Morphologic and Histologic Assessment of Porcine Model of Legg-Calve-Perthes Disease at a Clinically Relevant Time Point: A Comprehensive Evaluation of Osteonecrosis and Healing Parameters During Early-Fragmentation

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INTRODUCTION: Legg-Calve-Perthes Disease (LCPD) is a pediatric hip disorder causing ischemic osteonecrosis (ON) of the femoral head. The lack of blood flow produces permanent deformity of the femoral head and leads to osteoarthritis and disability. The porcine ON model is a well-established large animal model of LCPD [1]. In previous studies using the model, various treatments were instituted one week after the surgical induction of ON, which represents the initial avascular necrosis stage of LCPD [2,3]. However, patients commonly present to clinic in the late avascular necrosis stage or early fragmentation stage, during which important pathological changes such as subchondral fracture, initiation of mild femoral head deformity, and revascularization occur. In the porcine model, this critical time point of disease progression has not been well characterized. The purpose of this study is to assess the histologic and morphologic changes in the porcine model of LCPD at 3 weeks after the ON induction. We hypothesized that the 3-week time point better represents a clinically relevant, early fragmentation stage of LCPD.

METHODS: Eighteen piglets underwent a surgical procedure on the right femoral head to induce ischemic ON. Left un-operated femoral heads served as controls. At 3 weeks after ON induction, femoral heads were harvested. X-ray and histologic assessments were performed to determine and confirm a presence of subchondral fracture. X-ray images were also used to obtain the epiphyseal quotient (EQ), a measure of femoral head deformity calculated by dividing maximum femoral head height by its maximum diameter. H&E stained sections were used to quantify % revascularized area in four quadrants of the necrotic femoral head (medial periphery, lower central, upper central, lateral periphery). MacNeal tetrachrome stained sections were used to quantify osteoblast (Ob) and osteoclast (TRAP+ Oc) numbers, which were normalized by bone surface (BS). Furthermore, we flushed out cells from the femoral heads (n=11) and performed LIVE/DEAD® cell assay to quantify viable and non-viable cells present. Micro-CT was used to quantify trabecular thickness (Tb. Th), number (Tb. N), separation (Tb. Sp), and bone volume % (BV/TV). Paired *t*-test was used to compare values between unoperated left (control) and right (ON) sides. A p-value < 0.05 was considered significant.

RESULTS: We observed subchondral fractures in 7 out of 18 samples. The fractures were predominantly (6/7, 86%) seen in the lateral region, which represents the weight bearing region of the femoral head. Both the central and the medial regions contained a fracture in 43% (n=3 each) of the samples. The mean EQ of the ON group was significantly lower than the control group (ON 0.4 ± 0.04 vs. Control 0.5 ± 0.02, p=0.009), indicating a mild deformity. Further analysis demonstrated that the ON group with a subchondral fracture exhibited significantly higher deformity compared to the control group (p=0.02) than ON samples without a subchondral fracture did when compared to the control group (p=0.06). In the ON samples, mean revascularized area was 0.47 ± 0.36 with range from 0-100%. Three samples showed a complete absence of revascularization while the rest showed a variable degree of revascularization with the medial region being the most commonly revascularized region, observed in 14 out of 18 samples. A positive linear correlation ($R^2 = 0.78$) was found between the percentage of revascularization and the percentage of live cells detected by the live/dead assay of flush fluid from the ON femoral heads. Ob and Oc number was significantly lower in the ON group compared to the control group: Ob/BS (mm) (ON 1.8±3, Control 5.11±2.6, p=0.014); Oc/BS (mm) (ON 0.3±0.5, Control 2.3±0.9, p<0.001). Micro-CT measurements of trabecular thickness, trabecular number, and bone volume to tissue volume ratio showed no significant difference between ON and control groups. There was a small but significant difference in the trabecular separation between the ON and control groups: Tb. Sp (μm) (ON 352.7±83.9, Control 312.8±23.5, p=0.045), indicating early trabecular bone resorption.

DISCUSSION: To our knowledge, this is the first study to investigate femoral head ON at a delayed, 3-week time point in the porcine model of LCPD. The presence of a subchondral fracture, mild deformity, variable revascularization, and bone resorption indicate the transition into the early-fragmentation stage of ON, which is a clinically relevant time point when patients present to clinic with symptoms. It is important to note a wide variation in % revascularization between the samples, ranging from 0-100%, revealing vast individual differences in the angiogenic and healing responses despite receiving the same ON surgery. Reduced Ob and Oc numbers per bone surface and the absence of significant differences in the micro-CT trabecular parameters, except for the trabecular separation, are consistent with relatively early stage of ON, where excessive bone resorption, bone remodeling, and trabecular structural changes have not yet occurred.

SIGNIFICANCE/CLINICAL RELEVANCE: This study provides comprehensive morphologic and histologic assessment of the porcine model of LCPD at the early fragmentation stage of ON. This important baseline data will help develop and test new treatment strategies at clinically relevant time point. This study shows substantial individual variability in angiogenic and repair response to ON after three weeks, similar to the variability of femoral head perfusion seen in LCPD patients in the early stage of the disease using perfusion MRI [4]. A wide variability of revascularization and repair, even at the same disease stage of early-fragmentation, stresses the importance of assessing patients on an individual basis and devising individualized treatment plans that reflect the extent of revascularization and healing.

REFERENCES:

[1] Kim HK. Pathophysiology and new strategies for the treatment of Legg-Calvé-Perthes disease. JBJS 2012. PMID: 22488623. [2] Kim et al. Local administration of bone morphogenetic protein-2 and bisphosphonate during non-weight-bearing treatment of ischemic osteonecrosis of the femoral head. JBJS 2014. PMID: 25232075. [3] Kim et al. Minimally Invasive Necrotic Bone Washing Improves Bone Healing After Femoral Head Ischemic Osteonecrosis. JBJS 2021. PMID: 33877059. [4] Kim et al. Assessment of Femoral Head Revascularization in Legg-Calvé-Perthes Disease Using Serial Perfusion MRI. JBJS 2016. PMID: 27852906.

FIGURES:

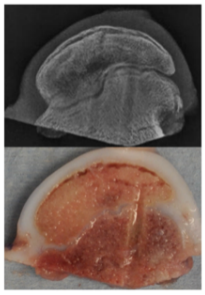

Fig. 1 X-ray and gross sections of a representative sample with a subchondral fracture.

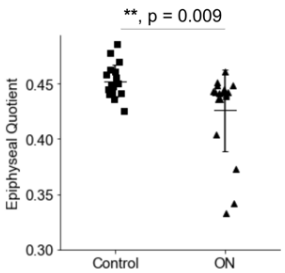

Fig. 2 Epiphyseal Quotient demonstrating increased femoral head deformity in the ON group.

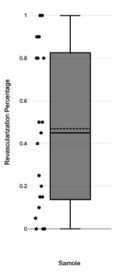

Fig. 3 Box-plot with raw scatter data showing wide variation in sample femoral head area that became revascularized within 3 weeks of ON induction.