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## Implantation of autologous bone marrow mononuclear cells as a minimal invasive therapy of legg-calve-perthes disease in the dog

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**Objective:** The cell therapy is at present considered a novel solution for the regeneration and also for the treatment of some disease of many tissues including bone and other mesenchymal derived tissues. The Legg-Calvè-Perthes (LCP) syndrome is a degenerative disease of the hip joint which is characterized by idiopatic avascular osteonecrosis of femoral head. This disease typically occurs in young children and for the distinguishing features it can be comparable to the LCP disease in young small breed dogs. In the present study, the preliminary results of the use of autologous Bone Marrow Mononuclear Cells (BMMCs) and cultured Bone Marrow Stromal Cells (cBMSCs) are reported as a possible minimal invasive therapeutic treatment of Legg-Calvè-Perthes disease in the dog.

Material and Methods: Twelve dogs of small size and different breed, six males and six females, aged from 8 to 15 months affected by monolateral LCP disease were enrolled in this study. The grade of the radiographic features of the disease was estimated according to the Ljunggren's scale. In eight dogs the BMMCs have been administered while in the other four the cBMSCs ones, after they were cultured for 3-4 weeks in COON's Medium at 37°C in a 5% carbon dioxide atmosphere. The bone marrow was collected from each patient from the iliac crest and the mononuclear fraction was separated by a gradient centrifugation at 2000 rpm for 30 minutes. The mean number of BMMCs was of 8.9x108±3.9x108 while the mean number of the cBMSCs was of 8.4x106±3.5x106. For the BMMCs the Colony Forming Unit (CFU) were evaluated and the mean number obtained was of 5.5x102±5.2x102/ml. The cells were suspended in fibrin glue just before the administration and then implanted by transcutaneous injection, under CT or RX guide, using a Jamshidi needle inserted through the femoral head and neck starting at the basis of the trochanter major.

Results: In ten of the treated dogs the disappearance of pain was observed starting from 3-4 weeks after the cells administration and also a gradual weightbearing on the affected limb up to a complete remission of the symptomatology. In the other 2 cases at ten weeks from the treatment a femoral head and neck ostectomy was performed because the recovery proceeded too slowly and the owners prefered to don't wait anymore. Histological and immunohistochemistry studies were then performed on these samples and had evidenced the presence of cartilage and subcondral bone of new formation in the area in which the cells have been implanted.

Conclusions: As to the results obtained, the cell therapy seems to be an effective and minimal.

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