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EXPERIMENTAL MODEL STUDY OF ISCHEMIC NECROSIS INDUCTION OF THE GROWING FEMORAL HEAD

ESTUDO DO MODELO EXPERIMENTAL DE INDUÇÃO DA NECROSE ISQUÊMICA DA CABEÇA FEMORAL EM CRESCIMENTO

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ABSTRACT

Many experimental models exist to better understand the necrosis of the femoral head etiology, both in terms of the species variety in which necrosis is induced and in the operative techniques used for treatment. Objective: This study has two main objectives, the first is to review the literature concerning experimental models of avascular necrosis of the growing femoral head, the second, to demonstrate the experimental pig model's reproducibility using a pilot study. Methods: This was a bibliographic review to describe the attempts over time to find the best species and technique for induction that would reproduce ischemic necrosis of the growing femoral head in humans. Simultaneously, a pilot study was performed to verify the replication of induction in pigs, the species that has more similarities with the human hip. The pilot's methodological analysis consists of conventional radiology and verification of possible anatomical, pathological changes. Results: In imaging exams; lateral sub-dislocation of the femur head and triangular appearance of the head were observed, characterizing its flattening; in macroscopic examination, the femoral head flattening with femoral neck widening and shortening was identified; in histology, the proliferation of articular cartilage with the presence of vascular granulation regenerative tissue, with osteoclasts and fibrocartilaginous tissue in the metaphyseal femoral neck region was identified. Conclusion: The experimental pig model can be used as a valuable tool for the reproducibility of anatomical, pathological changes in ischemic necrosis of the growing femoral head. The model is reproducible and feasible and can be beneficial for future studies on the anatomical pathology of necrosis of the growing femoral head. ***Level of Evidence III, Literature Review.***

Keywords: Femur Head Necrosis. Legg-Calvè-Perthes Disease. Models, Animal.

RESUMO

Na tentativa de compreender melhor a etiologia da necrose da cabeça femoral, existe uma diversidade de modelos experimentais tanto no que diz respeito à variedade das espécies em que é induzida a necrose quanto nas técnicas operatórias utilizadas para o tratamento. Objetivo: Este trabalho tem fundamentalmente dois objetivos: a revisão da literatura concernente aos modelos experimentais da necrose avascular da cabeça do fêmur em crescimento e demonstrar a reprodutibilidade do modelo experimental do suíno por meio de um estudo piloto. Métodos: Foi realizada uma revisão bibliográfica descrevendo as tentativas ao longo do tempo em buscar qual seria a melhor espécie e técnica para indução que reproduzisse a necrose isquêmica da cabeça do fêmur em crescimento nos humanos. Simultaneamente foi feito um estudo piloto para verificar a replicação da indução na espécie suína, o espécime cujo quadril tem mais similaridades com o humano. A análise metodológica do piloto consiste na radiologia convencional e verificação das possíveis alterações anátomo patológicas. Resultados: Nos exames por imagem, foram observadas sub-luxação lateral da cabeça do fêmur e aparência triangular da cabeça, caracterizando o achatamento da mesma; no exame macroscópico, identificamos o achatamento da cabeça femoral com alargamento e encurtamento do colo; na histologia, identificamos a proliferação da cartilagem articular com presença de tecido regenerativo vascular de granulação, com osteoclastos e tecido fibrocartilaginoso na região metafisária do colo femoral. Conclusão: Podemos inferir que o modelo experimental suíno pode servir como ferramenta valiosa para a reprodutibilidade das alterações anátomo patológicas da necrose isquêmica da cabeça femoral em crescimento. O modelo é reprodutível e factível, servindo para estudos futuros sobre a anátomo patologia da necrose da cabeça do fêmur em crescimento. ***Nível de Evidência III, Revisão da Literatura.***

Descritores: *Necrose da Cabeça do Fêmur. Doença de Legg-Calve-Perthes. Modelos Animais.*

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INTRODUCTION

Avascular necrosis of the femoral head is an orthopedic disease. When it affects the hip of the growing child it is denominated Legg Calvé Perthes disease (LCPD), due to the disease was reported almost simultaneously in 1910 by Legg, Calvé, and Perthes.[1-3]
The authors described an affection in the hip of the child different from joint tuberculosis, which was the disease most found at that time. During all these years, about 2,000 studies have been published on the subject; however, the etiology remains unclear, but certainly occurs a disturbance in the arterial circulation of the growing femoral head.[4-7]
To better understand the etiology of femoral head necrosis, we have a diversity of experimental models discussing both: the variety of species, in which necrosis is induced, and the techniques used for induction.[8]
In this study, we present an analysis of the literature in relation to the models idealized over time, and the description of an experimental model in the swine species in a pilot project, used for the study of ischemic necrosis of the growing femoral head.

MATERIALS AND METHODS

This study was approved by the Ethics Committee on the Use of Animals under protocol number no. 141/15.
To review the literature, the most relevant databases available on the Internet (PubMed, SciELO, LILACS, MEDLINE, Cochrane) from 1933 to the present. were used. The keywords used were the following: osteonecrosis, experimental model, femur head necrosis, Legg Calvè Perthes disease.
Simultaneously, a pilot induction study was conducted in the swine species that was subjected to methodological analysis with conventional radiology and analysis of possible anatomopathological changes.

Experimental model: pilot study

We used the experimental model in the growing swine. An animal with four weeks of life and weighting 8 kg was operated. We used the right coxofemoral joint for the operation, remaining the left coxofemoral joint as an unoperated control.

Anesthesia

The animal received preanesthetic medication with ketamine (3 mg/kg IM) and midazolam (0.4 mg/kg IM). Anesthesia induction was executed with isoflurane (3.5%) with mask aid and anesthesia maintenance was performed with isoflurane (2%) and fentanyl (0.2 μg/kg/h IV). For intubation, 5 mg/kg of propofol was administered. Immediately before the surgery, prophylactic antimicrobial therapy with cefazolin (30 mg/kg IV) was performed and non-steroidal anti-inflammatory drugs (meloxicam 0.4 mg/kg IM) were administered.
At the end of the surgical procedure, analgesia was performed with dipyrone (25 mg/kg IV), morphine (3 mg/kg IV), wound dressing in the surgical region, and a peripheral catheter for the administration of analgesic and anti-inflammatory drugs in the postoperative period.

Surgical procedure

After induction and anesthesia, a longitudinal incision was made in the right hip of the animal under sterile conditions (Figure 1). To access the joint capsule, the middle and superficial gluteus muscles are pulled with the aid of a Farabeuf retractor. Partial joint capsulotomy is executed, and longitudinal traction of the lower limb is performed to dislocate the joint. The round ligament is sectioned to facilitate looping a circumferential double suture in the femoral neck of the animal (Figure 2). Using a curvilinear instrument, type curved Mixter or with a tonsil needle, an absorbable vicryl suture 2 is positioned around the femoral neck and tied with greatest possible pressure to block the arterial circulation of the cervical ascending vessels that supply the proximal femoral epiphysis.

Figure 1. Surgical procedure: Longitudinal and cranial incision to the coxofemoral joint.

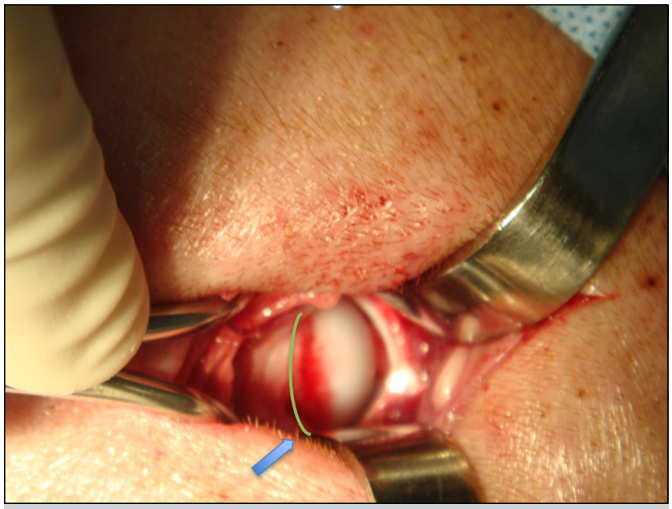

Figure 2. Surgical procedure: exposure and ligation of the femoral neck (the arrow points to ligation site).

Image evaluation

Imaging diagnosis was performed by fluoroscopy at the time of the operation of ischemic necrosis induction and six weeks postoperatively, at the time of euthanasia of the animal. The left hip serving as control.

Macroscopic and histological evaluation

The right and left hip of the animal were carefully dissected. Afterward, macroscopic and microscopic examinations of the samples were performed.
For histology, only the sample of the right hip (operated) of the animal was fixed in formalin at 10%, embedded in paraffin and cut into slices sized 6 μm.
The sections thus obtained were stained by Hematoxylin and eosin (HE) and examined under common optical microscopy. With microscopic evaluation we tried to answer the following questions:

- presence of an increased number of osteoclasts;
- increased bone resorption;
- presence of granulation vascular regenerative tissue;
- joint cartilage proliferation.

RESULTS

Figure 3 shows the result of radiographic evaluation at the time of euthanasia of the animal six weeks after ischemia induction

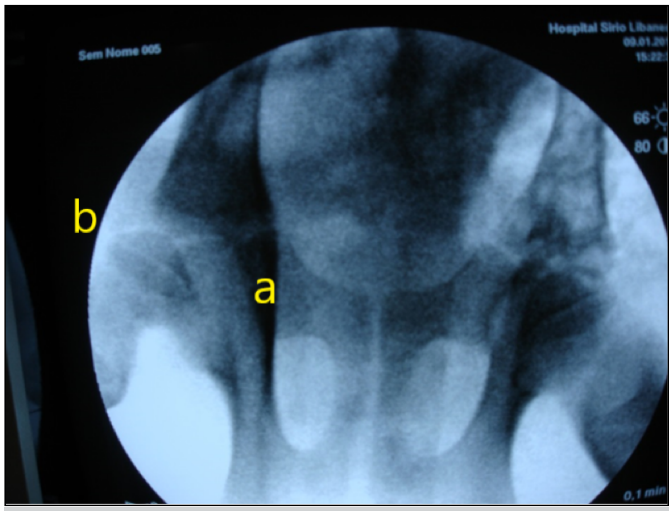

Figure 3. Fluoroscopy image after six weeks postoperatively (EUTHANASIA).

In "a", we observed sub dislocation of the femur head; in "b" triangular appearance of the femur head with flattening on it. The image of the normal left coxofemoral joint (control).

Macroscopy

Macroscopic analysis of the right and left hip samples was performed immediately after euthanasia (Figure 4). Note the flattening of the right femoral head with enlargement and shortening of the femoral neck, when compared with the control hip (left).

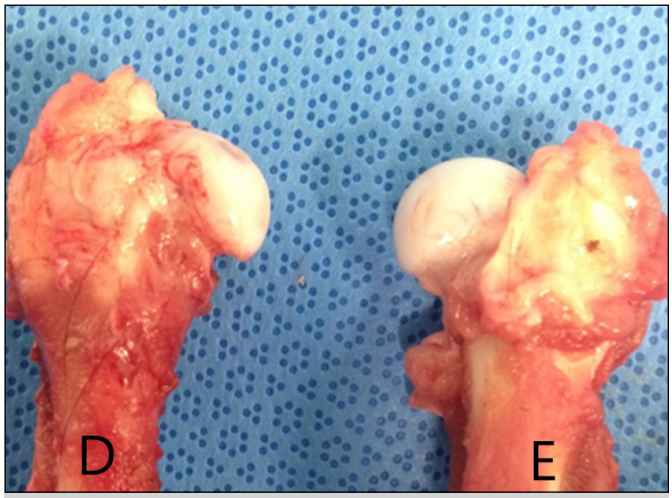

Figure 4. Macroscopic evaluation of the right and left femurs (control). Advisor source, advisor's personal file.

Histological evaluation

Figure 5 shows (a) the proliferation of articular cartilage; (b) presence of granulation vascular regenerative tissue in the metaphyseal region of the femoral neck; and (c) presence of fat cells in granulation tissue. Figure 6 complements our analysis showing (d) the presence of osteoclasts in the fibrocartilaginous tissue; and (e) chondrocytes.

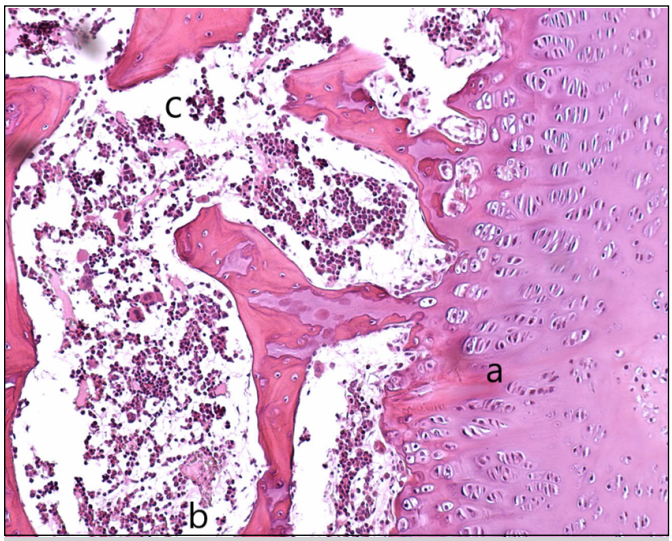

Figure 5. Histological cut of the sample with an increase of 20×, HE staining.

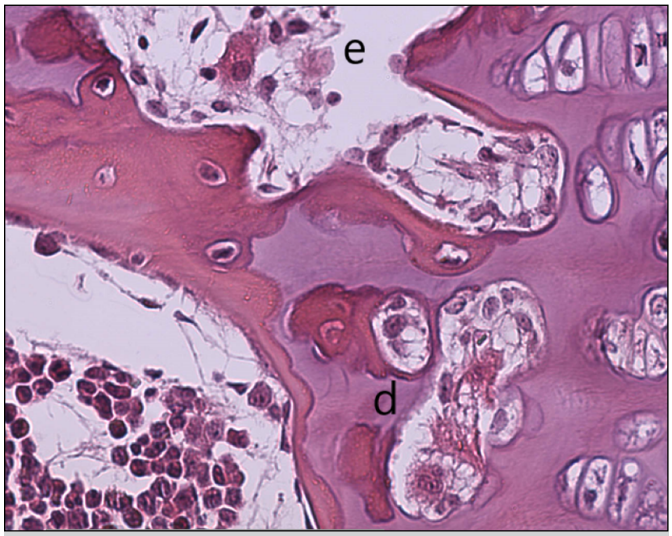

Figure 6. Higher increase 40× – osteoclasts + fibrocartilaginous tissue + chondrocytes (articular cartilage).

DISCUSSION

This study has two objectives: the review of the literature concerning the experimental models of avascular necrosis of the growing femoral head and the demonstration of reproducibility in the experimental swine model by a pilot study.

Most animal models for the study of ischemic necrosis focused on quadrupeds, including pigs, rabbits, dogs, rats, and goats. Biped investigation was limited to chicken and ema. The list of techniques that attempted to induce osteonecrosis of the femoral head is extensive.

Comparing the pig model with other species, we found rabbits' femoral neck to be short, hindering the correct application of the surgical technique of induction of avascular necrosis, causing difficulty for surgical ligation. As Robichon et al. refer, in dogs the collateral circulation allows a faster revascularization when compared to pig.[9]

We also note that the cycle of bone remodeling in pigs is more similar to that of humans when compared to the cycles of rats or mice.[10] Thinking about the biomechanics of the human hip, the Ema, for being biped, has a great advantage in the anatomical similarity in relation to quadrupeds, besides being an animal with a joint dimension similar to that of children.[11]

We agree with authors such as Salter[12] and Rang,[13] that induction by surgical ligation of the femoral neck is the most appropriate to reproduce the results observed in humans, being useful to elucidate the development of Legg Calvè Perthes pathogenesis, and also serving for prevention and therapeutic studies for the disease. Compared to noninvasive methods, the advantages of traumatic techniques are due to the femoral necrotic lesions concentrate on the femoral head, unlike non-traumatic techniques (corticosteroid overdose, autoimmune reactions) where the lesion reaches the femoral metaphysis and other bones when induced by a systemic reaction. Another advantage of invasive techniques is that we can determine the exact moment of the ischemic lesion, enabling the control of examinations by image and histology in all stages of the pathophysiological process, from the ischemic phase to the remodeling phase.[8,14]

This study showed similarity between induced osteonecrosis methods by femoral neck ligation described earlier in the literature review; the pilot model executed in our research using swine species was successful in inducing avascular necrosis, confirming that it is feasible in our institution, serving for future studies. Although it is a single piece, we found that the time of six weeks after surgical induction is able to induce osteonecrosis due to the various radiological, anatomical, and cellular alterations that we observed.

The absence of a fully satisfactory therapy for Legg Calvè Perthes disease and the morbidity associated with joint injury justify the maintenance of scientific research on the subject and even with the evolution of computer programs capable of copying pathological events, the maintenance of animal experimentation to study avascular necrosis of the femoral head is necessary. The canine species also develops aseptic necrosis of the femoral head without a treatment that shows complete efficacy, being only possible to perform resection of the femoral head or total prosthesis of the hip, procedures that can cause functional limitation.[15] Therefore, we advocate for the continuation of the experimental study of femoral head ischemia, and the swine species was the one that best reproduced the disease due to its anatomical similarity to humans, a relatively low maintenance cost, a species easy to deal, and a wide literature on the subject available .

CONCLUSION

We can infer that the experimental swine model can serve as a valuable tool for the reproducibility of pathological anatomic alterations of ischemic necrosis of the growing femoral head. The model is reproducible and feasible, serving for future studies on the pathology anatomic of necrosis of the growing femoral head.

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