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Surgical induction, histological evaluation, and MRI identification of cartilage necrosis in the distal femur in goats to model early lesions of osteochondrosis

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Abstract

Objective—Identify and interrupt the vascular supply to portions of the distal femoral articularepiphyseal cartilage complex (AECC) in goat kids to induce cartilage necrosis, characteristic of early lesions of osteochondrosis; then utilize MRI to identify necrotic areas of cartilage.

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Author contributions

All authors contributed significantly to the article formulation. FT drafted the primary manuscript with input from CSC. All authors were involved in critical revision of the manuscript and approved the final version to be published. Surgical procedures and histological evaluation were performed by FT and CSC. MRI was performed and interpreted by MJN, LW, and JME. Responsibility for the integrity of this work as a whole is assumed by Tóth (ftoth@umn.edu), and Carlson (carls099@umn.edu).

Conflict of interest

There are no conflicting interests to report.

Design—Distal femora were perfused and cleared in goat kids of various ages to visualize the vascular supply to the distal femoral AECC. Vessels located on the axial aspect of the medial femoral condyle and on the abaxial side of the lateral trochlear ridge were transected in eight 4-day-old goats to induce cartilage necrosis. Goats were euthanized 1, 2, 3, 4, 5, 6, 9, and 10 weeks post operatively and operated stifles were harvested. Adiabatic T1 ρ relaxation time maps of the harvested distal femora were generated using a 9.4T MR scanner, after which samples were evaluated histologically.

Results—Interruption of the vascular supply to the medial femoral condyle caused lesions of cartilage necrosis in 6/8 goat kids that were demonstrated histologically. Adiabatic T1 ρ relaxation time mapping identified these areas of cartilage necrosis in 5/6 cases. No significant findings were detected after transection of perichondrial vessels supplying the lateral trochlear ridge.

Conclusions—Cartilage necrosis, characteristic of early osteochondrosis, can be induced by interrupting the vascular supply to the distal femoral AECC in goat kids. The ability of high field MRI to identify these areas of cartilage necrosis in the AECC using the adiabatic T1 ρ sequence suggests that this technique may be useful in the future for the early diagnosis of osteochondrosis.

Keywords

cartilage necrosis; adiabatic T1 ρ ; MRI; osteochondrosis; goat

Introduction

Osteochondrosis (OC), or osteochondritis dissecans as it is often referred to in human medicine, is a developmental orthopedic disease that affects juvenile humans[1] and animals[2, 3]. In the veterinary medical field OC is defined as a focal failure of enchondral ossification[3], whereas in human medicine it is often considered an acquired, idiopathic lesion of the subchondral bone resulting in delamination and sequestration with or without articular cartilage involvement and instability[1]. In spite of differences in disease definition, a recent review focusing on the clinical aspects of the disease presents compelling evidence that OC affecting animals and humans is the same disease[4]. Predilection sites are often shared among species, e.g., human OC most frequently affects the femoral condyles, which are also commonly affected sites in pigs[5].

The etiology and pathogenesis of OC affecting humans are poorly understood; genetic factors, ischemic events, (repetitive micro) trauma, and ossification abnormalities all are considerations[1, 6, 7]. These uncertainties likely arise from the fact that OC in humans is rarely recognized before clinical signs become apparent. Thus, inferences regarding the etiology and pathogenesis of OC in humans are largely based on histological evaluation of osteochondral fragments removed from symptomatic joints[8]. Although these fragments accurately represent the end stage disease, they provide little information regarding its pathogenesis. This limited understanding of the pathogenesis of OC makes treatment selection and disease prognostication in humans challenging[6].

In children with open physes, stable OC lesions are known to heal in 50% of the cases by simply altering activity levels[9]. However, predicting which lesions will heal and which

will progress and require surgical treatment is difficult, and the disease is rarely detected before the onset of clinical signs. The ability of conservative treatment to succeed, if applied early during the course of the disease, provides a strong argument for development of diagnostic imaging methods capable of identifying these lesions during the early stages of their development. Additionally, novel imaging techniques capable of identifying and monitoring the development of early OC lesions will likely yield insight into the disease pathogenesis.

In contrast to humans, the pathogenesis of OC affecting animals has been described in detail[3]. Histological studies using samples obtained from predilection sites of OC in young, asymptomatic animals have identified changes characteristic of specific stages of the disease. First, ischemic necrosis of the epiphyseal cartilage subjacent to the articular cartilage (OC latens); followed by the associated delay in the progression of the ossification front (OC manifesta) after it reaches the area of necrotic cartilage. Finally, cleft formation through the area of necrosis (OC dissecans)[10, 11]. The presence of necrotic vascular channels located within or adjacent to the areas of necrosis provides strong evidence that vascular failure plays a central role in the development of cartilage necrosis, a hallmark of subclinical disease. However, the upstream events triggering ischemia in the epiphyseal cartilage in naturally occurring disease are yet to be identified. Recent work performed in horses[12] and pigs[13] demonstrated that surgical interruption of vascular supply to a portion of the articular-epiphyseal cartilage complex (AECC, immature joint cartilage at the ends of growing long bones) results in development of lesions identical to those present in naturally occurring OC. This ability to surgically induce OC-like lesions in animals suggests that development of a clinically relevant animal model of the disease is feasible. However, the high incidence of naturally occurring disease, costs associated with their housing, and their large size are challenges in using horses and pigs as animal models. Goats have the potential to be an ideal model for surgically-induced OC because of their low cost, long life span, absence of naturally occurring disease, and relatively small size that allows evaluation of lesions by *in vivo* magnetic resonance imaging (MRI).

Magnetic resonance imaging has the potential to play a central role in the early diagnosis of OC by allowing noninvasive, *in vivo* investigations. Our recent studies demonstrate that the vasculature of developing joints in pigs can be visualized using susceptibility weighted imaging [14, 15]. Thus, this technique might be utilized to image failure of vascular supply associated with OC. Relaxometry, a quantitative parametric MRI technique, is another method increasingly applied in the evaluation of cartilage[16, 17]. T1 ρ and adiabatic T1 ρ , two of these quantitative parameters, have received attention lately due to their sensitivity to changes in cartilage proteoglycan content[18-23]. Results from these studies suggest that cartilage necrosis and the associated decrease in proteoglycan content, characteristic features of early OC, might be utilized for early diagnosis of this disease using MRI.

In the present study, goat kids were used to study surgical induction of OC-like lesions. The objectives of the study reported here were to: 1) assess the vascular supply to the AECC of the distal femur in goat kids to identify vessels for surgical interruption, 2) induce cartilage necrosis in the AECC of the distal femur by surgically interrupting the blood supply to a specific area, and 3) image early changes within the AECC after interruption of the vascular

supply using high field MRI and validate these MRI findings by histological examination of the affected site.

Materials and methods

The study was conducted in two stages, which were approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC # 1207A18021).

First stage – vascular perfusion and clearing

Seven male dairy goat kids aged 4, 7, 14, 21, 28, 35, and 42 days were used to assess the vascular supply of the AECC of the distal femora. The goats were sedated with an intramuscular injection of 0.1 mg/kg xylazine, followed by 500 IU/kg heparin administered intravenously. Immediately after heparin administration the goats were euthanized and then eviscerated via a median laparotomy. The left external iliac artery was identified and catheterized with a red rubber catheter. The limb was then perfused with approximately 150 mL radiographic contrast media (200 g BaSO₄ [Sol-O-Pake] diluted to 1000 mL with 10% neutral buffered formalin). After completion of the perfusion, left distal femora were harvested, then cleared using the modified Spalteholz technique [24]. Briefly, samples were fixed in 10% neutral buffered formalin for 48 h then dehydrated by immersing them in sequentially increasing concentrations of ethyl alcohol (70%, 80%, 90%, 95% then 100%). Dehydrated samples were cleared by placing them into concentrated methyl-salicylate solution. Cleared specimens were visually inspected and then photographed to identify specific vessels as well as the overall vascular architecture supplying the AECC.

Second stage – surgical interruption of vascular supply

During the second stage of the study we sought to surgically interrupt the vascular supply to portions of the distal femur to induce OC-like lesions. Initially, perichondrial vessels were transected in six 14-day-old goats using the techniques described below; however, this did not result in OC-like lesion development identifiable histologically or by MRI over a period of 12 weeks. A review of the results obtained in the first stage of the study revealed that, by the age of 14 days, large numbers of vessels bridged the ossification front in the distal femoral epiphysis, providing an alternative blood supply to the perichondrial vessels that were surgically interrupted. Assuming that failure to induce necrosis in the 14-day-old goats was due to this alternative blood supply, we elected to repeat the experiment using 4- to 5-day-old goats, which had the most extensive perichondrial blood supply with only a limited number of vessels bridging the ossification front.

Eight 4- to 5-day-old male dairy goat kids were selected for surgical intervention. Briefly, goats were anesthetized with a combination of midazolam and ketamine administered intravenously and anesthesia was maintained by inhalation of sevoflurane vaporized in oxygen. The right pelvic limb was prepared for aseptic surgery and the stifle joint was approached via a lateral parapatellar arthrotomy. The patella was luxated medially, the femorotibial joint was brought into complete flexion, and the infrapatellar fat pad was retracted, exposing the intercondylar region of the distal femur cranial to the attachment of the cranial cruciate ligament. After transecting the perichondrial vessel that was consistently

present on the axial aspect of the medial femoral condyle (**MFC**) (figure 1) an additional ~3 x 4 mm (beaver blade, goats euthanized at 1, 2, 4, 6, and 10 weeks post operatively) or 5 x 5 mm (custom designed blade, goats euthanized 3, 5, and 9 weeks post operatively) incision extending into the AECC at the cranio-axial aspect of the MFC was created (figure 1) parallel to the articular surface to interrupt any blood supply to the AECC traversing the ossification front. We also attempted to induce OC-like lesions in the proximal portion of the femoral trochlea by transecting perichondrial vessels that were consistently present on the proximo-abaxial aspect of the lateral trochlear ridge (figure 1). These vessels were transected in two locations 3-4 mm apart. Upon completing the procedure, the joint capsule, subcutaneous tissues, and skin were closed in layers. After assisted recovery, goats were housed together in an approximately 6 x 6 m pen until euthanasia. Goats were euthanized 1, 2, 3, 4, 5, 6, 9, and 10 weeks post operatively and distal femora were harvested and photographed.

MRI

MR imaging of the specimens was conducted on a 9.4 Tesla scanner driven with VnmrJ software (VnmrJ, version 3.2A, Agilent Technologies, Santa Clara, CA, USA), using a quadrature volume transceiver coil (Millipede, Varian NMR Systems, Palo Alto, CA) within six hours of euthanasia. The specimens were placed in flexible latex containers for optimal fitting in the coil and immersed in perfluoropolyether for clean and susceptibility matched background. Three-dimensional gradient recalled echo images were acquired to localize the operated areas within the MFC and the lateral trochlear ridge. Subsequently, adiabatic T1 ρ relaxation time[23, 25] of the specimens was measured in a single axial slice covering the operated area as determined from 3-D gradient recalled echo images. The relaxation time was measured using a magnetization preparation block consisting of 0, 4, 8, 12 and 16 adiabatic full passage pulses from the hyperbolic secant family[18] of 6 ms duration at peak power $\gamma B_{1,max} = 2.5$ kHz coupled to a fast spin echo readout sequence[23, 25]. Slice thickness was 1 mm with imaging matrix of 256 x 256; echo train length was 8; echo spacing was set to minimum (~5 ms) and a repetition time of 5 s was used. The final imaging resolution was dependent on the sample size and was approximately 150 – 165 μ m in the image plane. The adiabatic T1 ρ relaxation time was calculated using monoexponential 2-parameter fit to the measured data in Matlab (MATLAB R2011b, The MathWorks, Natick, MA, USA).

Histology

At the conclusion of the MRI procedures, distal femora from each goat were placed in 10% neutral buffered formalin for 48 hours followed by decalcification using 10% EDTA. Decalcified samples were divided to separate the femoral trochlea from the condyles and then were trimmed into 2 to 3mm thick slabs in the coronal plane, yielding 5 slabs from each condyle and trochlea. Slabs were routinely processed into paraffin blocks and five mm thick sections were obtained from the surface of each slab and stained with hematoxylin and eosin. Blocks containing the surgical incision were thoroughly explored. If necrotic cartilage canals and/or areas of cartilage necrosis were identified in any of the sections, additional sections were obtained in 200-500 μ m intervals adjacent to the original section until the lesion was no longer present. Selected sections containing necrotic cartilage or cartilage canals

were also stained with safranin O and toluidine blue to demonstrate changes in the extracellular matrix. Histological sections corresponding with the MRI slice in which the adiabatic T1 ρ relaxation time was measured were identified and changes in the cartilage maps corresponding with changes noted in histological sections were compared. For both histological and MRI evaluations, corresponding areas of the lateral femoral condyle (LFC) and the medial trochlear ridge within the same stifle joint served as control sites.

Results

During the first stage of the study, perfusion and subsequent clearing of the left pelvic limb in seven goats enabled successful visualization of the vascular supply to the AECC. Perichondrial vessels that were consistently present at the axial aspect of the MFC (figure 1) and the abaxial aspect of the proximolateral femoral trochlea (figure 1) were designated for transection in the next stage of the study. The most abundant perichondrial blood supply, including small branches extending into the distal femoral AECC, was noted in the 4-day-old goat. Although perichondrial blood supply remained apparent in older goats, vessels crossing the ossification front became more prominent as the animals aged.

Surgical procedures to interrupt blood supply to the distal femoral AECC were completed in eight 4- to 5-day-old goats without any complications. After recovery, all animals exhibited non-weightbearing lameness on the operated limb for 48 to 72 hours which was treated with an NSAID (Flunixin meglumine 1.1 mg/kg sc) for 3 days and gradually resolved by the 10th post operative day. The goat that was euthanized 1 week post-operatively developed a seroma on the lateral side on the operated femorotibial joint which persisted until euthanasia. The goat that was euthanized 2 weeks post-operatively developed a hard, fibrous, pea-sized swelling on the lateral side of the femorotibial joint involving the periarticular soft tissues. The remaining six goats exhibited no complications prior to euthanasia at 3, 4, 5, 6, 9, and 10 weeks post-operatively.

Histological and MRI findings

Histological changes (Table 1) in the goat euthanized 1 week post-operatively were restricted to the AECC immediately adjacent and distal to the surgical incision created in the axial aspect of the MFC. In the hematoxylin and eosin stained sections, the surgical incision extending into the AECC was clearly visible and a thin zone of necrotic chondrocytes was present on either side of the incision. Cartilage canals located between the incision and the articular surface appeared necrotic, characterized by loss of endothelium, absence of erythrocytes, and a lumen filled with amorphous eosinophilic material. Conversely, cartilage canals located outside this region lacked these features and appeared to be viable (supplementary figure 1). Due to a mistake during setup, the MRI study missed the area of this lesion; thus, no MRI data were available for this sample. No pathological changes were seen in the corresponding control sections from the LFC.

More extensive histological changes were noted in the goats euthanized 2, 3, 4 and 5 weeks post-operatively (table 1). A portion of the epiphyseal cartilage that was located between the incision and the axial articular surface of the MFC was necrotic in these animals, characterized by shrunken chondrocytes with pyknotic nuclei. Pallor of the extracellular

matrix was also apparent in multiple adjacent sections (both hematoxylin and eosin and special stains). These findings are consistent with OC latens[26]. In the MRI images, a mild (~250 ms) to moderate (~300 ms) focal increase in adiabatic T1 ρ relaxation time was observed in the epiphyseal cartilage corresponding with the area of cartilage necrosis in goats euthanized 2 (figure 2), 3, and 5 (figure 3) weeks post operatively. In the remainder of the MFC adiabatic T1 ρ relaxation time was similar to that observed in the LFC. In the goat euthanized 4 weeks post operatively adiabatic T1 ρ relaxation times of the lateral and medial femoral condyles appeared similar (~150 ms) (supplementary figure 2).

Histological results of the MFC in the goat euthanized 6 weeks post-operatively indicated that the surgical incision in this animal was created too close to the articular surface, entering approximately at the border between the articular and epiphyseal cartilage. Deeper portions of the surgical incision were filled with granulation tissue delaying the advancement of the surrounding ossification front. The superficial portion of the incision persisted with no or minimal signs of healing, and appeared similar to that present in the goat euthanized 1 week post-operatively. In the MRI images of the MFC an irregularity at the cartilage-bone interface (ossification front) was clearly apparent, but adiabatic T1 ρ relaxation times were no different in the AECC between the MFC and LFC (~150 ms) (supplementary figure 3).

Histological findings obtained from the goat euthanized 9 weeks post operatively were consistent with an OC manifesta lesion. Progression of the ossification front in the axial portion of the MFC was delayed by the presence of a necrotic island (5.3 mm²) of epiphyseal cartilage. This island of necrotic epiphyseal cartilage was clearly present in the MRI image as a focal area of markedly (~350ms) increased adiabatic T1 ρ relaxation time adjacent to the ossification front (figure 4). No pathologic changes were noted in the control LFC.

An island composed of necrotic cartilage was identified within the subchondral bone in multiple adjacent sections obtained from the MFC of the goat euthanized 10 weeks postoperatively. The appearance and location of this lesion, apparently bypassed by the ossification front, was consistent with a healing OC manifesta lesion. In the MRI images the epiphyseal cartilage appeared to have nearly identical adiabatic T1 ρ relaxation times in the lateral and medial femoral condyles. In the subchondral bone of the MFC, however, there was a well-defined area with mildly (~250 ms) increased adiabatic T1 ρ relaxation time corresponding with the island of necrotic cartilage seen histologically (figure 5). No pathologic changes were noted in the control LFC.

No significant lesions were noted in any of the sections obtained from the trochlear ridges in any of the goats.

Discussion

In the study reported here, surgical interruption of the vascular supply to a portion of the axial aspect of the MFC resulted in focal necrosis of the epiphyseal cartilage in 6/7 goats operated at the age of 4 to 5 days. In the remaining goat, euthanized 1 week after the

surgery, cartilage canal necrosis, a precursor of cartilage necrosis, was noted. Necrotic islands of epiphyseal cartilage were differentiated from surrounding normal tissues *ex vivo* using adiabatic T1 ρ relaxation time maps generated using a 9.4 T magnet in 5/6 cases.

Vascular perfusion and clearing allowed visualization of the vasculature supplying the distal femoral AECC in goats, enabling identification of perichondrial vessels for surgical interruption. Interestingly, the vessels designated for transection on the abaxial aspect of the lateral trochlear ridge had a nearly identical appearance and location to those which were interrupted in horses in another study[12] in which OC-like lesions of the lateral trochlear ridge were successfully induced. Conversely, interruption of these vessels in goats failed to produce OC-like lesions in any of the operated animals. This difference between species is likely explained by variations in the blood supply to the distal femoral AECC. Indeed, re-evaluation of the perfused specimens obtained from goats ranging from 1 to 6 weeks old demonstrated a large number of small vessels bridging the ossification front, with some of these vessels already present in the 4-day-old goat. These bridging vessels are particularly important, because they provide an alternative blood supply to the AECC, thereby decreasing its dependence on the perichondrial vessels.

This apparent difference in blood supply prompted a minor alteration in the surgical technique employed in the 4- to 5-day-old goats whereby, in addition to interrupting the perichondrial vasculature (the only procedure performed in the 14-day-old goats), a horizontal incision extending into the AECC of the MFC was also created to interrupt the vessels bridging the ossification front. It is likely that this additional step was critical in the successful induction of cartilage necrosis in six of the goats, as none of these animals developed a lesion in the trochlear ridges where only the perichondrial vessels were transected. These findings are different from those obtained in 2-week-old horses[12] and pigs up to 6 weeks old[13], where cartilage necrosis was consistently induced by interruption of the perichondrial blood supply. This species difference in dependence on perichondrial blood supply of the AECC at older ages might provide a partial explanation for the high incidence of OC in pigs[27] and horses[20] and the lack of reports in the literature describing OC in goats.

Our results, along with data obtained from studies using imaging and clearing techniques of the distal femora in pigs[11] and horses[28, 29], indicate that changes in the vascular architecture of the AECC occur at different times in various species. It is possible that goats have already passed a 'threshold' in joint development at the time of birth, and the vascular architecture of their AECC both makes them resistant to naturally-occurring OC and hampers surgical induction of OC-like lesions. This is in contrast to humans, where development of joints is still ongoing a decade after birth, and may explain in part why OC lesions are diagnosed at a more advanced age in humans than in animals[6, 30]. It would be ideal to time the surgical intervention to induce OC-like lesions in any species based on the degree of skeletal development as determined by the degree of closure of the epiphyseal growth cartilage, however this information is currently unavailable. Based on the volume ratio of the epiphyseal cartilage to the secondary ossification center (unpublished data) joints of goats appear to be more developed (increased volume of secondary ossification center

compared to epiphyseal cartilage) than pigs or humans at the time of birth, which might also contribute to their apparent resistance to OC.

Another important finding of the present study is the remarkable ability of extensive lesions affecting the epiphyseal cartilage in goats to heal, demonstrated by the fact that none of the goats developed clinically apparent disease (osteochondrosis dissecans). Indeed, in the goat euthanized 10 weeks post-operatively, the ossification front had completely bypassed the necrotic cartilage, leaving it surrounded by bone. Rapid replacement/bypass of the necrotic portion of the epiphyseal cartilage by bone appears to be an effective strategy in preventing the progression of OC manifesta lesions. Studies of spontaneous disease in swine suggest that areas of necrotic cartilage surrounded by bone gradually decrease in size and eventually disappear [3, 13].

Quantitative mapping of adiabatic T1 ρ relaxation time identified areas of cartilage necrosis within the AECC or the subchondral bone in 5/6 cases. One plausible explanation for missing the lesion in the goat examined 4 weeks post-operatively in the MRI study is the substantial difference in average slice thickness between histology (5 m) and the MRI (1 mm). Due to the thicker slices, MRI may occasionally miss (“average out”) a lesion that is only a couple hundred m in size. Alternatively, a small lesion could also be missed due to operator error upon slice selection for adiabatic T1 ρ imaging, as was demonstrated by our unfortunate failure to identify the correct region for MRI in the goat euthanized 1 week post-operatively. Nevertheless, results obtained from the remaining 5 goats suggest that MRI allows identification of lesions within cartilage. Longitudinal monitoring of the developing OC lesions might also become possible after the above sequences are tested *in vivo* at a clinically applicable field strength.

An important limitation of our study is its inability to assess biological variability due to our results being derived from single observation per time point. Also, it appears that goats receiving an incision measuring exactly 5 x 5 mm (created with the custom designed blade) developed more uniform lesions than those operated using the beaver blade; therefore, future studies would ideally use this larger, more standardized incision.

While initial results are promising, further refinement of the surgical technique presented here, as well as evaluation of additional joints, should be performed before recommending the use of goats as a model of surgically-induced OC. In its current stage the model is best suited to evaluate repair mechanism of ischemic cartilage necrosis rather than studying clinically apparent disease, i.e., OC dissecans. Our findings do suggest an explanation as to why goats appear to be free of naturally occurring OC, and may provide further insight into general disease pathogenesis. The ability of ultra high field MRI using the adiabatic T1 ρ sequence to identify areas of necrotic cartilage within the AECC is an important finding, which is currently being evaluated at clinically relevant field strengths.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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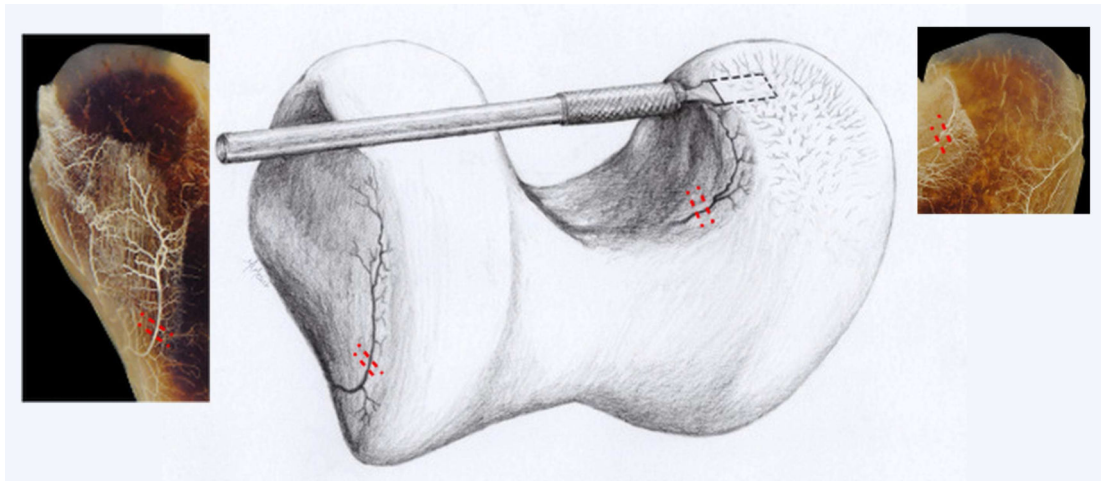


Figure 1.

Drawing demonstrating the surgical procedure used to interrupt the vascular supply to the distal femoral AECC. Dashed lines indicate location of incisions used to interrupt perichondrial vessels on the axial aspect of the medial femoral condyle (right) and lateral trochlear ridge (left). The incision extending into the epiphyseal cartilage is indicated by the beaver blade entering the medial femoral condyle. Perichondrial vessels interrupted in the axial aspect of the medial femoral condyle (right inset) and the abaxial aspect of the lateral trochlear ridge (left inset) are also shown in perfused, cleared specimens obtained from a 2-week-old goat.

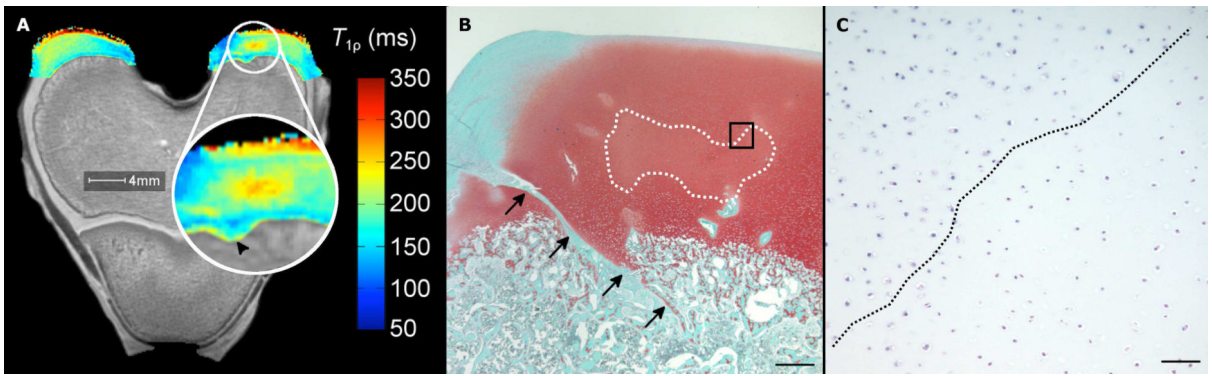


Figure 2.

Adiabatic $T_{1\rho}$ relaxation time map (panel A) obtained from the distal femur of a goat 2 weeks post operatively with the affected area enlarged, demonstrating mildly increased relaxation time in the area corresponding with the area of decreased proteoglycan content in panel B. Arrowhead indicates minor alterations in the advancement of the ossification front. Photomicrograph of a safranin-O stained section (panel B) obtained from the axial aspect of the medial femoral condyle of the same specimen (scale bar = 500 μ m). The surgical incision is partially healed (black arrows in panel A). Faint staining marked by a dotted line indicates decreased proteoglycan content in the area of cartilage necrosis. Panel C depicts high magnification image (scale bar = 50 μ m) of the area marked by the black box in panel B in a hematoxylin and eosin stained section. Chondrocytes to the right of the dotted diagonal line are necrotic, characterized by pyknotic nuclei and shrinkage.

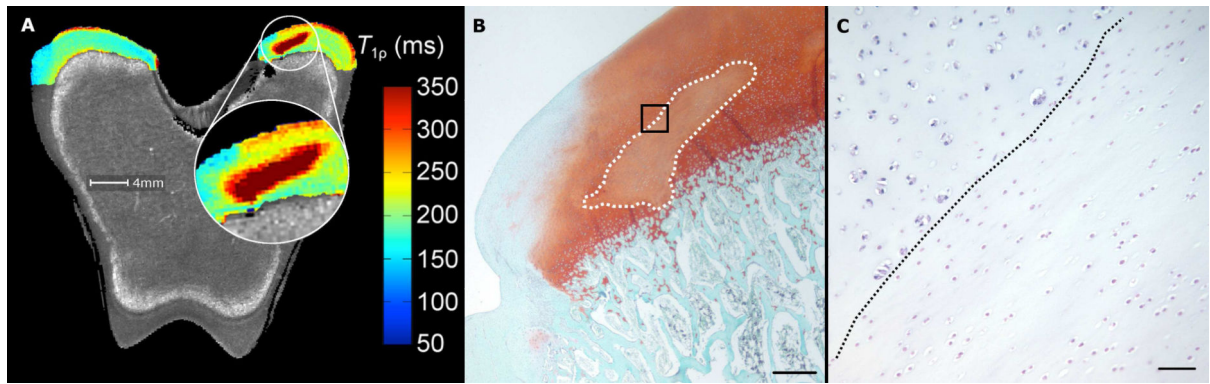


Figure 3.

Adiabatic $T_{1\rho}$ relaxation time map (panel A) obtained from the distal femur of a goat 5 weeks post operatively with the affected area enlarged, demonstrating markedly increased relaxation time in the area corresponding with the area of decreased proteoglycan content in panel B. Photomicrograph of a safranin-O stained section (panel B) obtained from the axial aspect of the medial femoral condyle of the same specimen (scale bar = 500 μ m). Faint staining marked by a dotted line indicates decreased proteoglycan content in the area of cartilage necrosis. Panel C depicts high magnification image (scale bar = 50 μ m) of the area marked by the black box in panel B in a hematoxylin and eosin stained section. Chondrocytes to the right of the dotted diagonal line are necrotic, characterized by pyknotic nuclei and shrinkage.

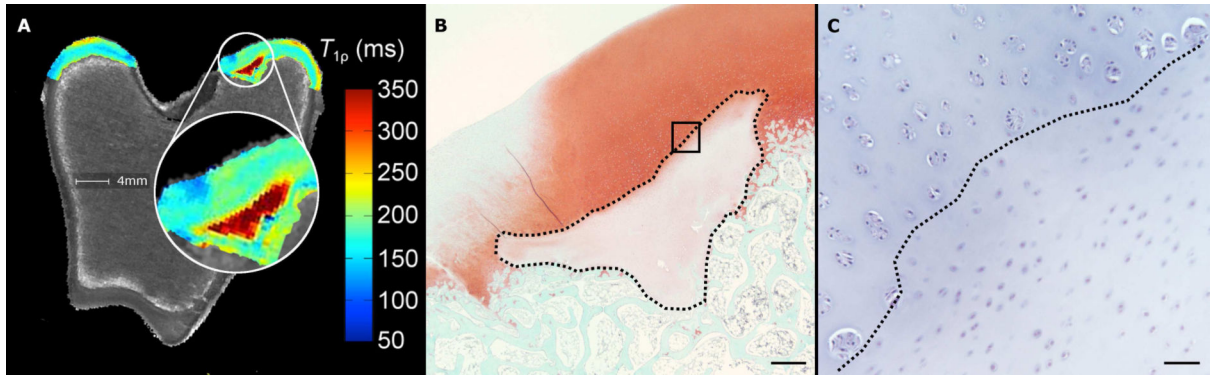


Figure 4.

Adiabatic $T_{1\rho}$ relaxation time map (panel A) obtained from the distal femur of a goat 9 weeks post operatively with the affected area enlarged, demonstrating markedly increased relaxation time in the area corresponding with the area of decreased proteoglycan content in panel B. Photomicrograph of a safranin-O stained section (panel B) obtained from the axial aspect of the medial femoral condyle of the same specimen (scale bar = 500 μ m). Faint staining marked by a dotted line indicates decreased proteoglycan content in the area of cartilage necrosis. Delayed progression of the ossification front in the area of necrotic cartilage is also apparent, which is consistent with an OC manifesta lesion. Panel C depicts high magnification image (scale bar = 50 μ m) of the area marked by the black box in panel B in a hematoxylin and eosin stained section. Chondrocytes to the right of the dotted diagonal line are necrotic, characterized by pyknotic nuclei and shrinkage. Chondrocyte clones are present in the viable cartilage to the left of the dotted diagonal line.

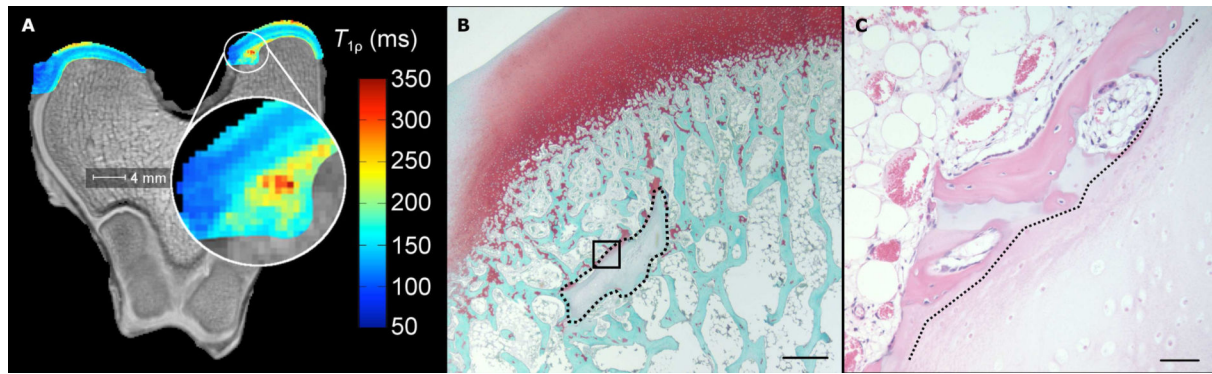


Figure 5.

Adiabatic $T_{1\rho}$ relaxation time map (panel A) obtained from the distal femur of a goat 10 weeks post operatively with the affected area enlarged, demonstrating moderately increased relaxation time in the area corresponding with the area of necrotic cartilage in panel B. Photomicrograph of a safranin-O stained section (panel B) obtained from the axial aspect of the medial femoral condyle of the same specimen (scale bar = 500 μm). An island composed of necrotic cartilage (marked by the dotted line), characterized by markedly decreased staining relative to the epiphyseal cartilage, is retained in the subchondral bone. Necrotic cartilage that was initially surrounded by epiphyseal cartilage (as shown in Figure 3) has become incorporated in subchondral bone as the animal aged and the ossification front progressed towards the articular surface. Panel C depicts high magnification image (scale bar = 50 μm) of the area marked by the black box in panel B in a hematoxylin and eosin stained slide. Tissue to the right of the dotted diagonal line is composed of necrotic cartilage.

Table 1

Histological and MRI findings obtained from eight goat kids after interruption of the vascular supply to portions of the distal femur.

	Goat age at the time of euthanasia									
	1 week	2 week	3 week	4 week	5 week	6 week [#]	9 week	10 week		
Incision										
Original size (mm)	~3 × 4	~3 × 4	5 × 5	~3 × 4	5 × 5	~3 × 4	5 × 5	~3 × 4		
Healing	No	Deep portion is forming granulation tissue	Deep ~50% is healed	Deep ~75% is healed	Deep ~90% is healed	Deep portion is forming granulation tissue	100% healed	100% healed		
Cartilage canals	Necrosis distal to incision	Necrosis in axial ~50% of condyle	Absent in axial ~50% of condyle	Absent in axial ~20% of condyle	Absent in axial ~50% of condyle	Absent in axial ~50% of condyle	Low numbers in abaxial 50% of condyle	Absent or chondrified throughout		
Chondrocyte	Adjacent to incision	1.9 mm ² area in AECC	1.8 mm ² area in AECC	0.5 mm ² area in AECC	1.2 mm ² area in AECC	Not apparent	5.3 mm ² area a ossification front	0.6 mm ² area in subchondral bone		
Morphology	Shrinkage, pyknosis	Shrinkage, pyknosis	Shrinkage, pyknosis	Shrinkage, pyknosis	Shrinkage, pyknosis	Normal	Shrinkage, pyknosis	Shrinkage, pyknosis		
ECM [§] staining	Retained	Mildly decreased	Markedly decreased	Mildly decreased	Markedly decreased	Retained	Markedly decreased	Nearly absent		
T1rho relaxation time in necrotic cartilage	N/A [*]	Mildly increased (~250 ms)	Moderately increased (~300 ms)	No changes noted (~150 ms)	Markedly increased (~350 ms)	No changes noted (~150 ms)	Markedly increased (~350 ms)	Mildly increased (~250 ms)		
OC classification	N/A	OC latens	OC latens	OC latens	OC latens	N/A	OC manifesta	Healing OC manifesta		

[#] In the 6-week-old goat the incision into the articular-epiphyseal cartilage-complex was created too superficially relative to the rest of the animals.

[§]ECM = extracellular matrix

^{*} MRI study missed the area of lesion