Rev Esp Cir Ortop Traumatol. 2014;58(2):68–77

Revista Española de Cirugía Ortopédica y Traumatología

www.elsevier.es/rot

RESEARCH

Experimental induction of Perthes disease in lambs☆,☆☆

S. Martínez-Álvarez[a,*], T. Epeldegui-Torre[a], G. Manso-Díaz[b], A. Rodríguez-Bertos[c], F. Forriol[d]

[a] *Servicio de Cirugía Ortopédica y Traumatología Infantil, Hospital Universitario Infantil Niño Jesús, Madrid, Spain*
[b] *Departamento de Medicina y Cirugía Animal, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain*
[c] *Departamento de Anatomía Patológica, Facultad de Veterinaria, Universidad Complutense, Madrid, Spain*
[d] *Facultad de Medicina, Universidad San Pablo CEU, Campus de Montepríncipe, Madrid, Spain*

Received 21 September 2013; accepted 3 November 2013

KEYWORDS
Leg-Calvé-Perthes disease;
Avascular necrosis;
Lamb;
Bone ischemia

Abstract
Objective: To establish a simple, reproducible and safe experimental model, for the development of ischemic vascular necrosis of the hip in the lamb.
Material and methods: We used 15 lambs (10 males and 5 females) aged four weeks, divided into a control group (7 animals) and an experimental group (8 animals) producing ischemia in the proximal femur. Standard radiography and MRI were performed. The animals were euthanised at the 4th, 8th and 12th weeks after surgery. The femoral heads were extracted and measured and a histological analysis was performed with hematoxylin–eosin staining.
Results: Decreased height and increased width of the femoral head was observed in the x-rays, particularly after the 4th week. We did not observe any changes in the height of the lateral pillar or trochanteric distance. The experimental group showed macroscopical hypertrophy and progressive flattening of the head. At 4 weeks necrotic areas in articular cartilage were observed, bone marrow was dense and the growth cartilage height was lower. The vessels were thickened by proliferation of the medial and adventitia layers. At 8 weeks, we found fibrosis in the subchondral bone with thinned and devitalized angiogenesis fat areas. The articular cartilage showed irregularities. At 12 weeks the closure of the physis was noted, as well as chondral areas in the trabecular bone and fat cells in the methaphysis.
Conclusion: Although the histological changes are consistent with necrosis of the femoral head, the images obtained did not resemble Perthes disease, so we do not advise this experimental model for the study of this disease.

☆ Please cite this article as: Martínez-Álvarez S, Epeldegui-Torre T, Manso-Díaz G, Rodríguez-Bertos A, Forriol F. Inducción experimental de la enfermedad de Perthes en corderos. Rev Esp Cir Ortop Traumatol. 2014;58:68–77.
☆☆ Antonio and Mauricio Riosalido Award 2013 for works related to Orthopedic and Traumatology surgery.
* Corresponding author.
E-mail address: smartinez@salud.madrid.org (S. Martínez-Álvarez).

1988-8856/$ - see front matter

PALABRAS CLAVE
Enfermedad de Leg-Calvé-Perthes;
Necrosis avascular del fémur;
Cordero;
Isquemia ósea

Inducción experimental de la enfermedad de Perthes en corderos

Resumen
Objetivo: Establecer un modelo experimental sencillo, reproducible y seguro para conocer el desarrollo de la necrosis vascular isquémica de la cadera en el cordero.
Material y metodología: Utilizamos 15 corderos (10 machos y 5 hembras) de 4 semanas de edad, divididos en un grupo control (7 animales) y otro grupo experimental (8 animales), a los que se provocó la isquemia de la extremidad proximal del fémur. Se efectuaron radiografía convencional y resonancia nuclear magnética. Tras el sacrificio de los animales, a la 4.ª, 8.ª y 12.ª semanas poscirugía, extrajimos y medimos la cabeza femoral. Una vez fijada la pieza obtuvimos cortes histológicos de diferentes zonas que se tiñeron con hematoxilina-eosina.
Resultados: Radiográficamente disminuyó la altura y aumentó la anchura de la cabeza femoral, más evidente a partir de la 4.ª semana. No objetivamos cambios en la altura del pilar lateral ni en la distancia artículo-trocantérea. El grupo experimental macroscópicamente demostró hipertrofia y aplanamiento progresivo de la cabeza. A las 4 semanas de la cirugía aparecieron zonas de necrosis en el cartílago articular, una médula ósea más densa y menor altura de la fisis. Los vasos estaban engrosados por proliferación de la capa media y de la adventicia. A las 8 semanas encontramos una fibrosis subcondral, con un cartílago articular irregular, adelgazado y desvitalizado, y áreas de angiogénesis con grasa en el hueso subcondral. A las 12 semanas apreciamos el cierre de la fisis, áreas condrales en las trabéculas óseas y células adiposas en la médula diafisaria.
Conclusión: Aunque los cambios histológicos son compatibles con necrosis de la cabeza femoral, las pruebas de imagen obtenidas no se asemejan a la enfermedad de Perthes, por lo que desaconsejamos este modelo experimental para el estudio de esta entidad.

Introduction

The clinical relevance of Leg-Calvé-Perthes disease (LCPD) is based on the progressive deformity of the immature femoral head, which causes premature degeneration of the joint.[1] Several works have discussed the etiopathogenesis of idiopathic avascular necrosis in humans and the different etiopathological mechanisms involved, such as ischemia secondary to fatty microemboli from the bone marrow,[2] intravascular coagulation[3] and retrograde embolization of bone marrow fat.[4] The theory of accumulated cellular pressure as the cause of ischemic phenomena[5] has also been reported. This theory postulates that the cells would be exposed to multiple pressures and aggressions which would result in cell death. Thus, osteonecrosis would be caused by a disease of bone cells or mesenchymal stem cells (MSCs), suggesting that the cause is not only vascular. In support of this theory, it has been observed that the degree of activity and the number of MSCs in patients with osteonecrosis of the femoral head is below normal,[6] as is also the case with the proliferation capacity of osteoblastic cells.[7]

Necrotic lesions are characterized by the apoptosis of osteocytes and lining cells of the trabecular bone in the femoral head, which can distantly affect the proximal femur bone.[5] Kim[8] showed the mechanism of cell death secondary to the process of ischemic injury of the femoral head in pigs.

The regenerative process which follows ischemic necrosis takes place by deposition of new bone layers from the necrotic bone itself. Studies conducted on human femoral heads[6,9,10] and an experimental study on rabbits[11] have shown that tissue regeneration takes place through the penetration of MSCs and capillaries into the necrotic bone from areas which are not affected by ischemia. These studies also observed that MSCs located near the surface of the necrotic bone were differentiated into osteoblasts, which, in turn, formed bone by apposition on the surface of the trabecular necrotic bone and eventually coated the surface of the femoral head. Subsequently, the central necrotic area was resorbed by the osteoclasts and replaced by newly formed bone. This explains the changes observed during necrosis of the femoral head, such as the widening of the trabeculae, increase of bone mass per volume and density increase in the areas being repaired. The newly formed bone has less mechanical rigidity and plasticity, so the axial load and repeated trauma cause a progressive flattening and deformity of the femoral head. Koob et al.[12] observed that the alteration of the mechanical properties of epiphyseal bone and cartilage were associated with pathogenesis of the deformity of the femoral head.

The etiopathogenic mechanism of LCPD is unknown, and its anatomopathological evolution is difficult to assess clinically. Despite employing four-legged animals which do not much resemble humans, experimental models are very useful. Various animal models, such as rabbits,[11] dogs,[13,14] goats,[15,16] and, particularly, pigs,[14,17–22] have been used for the experimental study of LCPD. However, the macroscopic and radiographic results obtained have not been able to explain the pathogenesis of femoral head deformity. Works using lamb models are scarce despite being simple to obtain, work with and manage. Although it has been used in works studying the development of femoral head pathology, these works have never been conducted by isolated alteration of vascularization.[23–25] Our hypothesis is that the alteration of isolated vascularization causes an alteration in the proximal