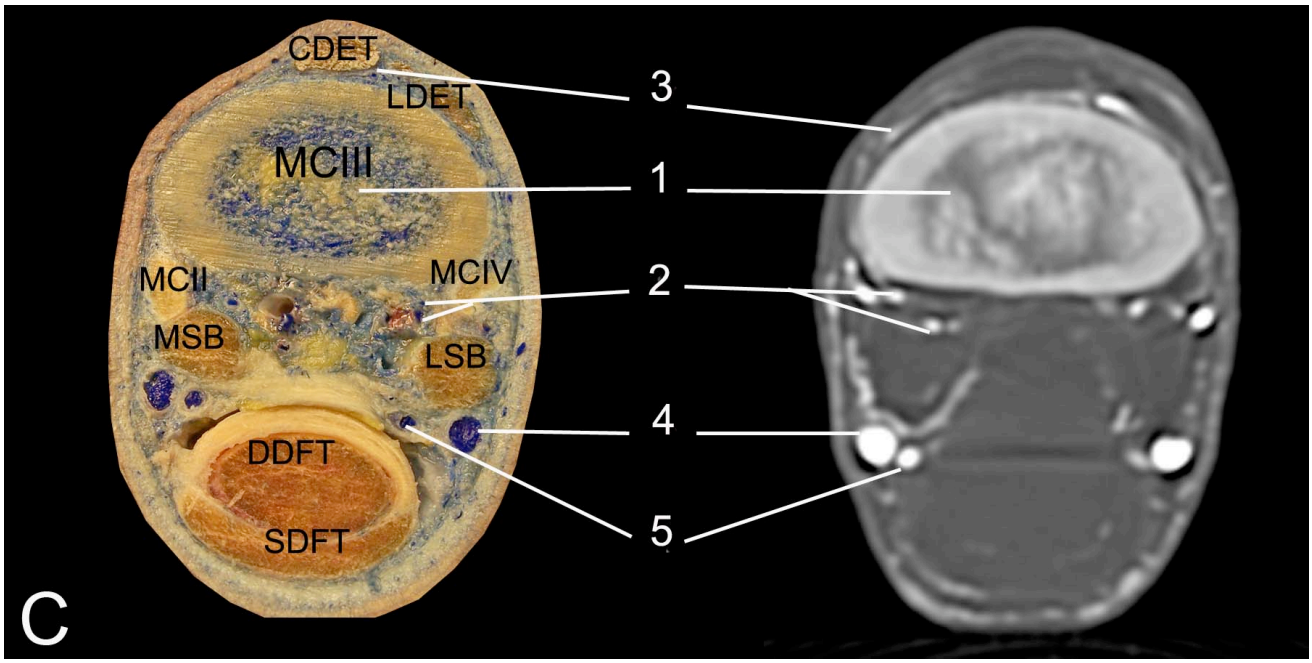
Figure 8 (A,B,C). Latex-perfused specimens transected in transverse plane and paired with corresponding CTA image at three locations: (A) distal interphalangeal joint; (B) middle of the proximal phalanx; (C) distal MCIII.



LEGEND

1. Medullary cavity of MCIII	**P3 = distal phalanx**
2. Palmar metacarpal vessels	**P2 = middle phalanx**
3. Dorsal MCIII periosteal vessels	**Nav = navicular bone**
4. Proper palmar digital veins	**DDFT = deep digital flexor tendon**
5. Proper palmar digital arteries	**DC = digital cushion**
6. Dorsal vessels of the proximal phalanx	**CDET = common digital extensor tendon**
7. Coronary vein and dorsal branch of P2	**LDET = lateral digital extensor tendon**
8. Bulbar branch artery and vein	**P1 = proximal phalanx**
9. Dorsal vascular plexus of laminar corium	**ODSL = oblique distal sesamoidean lig.**
10. Terminal arch vessels	**SDSL = straight distal sesamoidean lig.**
11. Solar marginal vessels	**SDFT = superficial digital flexor tendon**
12. Bulbar plexus	**MSB = medial branch of suspensory lig.**
13. Parietal plexus	**LSB = lateral branch of suspensory lig.**





Legend

MCIII = 3rd metacarpus **MCII = 2nd metacarpus** **MCIV = 4th metacarpus**

Figure 9. Transverse CTA image of the proximal metacarpus at the level of the intraosseous cannula. A) Specimen prior to perfusion. B) Specimen following IORP through the intraosseous cannula using iodinated contrast medium. Note the presence of contrast medium in the medullary cavity and periosteal area. Note the radiating bands of contrast medium throughout the cortex.

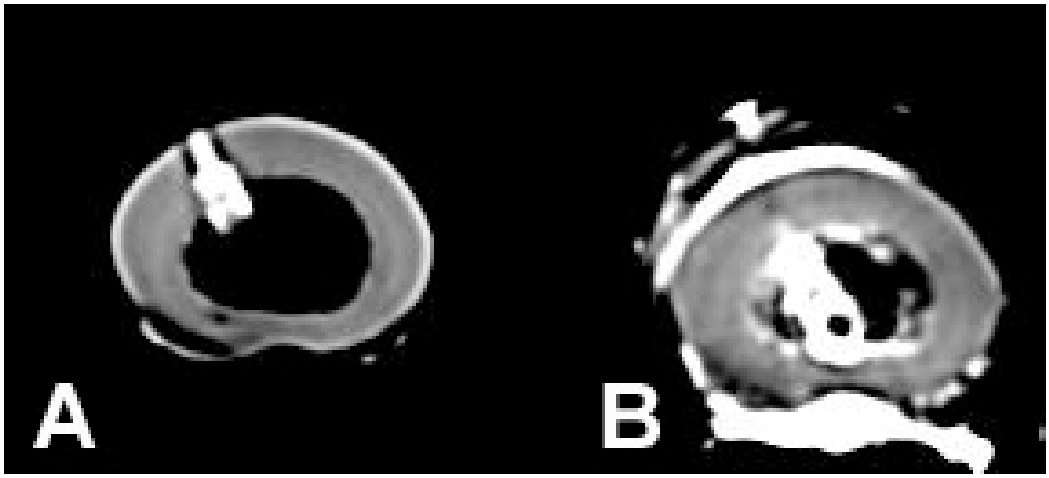


Figure 10. Sagittal section of MCIII and distal row of carpal bones from a latex-perfused and dissected specimen. White arrows identify proximal and distal extent of the tourniquet. Black arrow identifies the location of intraosseous cannula. An asterisk identifies the distal row of carpal bones.

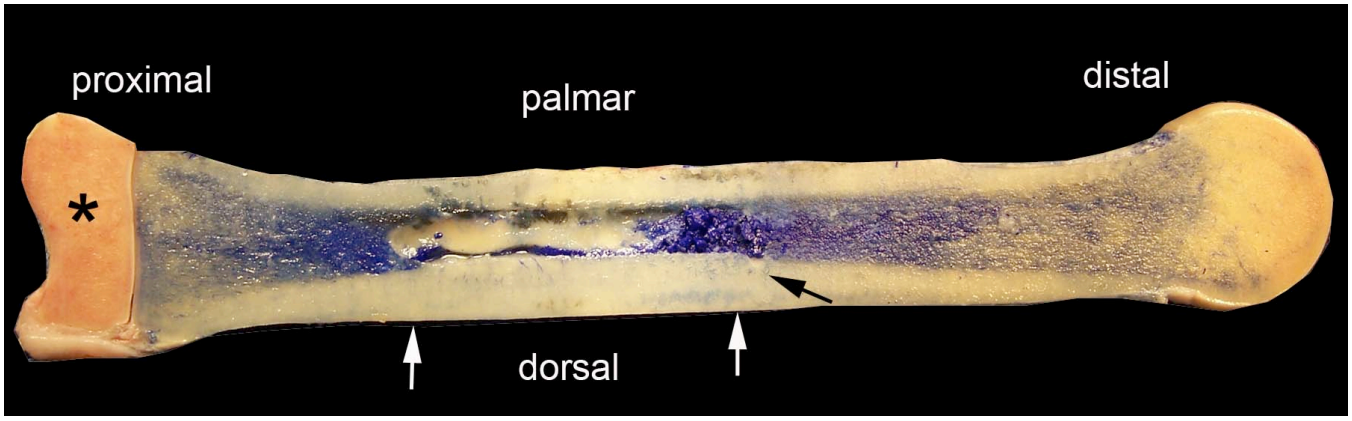
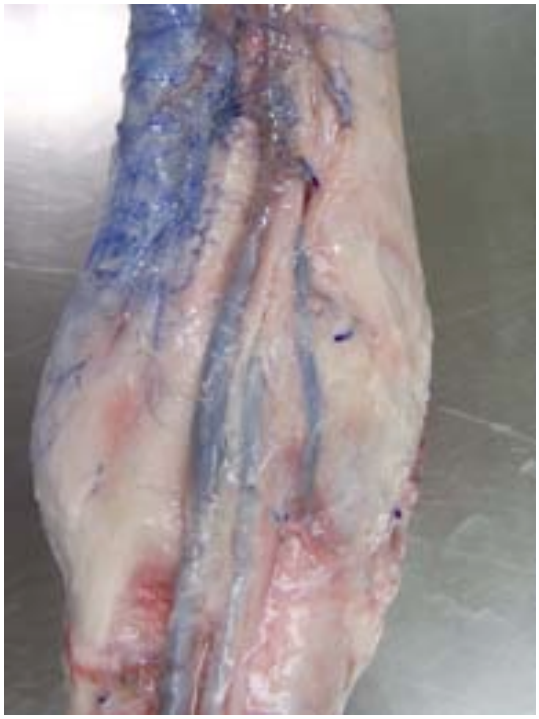


Figure 11. Dissection photographs of the latex-perfused specimen: lateral view of the metacarpophalangeal joint, proximal is to the top of the figure, dorsal is to the left of the figure. Note the uptake of blue latex in both the common palmar vein and artery, as well as the periosteal vessels of the dorsal MCIII. Palmar artery (PA), Palmar vein (PV).



PV PA

Figure 12. Dissection photographs of the foot of the latex-perfused specimen following removal of the hoof. (A) Lateral view of the hoof demonstrating the latex uptake in the coronary plexus and bulbar vessels. (B) Solar view of the same specimen. Note the extensive uptake of latex in the vessels of the frog.

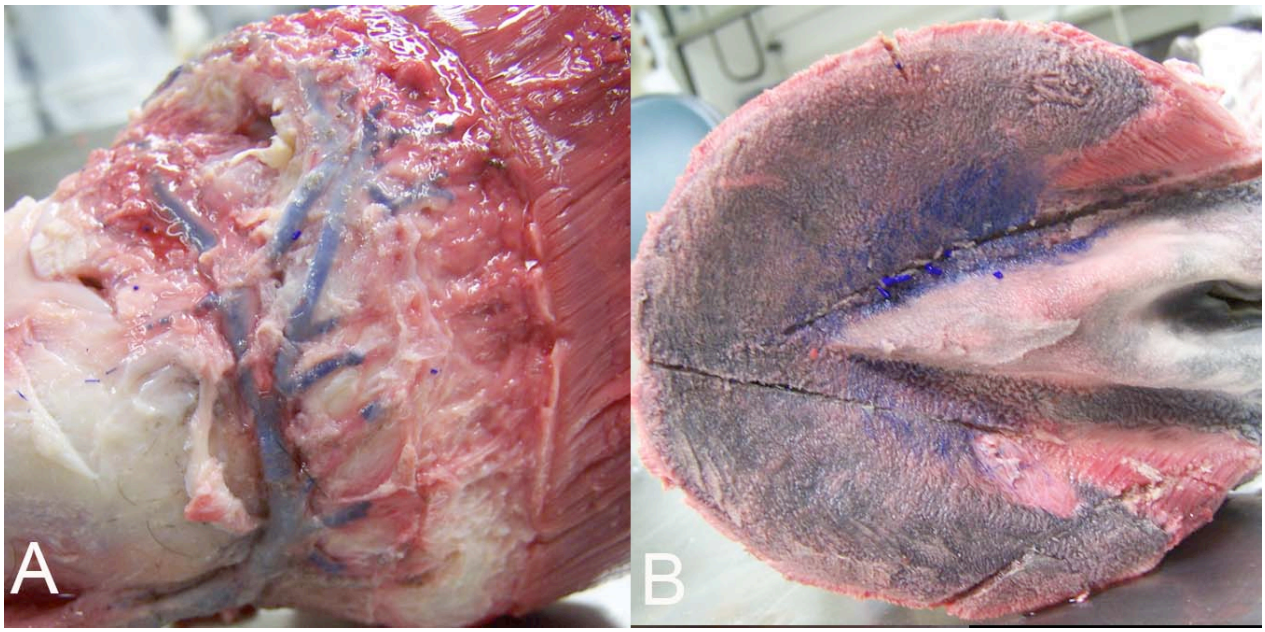


Figure 13. A) Dissection photograph of the latex-perfused vessels of the deep digital flexor tendon at the level of the fetlock joint along the palmar aspect of the distal limb. The palmar annular ligament has been transected and reflected. The superficial digital flexor tendon has been excised. B) Distal and palmar articular margin of MCIII. Notice no latex in the articular space, but dramatic uptake of latex in periosteal vessels, palmar metacarpal vessels, and joint capsule of MCIII.

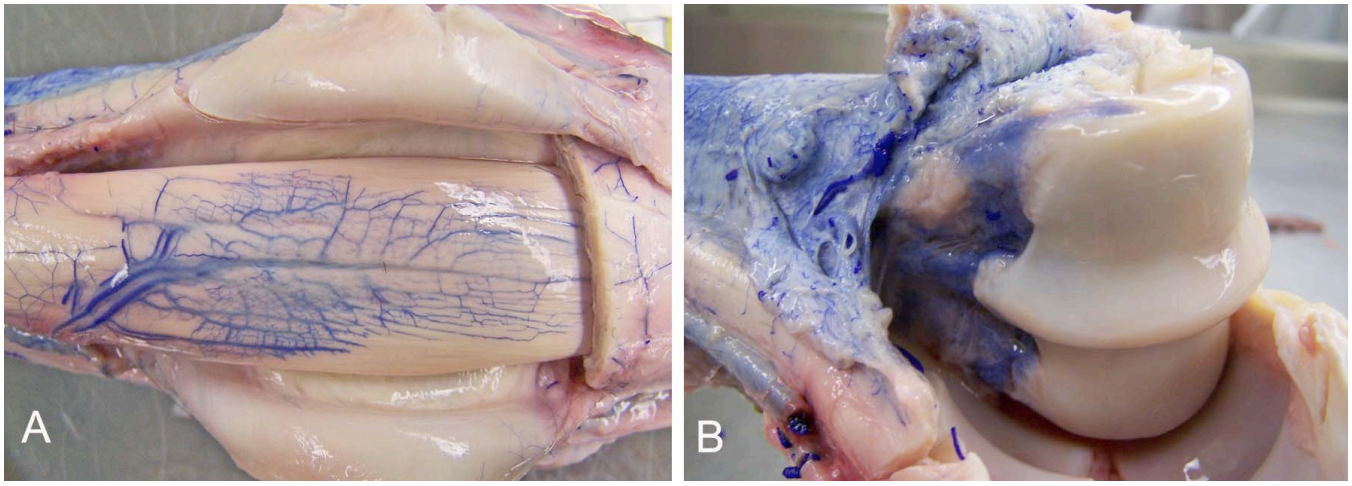


Figure 14. Percentage of ROI's opacified with contrast medium during IORP for time periods 1, 2, and 6 minutes. See text for details.

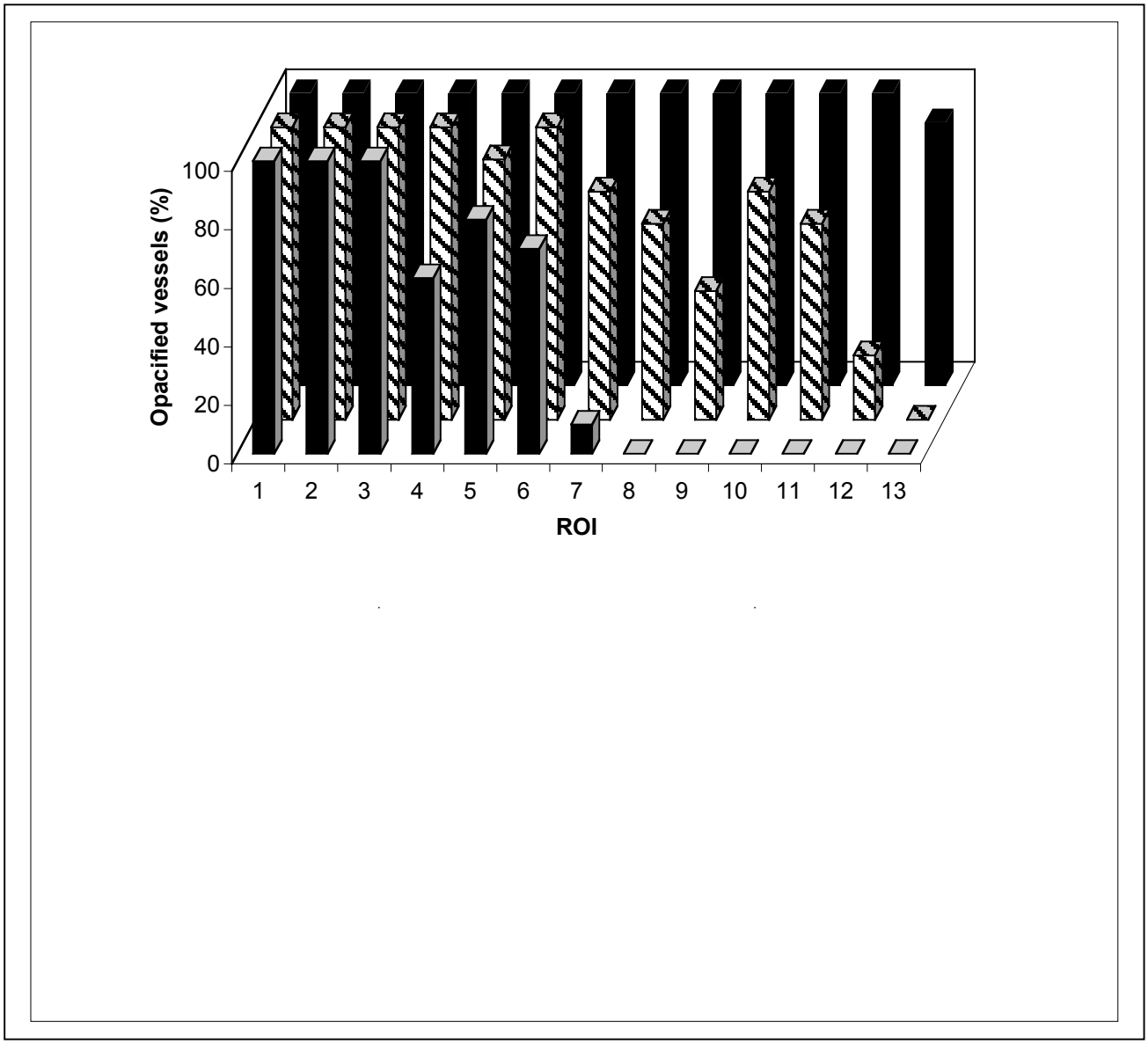


Table 1. Parameters and volumes of perfusate used in each horse.

Horse #	AGE	WEIGHT KG	VOLUME PERFUSATE (MLS)	LIMB	STUDY
1	23	400	60	RIGHT	SR
2	2	400	60	LEFT	SR
3	16	475	71.3	LEFT	SR
4	8	520	78.1	RIGHT	SR
5	22	462	69.3	RIGHT	SR
6	11	540	81	LEFT	SR
7	2	870	59.3	RIGHT	SR
8	11	449	67.3	LEFT	SR
9	8	529	80	LEFT	SR
10	25	641	96.2	LEFT	SR
11	7	514	77.1	RIGHT	LATEX
12	20	405	60.7	RIGHT	LATEX
13	20	405	60.7	LEFT	CTA

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