

# A new preclinical femoral head osteonecrosis model in sheep

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## Abstract

**Introduction** Animal models have been used as insight into the pathogenesis of osteonecrosis, even though most have failed to reproduce all stages of human disease, limiting progression in experimental treatment modalities. A new surgically induced animal model of femoral head osteonecrosis in sheep is presented.

**Method** Osteonecrosis was achieved using an improved method of intracephalic cryogenic lesion by means of a cryoprobe and vascular ligation.

**Results** Histologic findings at 6 and 12 weeks showed progression to advanced stage osteonecrosis. MRI, the gold

standard in diagnosis and follow-up in human osteonecrosis showed concordant results with histology.

**Conclusion** Our model can therefore be used as a preclinical femoral head osteonecrosis model in an easily accessible animal to assess biological treatments with MRI.

**Keywords** Preclinical model · Sheep · Femoral head osteonecrosis · MRI

## Introduction

Osteonecrosis of the hip typically affects young adults and frequently follows an unrelenting course resulting hip pain and loss of function [1, 5]. It is thought to be caused by impaired microcirculation to a section of the femoral head, and its etiology is associated with a wide range of precipitating factors [2, 3]. Most cases progress to structural collapse, leading to articular incongruence and coxarthrosis. It is estimated that 10,000–20,000 new cases of osteonecrosis of the femoral head are diagnosed each year in the US and that 5–18% total hip replacements performed each year are done to treat this diagnosis [4]. Currently, there is no clear-cut indication for a specific treatment for early stage (pre-collapse) femoral head osteonecrosis, and late stage (post-collapse) is best treated by reconstructive procedures [1]. With limited effectiveness of precollapse treatment and higher failure rates of arthroplasties within femoral head osteonecrosis patients there is great need in recognizing effective treatments to reliably prevent femoral head collapse [6].

Animal models have been used as insight into the pathogenesis of osteonecrosis [7–12]. Experimental treatment modalities in these animal models have had limited progression due to the fact that animal models were not able to

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reliably reproduce all the stages of human disease [13]. In 2002, a new animal model of femoral head osteonecrosis in emus was introduced [14]. The model utilized intracapsular and periarticular administration of liquid nitrogen combined with vascular ligation which induced consistent necrotic lesions that progressed to end-stage osteonecrosis. This model could still be improved in several aspects, like reducing subcapital fractures due to the freeze insult, reducing lesion size to more human segmental type lesions, and quantifying liquid nitrogen administration and intracapsular temperature [14, 15].

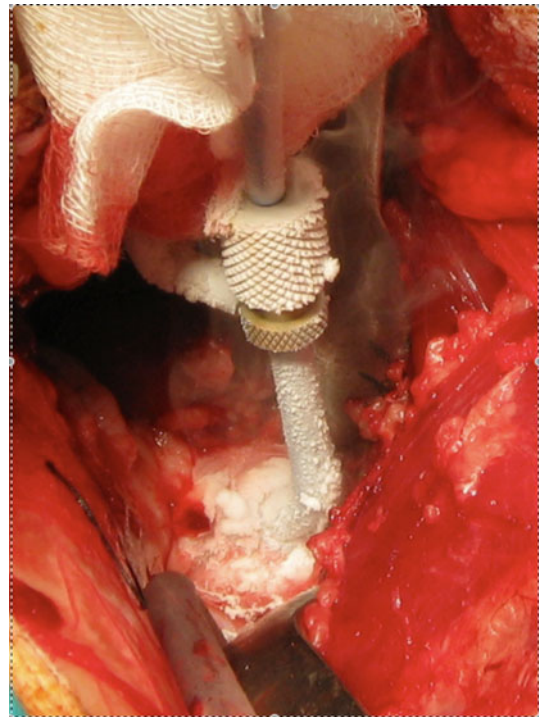
The purpose of this study was to create an animal model that reproduces the evolution, including all the progression stages of femoral head osteonecrosis in a quadruped that is easily accessible like the sheep. We also wanted to evaluate the correlation between histological and MRI changes, since MRI is the gold standard for assessing patients with suspected or established hip osteonecrosis [1].

## Materials and methods

The Institut de Recerca ethics committee and the Hospital Universitari Vall d'Hebron (Universitat Autònoma de Barcelona) ethics committee approved the experimental protocol. All animals received care in compliance with the Principles of Laboratory Animal Care formulated by the 50 National Society for Medical Research and the Guide for Care and Use of Laboratory Animals prepared by the National Institutes of Health (publication no. 80–23, revised 1985) and the Spanish law of protection of experimental animals (Real Decreto 223, 1988).

Ten sheep, 20 weeks old, were used in the study. The sheep were administered buprenorphine 30 min preoperatively, and then were induced to anesthesia with intravenous propofol. After intubation, anesthesia was maintained with flurane and oxygen. The sheep were placed in decubitus supine and the right hip was aseptically prepared. A 15-cm incision was made over the inguinal sulcus. Deep to the pectineus muscle, the lateral circumflex artery and vein were identified and ligated. Medial to the proximal pectineus muscle origin the medial circumflex artery and vein were identified, ligated and cut. The hip joint was then exposed and the hip capsule was removed with electrocautery. A 4.5-mm drill bit was used to create a transcortical access to the inferomedial aspect of the femoral head.

A 2-mm cryoprobe (Brymill Cry AC) was introduced into the cortical orifice and advanced to the anterosuperior region of the femoral head. Correct placement was checked with an intraoperative image intensifier. Then the cryoprobe was attached to a 500-cc liquid nitrogen gun (Brymill Cry AC) (Fig. 1). The induction of the cryo-insult was done

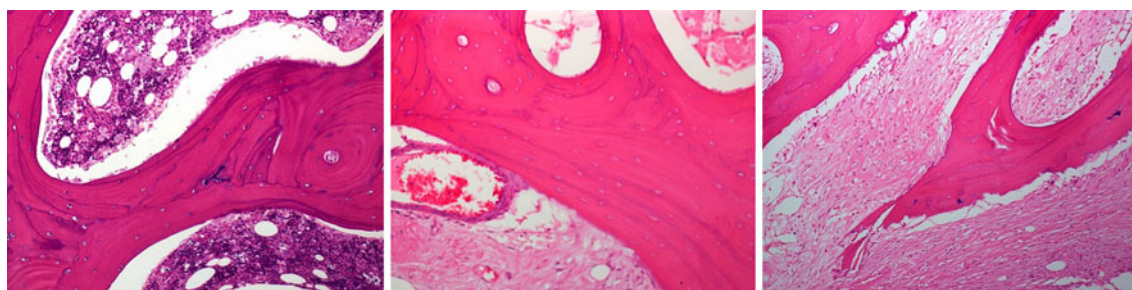
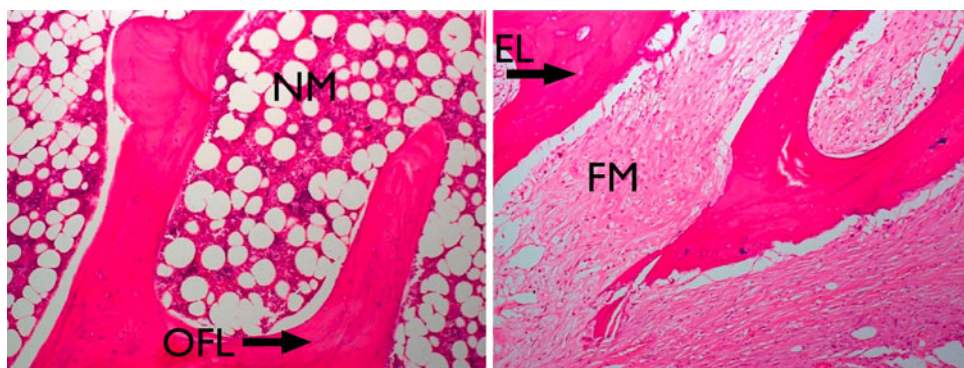


**Fig. 1** Cryogenic lesion using an intracapsular liquid nitrogen cryoprobe

with three 9-min cycles of freezing alternated with surgical field lavage with 500 cc of sterile saline at 50°C. The cryoprobe was removed and the cortical hole was sealed with bone wax. The incision was closed in anatomical layers. All animals received Duphamox antibiotic. During the postoperative period the sheep were left in a laboratory enclosure for 6 weeks and then in an outdoor enclosure for another 6 weeks.

To study the osteonecrotic progression and MRI changes the sheep were distributed in the following manner. Five sheep had MRI of the affected hip at 6 weeks and then were sacrificed. The remaining five sheep had MRI at 6 and at 12 weeks and were sacrificed afterwards. MR imaging was performed using a 0.2-T system (VET-MR; Esaote, Genoa, Italy). We examined the hip under general anesthesia using the same anesthesia protocol as for the osteonecrosis inducing procedure. The imaging protocol in an axial oblique view included the following sequences: intermediate-weighted fast spin-echo localizing images (repetition time in ms/effective echo time in ms; 1,300/11), standard spin-echo T1-weighted images (650/26) and fast spin-echo T2-weighted images (2,800/90). Imaging parameters included the following: field of view, 12–14 cm; section thickness, 4.0 mm; matrix size, 256 × 192. At the time of death the proximal femurs were harvested and fixed in 10% formaldehyde. Histological analysis by the standard hematoxylin-eosin stain was performed.

**Fig. 2** *Left* Histologic control specimen with normal marrow (NM) and osteocyte-filled lacunae (OCL). *Right* Histologic specimen obtained at 6 weeks with fibrotic marrow (FM) and empty lacunae (EL). Hematoxylin–eosin,  $\times 10$



**Fig. 3** *Left* Histologic control specimen. *Middle and right* Histologic specimens at 6 and 12 weeks showing progression of osteonecrosis and no evidence of bone regeneration

## Results

After the first 48 h after surgery all ten sheep walked without evidence of limp. No surgical complications were evidenced during the period of study. Only during the first 48 h did the sheep show some type of protected weight bearing.

Control specimens showed typical femoral head histology. Specimens obtained at 6 weeks showed bone marrow replaced by fibrotic marrow, areas of resorption and evidence of trabecular micro fracture. Lacunae were devoid of osteocytes (Fig. 2).

The five specimens obtained at 12 weeks had typical appearance of advanced stage osteonecrosis, with an area of spherical sequestrum made up of dead trabeculae, with empty osteocyte lacunae. The bone marrow showed a loss of haematopoietic cells, and fat cells with absent nuclei and ruptured cell walls. This necrotic zone was situated under the subchondral end plate. In the reparative zone, there was fibrovascular proliferation; dead trabeculae were completely or partially resorbed by osteoclasts, and replaced or covered with viable appositional bone by osteoblasts. Collapse and fragmentation led to articular cartilage incongruence and loss of sphericity in two cases. The cartilage surface showed no evidence of chondrolysis (Fig. 3).

Hip MRI at 6 and 12 weeks showed typical osteonecrotic changes for all sheep. On T1-weighted images there were geographic lesions of variable signal intensity out-

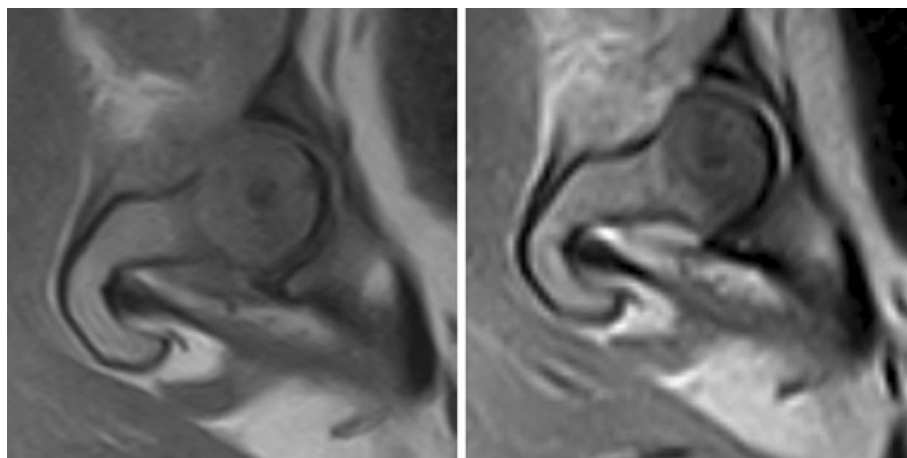
lined by a low signal rim. Two cases at 6 weeks and 3 cases at 12 weeks showed more specific imaging of femoral head osteonecrosis consisting of the double line sign that is comprised of an outer low-intensity rim and an inner high-intensity band on T2-weighted images (Figs. 4, 5) [16]. Two cases showed femoral head collapse and articular incongruence. There was no evidence of joint space narrowing or signs of osteoarthritis.

## Discussion

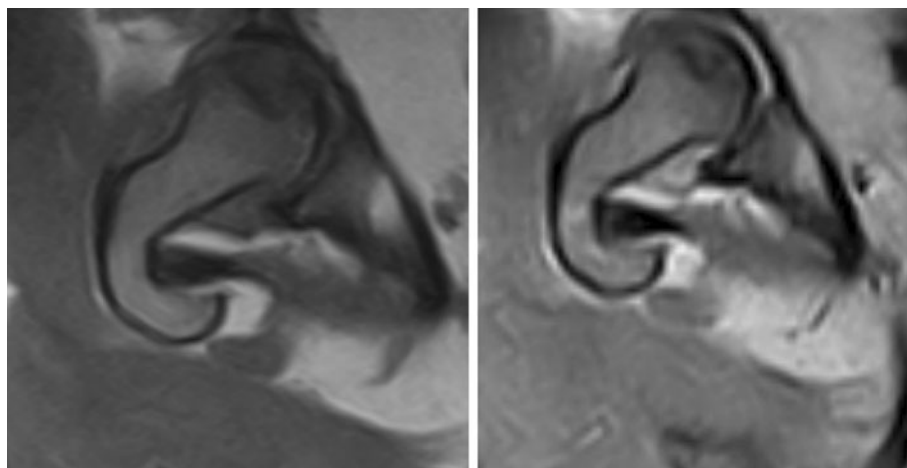
A new animal model that shows characteristic human femoral head osteonecrosis changes in sheep was established through this study. Until recently experimental animal models were not successful in reliably reproducing all stages of human disease. Most trials included many species of quadrupeds and a variety of physical, pharmacological, and mechanical insults [13]. An animal model in emus that used vascular ligation and cryogenic liquid nitrogen insult was very effective in inducing osteonecrosis, but it also had some setbacks [14]. Emus are uncommon experimental animal model that can be even hazardous to handle when awake. Also the method for administering the cryogenic insult was imprecise [14]. In order to improve freeze insult quantification, the same study group has published data on focal cryogenic insults induced by a specifically made cryoprobe in a finite model to specify dosage, critical isotherm



**Fig. 4** MRI at 6 weeks. T1-weighted image (*left*) shows fat-signal loss within the infarcted bone marrow and the T2-weighted image (*right*) shows some diffuse signal loss and very mild subcortical edema



**Fig. 5** MRI at 12 weeks. T1-weighted image (*left*). T2-weighted images (*right*) with the typical double line sign



and lesion size, but this cryoprobe has not been tested in vivo [15, 17]. We used an easily accessible animal for experimental research, the sheep, in a cryogenic-induced osteonecrosis model, using an of the shelf cryoprobe system.

Sheep are docile in nature and large, which facilitates surgical manipulation, and their physiology is also similar to humans. They have a long history in experimental animal investigation and continue to be used to study in an ever-increasing array of diseases, therapies, and surgical techniques [18]. It has been postulated that quadrupeds fail to progress to end-stage femoral collapse (protected weight bearing ability) and this could have limited our study in sheep [9]. But as the results show, the severe hypoxic and cryogenic insult causes irreversible damage and progression independent of weight bearing. This coincides with the human progression in that over 80% of patients treated with some sort of protected weight bearing develop end-stage osteonecrosis [19].

Almost all of the theories regarding the pathophysiology of osteonecrosis consider alterations in blood flow, local cellular toxicity, and even impaired mesenchymal cellular differentiation as potential pathogenic causes [3, 20]. It is

not clear which pathologic mechanism predominates, but sometimes there are factors like alcohol abuse, corticosteroids, lupus erythematosus, HIV-AIDS, chemotherapy and coagulopathies that are implied [21]. It is clear therefore that without more investigation leading to clear pathomechanisms in osteonecrosis, animal models of osteonecrosis will still only mimic the later stages necrosis rather than all the sequence of events in the disease [24]. Due to the similarity in disease progression and end-result independent of the etiology current animal models are still useful in studying new therapies.

Our animal model is an improvement compared to previous reports in many aspects, and there are others that can be perfected. The cryoprobe was fundamental in various aspects of technique refinement and objectivity. The cryoprobe in itself permitted correct placement and verification with image intensification intraoperatively. Using data from focal cryogenic insults testing in vitro, we objectified dosage using standard duration of administration of liquid nitrogen to ensure cell death and a volumetric lesion similar to human osteonecrosis [15]. No periarticular liquid nitrogen was administered to reduce the probability of fracture, which gave good results. It would probably be

recommended to measure intracranial temperature, but since results of necrosis are homogenous, we can assume freeze temperature achieved is also homogenous. Until further comparative studies are done, vascular ligation with its associated morbidity is still necessary to insure an irreversible lesion.

The documentation of histological changes is the basis for most of the previous femoral head osteonecrosis animal models [8, 9, 13, 14]. In our study histology at 6 weeks showed necrotic changes and at 12 weeks typical advanced osteonecrotic appearance. However, in humans histology is rarely used as a diagnostic tool due to its invasiveness. It cannot be used to measure response to treatment, leaving it only as a confirmatory test after a total hip arthroplasty. Fortunately, hip MRI with 99% sensibility and specificity serves as an excellent diagnostic and follow-up tool [3, 16].

MRI can also serve as a prognostic tool, quantifying risk of disease progression and risk femoral head collapse [22, 23]. It is therefore crucial to use MRI as a diagnostic, follow-up, and prognostic tool in femoral head osteonecrosis animal models. Our results showed good correlation between histological changes and MRI findings. With this data, future studies can analyze treatment response in animals in various stages before the definitive final histological result. It should be noted, however, that we did not dispose of high-definition MRI, so there is room for improvement.

Our model creates osteonecrosis of the femoral head in a reliable and standardized way in sheep, an accessible research animal that has similar human physiologic characteristics. We continued to refine an ischemic insult with an off-the-shelf cryoprobe that adds objectivity and precision, which helped us obtain irreversible necrosis in a quadruped with concordant histological and MRI results.

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**Conflict of interest statement** The authors have no conflict of interest.

## References

- Petrigliano FA, Lieberman JR (2007) Osteonecrosis of the hip. Novel approaches to evaluation and treatment. *Clin Orthop Relat Res* 465:53–62
- Aldridge JM 3rd, Urbaniak JR (2004) Avascular necrosis of the femoral head: etiology, pathophysiology, classification, and current treatment guidelines. *Am J Orthop* 33:327–332
- Lieberman JR, Berry DJ, Mont MA, Aaron RK, Callaghan JJ, Rajadhyaksha AD, Urbaniak JR (2003) Osteonecrosis of the hip: management in the 21st century. *Instr Course Lect* 52:337–355
- Vail TP, Covington DB (1997) The incidence of osteonecrosis. In: Urbaniak JR, Jones JP (eds) *Osteonecrosis: etiology, diagnosis, treatment*. American Academy of Orthopaedic Surgeons, Rosemont, IL, pp 43–49
- Mankin HJ (1992) Non-traumatic necrosis of bone (osteonecrosis). *N Engl J Med* 326:1473–1479
- McGrory BJ, York SC, Iorio R, Macaulay W, Pelker RR, Parsley B, Teeny SM (2007) Current practices of AAHKS members in the treatment of adult osteonecrosis of the femoral head. *J Bone Jt Surg Am* 89:1194–1204
- Iwasaki K, Hirano T, Sagara K, Nishimura Y (1992) Idiopathic necrosis of the femoral epiphyseal nucleus in rats. *Clin Orthop Relat Res* 277:31–40
- Kawai K, Tamaki A, Hirohata K (1985) Steroid-induced accumulation of lipid in the osteocytes of the rabbit femoral head: a histochemical and electron microscopic study. *J Bone Jt Surg Am* 67:755–763
- Malizos KN, Darryl L, Quarks AV, Seaber WS, Rizk JR (1993) An experimental canine model of osteonecrosis: characterization of the repair process. *J Orthop Res* 11:350–357
- Seiler JG 3rd, Kregor PJ, Conrad EU 3rd, Swiontkowski MF (1996) Posttraumatic osteonecrosis in a swine model: correlation of blood cell flux, MRI, and histology. *Acta Orthop Scand* 67:249–254
- Wang GJ, Sweet DE, Reger SI, Thompson RC (1977) Fat-cell changes as a mechanism of avascular necrosis of the femoral head in cortisone-treated rabbits. *J Bone Jt Surg Am* 59:729–735
- Yamamoto T, Hirano K, Tsutsui H, Sugioka Y, Sueishi K (1995) Corticosteroid enhances the experimental induction of osteonecrosis in rabbits with Shwartzman reaction. *Clin Orthop Relat Res* 316:235–243
- Conzemius M, Brown T (2001) Animal models in osteonecrosis. *Tech Orthop* 16:90
- Conzemius M, Brown T, Zhang Y, Robinson R (2002) A new animal model of femoral head osteonecrosis: one that progresses to human-like mechanical failure. *J Orthop Res* 20:303–309
- Reed KL, Brown TD, Conzemius M (2003) Focal cryogenic insults for inducing segmental osteonecrosis: computational and experimental assessments of thermal fields. *J Biomech* 36:317–326
- Coleman BG, Kressel HY, Dalinka MK (1988) Radiographically negative avascular necrosis: detection with MR imaging. *Radiology* 168:525–528
- Goetz JE, Pedersen DR, Robinson DA, Conzemius MG, Baer TE, Brown TD (2008) The apparent critical isotherm for cryoinsult-induced osteonecrotic lesions in emu femoral head. *J Biomech* 41:2197–2205
- Scheerlinck JP, Snibson KJ, Bowles VM, Sutton P (2008) Biomedical applications of sheep models: from asthma to vaccines. *Trends Biotechnol* 26:259–266
- Mont MA, Jones LC, Hungerford DS (2006) Current concepts review: non-traumatic osteonecrosis of the femoral head: ten years later. *J Bone Jt Surg Am* 88:1117–1132
- Suh KT, Kim SW, Roh HL, Youn MS, Jung JS (2005) Decreased osteogenic differentiation of mesenchymal stem cells in alcohol-induced osteonecrosis. *Clin Orthop Relat Res* 431:220–225
- Glueck CJ, Freiberg RA, Boppana S, Wang P (2008) Heritable thrombophilia-hypofibrinolysis and osteonecrosis of the femoral head. *Clin Orthop Relat Res* 466:1034–1040
- Ito H, Matsuno T, Minami A (2006) Relationship between bone marrow edema and development of symptoms in patients with osteonecrosis of the femoral head. *AJR Am J Roentgenol* 186:1761–1770
- Ha YC, Jung WH, Kim JR, Seong NH, Kim SY, Koo KH (2006) Prediction of collapse in femoral head osteonecrosis: a modified Kerboul method with use of magnetic resonance images. *J Bone Jt Surg Am* 88:35–40