50th Annual Veterinary Orthopedic Society Conference

March 11-18, 2023, Big Sky, Montana

Autologous micro-fragmented adipose tissue augmented by platelet-rich plasma in non-responsive distal interphalangeal joint osteoarthritis in horses: A case series of eight horses.

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Introduction: Osteoarthritis of the coffin joint (DIP-OA) is a very common cause of lameness in sport horses (1). Popular treatments include intra-articular medication of hyaluronic acid and cortisone (H+C), autologous conditioned plasma (ACP), autologous conditioned serum (ACS) and polyacrylamide hydrogel 2,5% (PAAG 2,5%) (2). In the previous years a commercially available, in-one step technique, which provides micro-fragmented and minimally manipulated adipose tissue without expansion or enzymatic treatment, has shown encouraging results, even in diffused chondropathy in human knee and shoulder joint (3,4,5). Additionally, previous studies have shown that the presence of platelets enhances the proliferation and immunomodulation in regenerative cells (6,7). This retrospective case series demonstrates the outcomes of the DIP-OA treatment with autologous micro-fragmented adipose tissue (AMFAT) combined with platelets rich-plasma (PRP) in horses with history of failed previous treatments.

Material and Methods: Inclusion criteria of the study were non-racing warmblood horses (with front feet distal interphalangeal joint osteoarthritis (DIP-OA) and positive intra-articular analgesia diagnostic imaging with ultrasound, radiography and standing MRI examination, arthroscopy, and failure of at least two previous intra-articular treatments. The latter included H+C, ACP, ACS and PAAG 2,5%. As failure was determined either the non-responsive to treatments lameness or re-appearance of the lameness within 12 weeks post-treatment due to DIP-OA. Horses with DIP-OA due to subchondral cyst-like lesions or fractures were excluded. Unless an additional lesion was detected in the foot, the horse was included in the study.

The harvesting of the adipose tissue was performed in the standing sedated patient. Regarding the pain management all horses received flunixin meglumine (1.1 mg/kg, I.V.) and metamizole sodium (30mg /kg, I.V.) simultaneously with the sedation. The tail head region was clipped, and, after usual surgical preparation, local anesthesia was performed. The liposuction was performed through mechanical disruption solely. Approximately 30 - 60 ml lipoaspirate was obtained and transferred into a disposable, closed device through a filter. The device enclosed five stainless steel marbles and through shaken fat material is disaggregated producing cell clusters and micro-fragmented fat tissue. Blood contaminating cells and undesired fat residues were removed by a gravity counterflow of saline solution. When the solution inside the device appeared clear, the device was turned upside down and a second micro-fragmentation of the tissue was obtained by pushing the adipose clusters with a syringe through a size reduction filter. The AMFAT product was then ready for use. The whole procedure had a duration approximately sixty minutes. The amount of end-product ranged from 5 – 10 ml, depending on the number of the treated joints. The PRP was produced as it has been described previously (8). Briefly, 55 ml blood mixed with 25.000 IE heparin underwent a double centrifugation. In the final product the concentration of platelets ranged from 4,2 to 5,5 times higher than the platelet blood count, and the leukocyte concentration ranged from 0,7 – 1,8 times higher than the initial one from the blood. The coffin joint was prepared for the injection aseptically. After the injection, the rehabilitation included one day box confinement and 5 weeks hand- or riding-walk. No major adverse effects were raised except of a temporary stiffness and effusion in 10 out of 13 joints, which were gradually diminished within two weeks by cooling the leg and without any medication.

Results: Eight client-owned horses fulfilled the inclusion criteria. The last unsuccessful treatment was conducted at least 4 months prior to the examination in all horses. 5 horses had bilateral front feet DIP-OA and 3 horses unilateral, two right front and one left front. All horses were warmbloods, and the mean age was 10 years old (6 – 17). The mean lameness duration was 10 months (6 – 15 months). Previous treatments were included: corticosteroids (8/8 horses: 5 triamcinolone, 2 betamethasone, 1 dexamethasone) with hyaluronic acid (HA). Additionally, 4 horses with ACS (5 injections once/weekly), 5 horses with PAAG 2,5%, and 3 horses with ACP (3 injections once/weekly).

Ultrasonographical findings included synovitis (13 joints), and tear of the dorsal capsule (9 joints) (Figure 1). Additionally, six joints emerged dorsal new bone formation and osteophytes. All but two joints in the MRI examination revealed osteochondral lesions with involvement of the subchondral bone (Figure 1 and 2). Subsequently, the dorsal and palmar recess of the coffin joint were inspected arthroscopically. All joints enclosed focal (10 joints) or diffuse (3 joints) articular cartilage defects with or without chondromalacia (Figures 3, 4, 5 and 6). Additional findings during the surgery were observed such as torn capsular tissue and proliferation of the villi (Figure 6). Seven out of eight horses were sound six weeks after the treatment. In the follow-up twelve months' post-treatment 5/8 (62,5%) horses were still sound and 4/8 (50%) returned to their previous performance.

Discussion: In this study AMFAT augmented with PRP was applied in horses with non-responsive treatment OA-DIP. These patients have been selected, since any other treatment has been failed. AMFAT has been used successfully in osteoarthritis in human patients, particularly in moderate and severe cases (3,4,5). AMFAT consists of many cell populations such as vascular endothelial cells, adipose mesenchymal stromal cells, monocytes and pericytes (9). Additionally, more than 870 proteins in AMFAT participate in inhibition of downregulation paths and in stimulation of upregulated paths (9,10). Moreover, analysis of extracellular vesicles highly expressed miRNAs detection for chondroprotective role in OA and M2 anti-inflammatory macrophage polarization (11). Due to minimal manipulative technique, minimal changes into microenvironment of the regenerative cells and autologous nature of the therapy, the perseverance of the viability of the cells is maximised and warrants the retaining regenerative cell function (11). Therefore, there is a) a strong anti-inflammatory and immunomodulatory potential effect, b) chondroprotection, c) secretion of multiple paracrine growth factors and cytokines and d) structural extracellular adipose matrix in the defects. The main limitation of the current case series is the absence of additional groups with AMFAT and PRP as sole treatments, since the contribution of PRP to the results of these treatments remains unknown. Another limitation is the low number of horses treated.

Conclusion: AMFAT combined with PRP is an alternative promising option in the management of non-responsive to conventional treatments DIP-OA.

FIGURES:

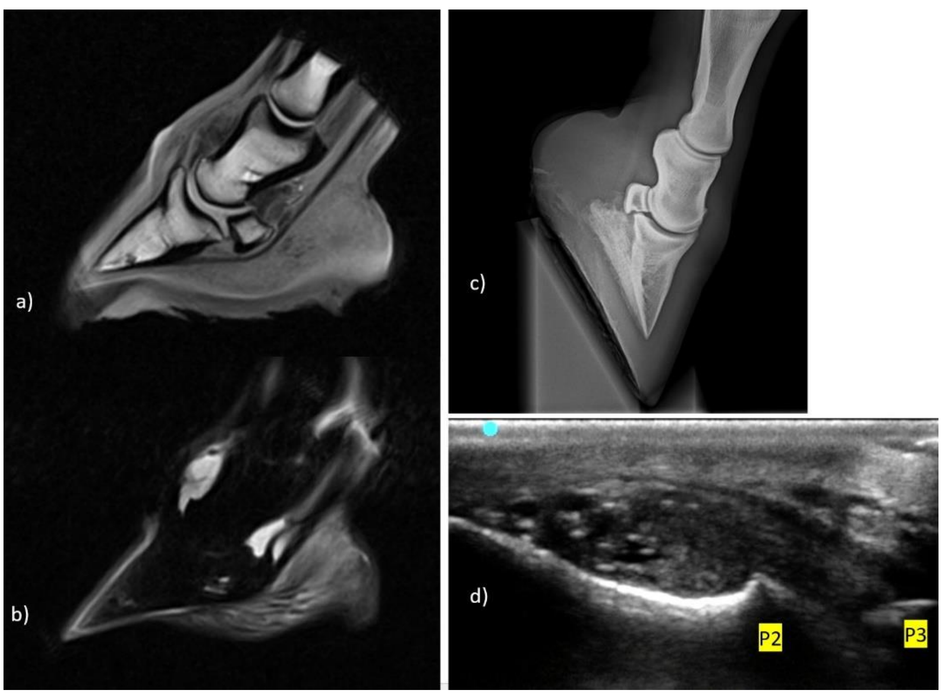

Figure 1. Diagnostic imaging modalities. a) MRI T1W GRE and b) MRI STIR FSE FAST showed a proliferative synovitis with moderate to severe effusion of the coffin joint. c) Lateromedial radiographic appearance of the joint. d) Ultrasound examination revealed numerous hyperechogenic ‘’spots’’ inside the joint and thickening of the joint capsule.

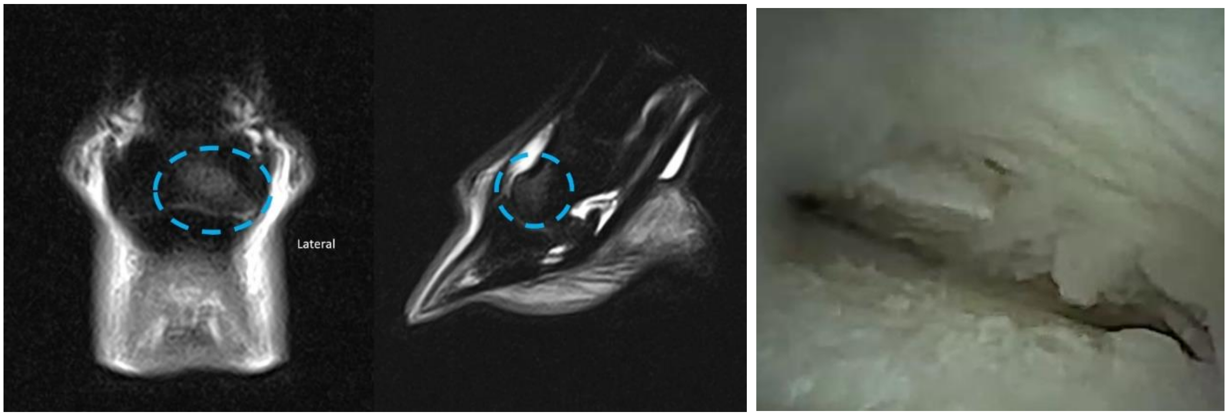

Figure 2. MRI examination STIR FSE FAST, where a clearly distinguish subchondral bone lesion appeared in the lateral aspect of the distal pastern bone. This lesion usually correlates with articular cartilage defect (osteochondral defect). On the right image the defect arthroscopically. Diffuse cartilage defects are recognized. This horse returned to his previous performance.

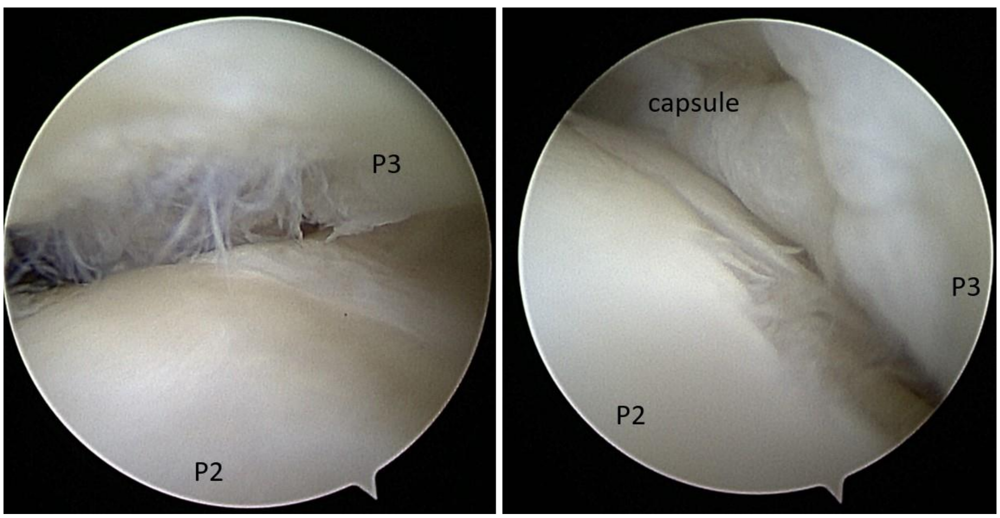

Figure 3. Arthroscopic findings of the dorsal pouch of coffin joint of figure 1. There is a large fibrillation area in the distal part of the extensor process (left picture) and a full-thickness articular cartilage defect in the pastern bone (right picture). Additionally, the synovium is thick and protrudes into joint. This horse returned to the previous performance. P2= middle phalanx, pastern bone. P3= distal phalanx, pedal bone.

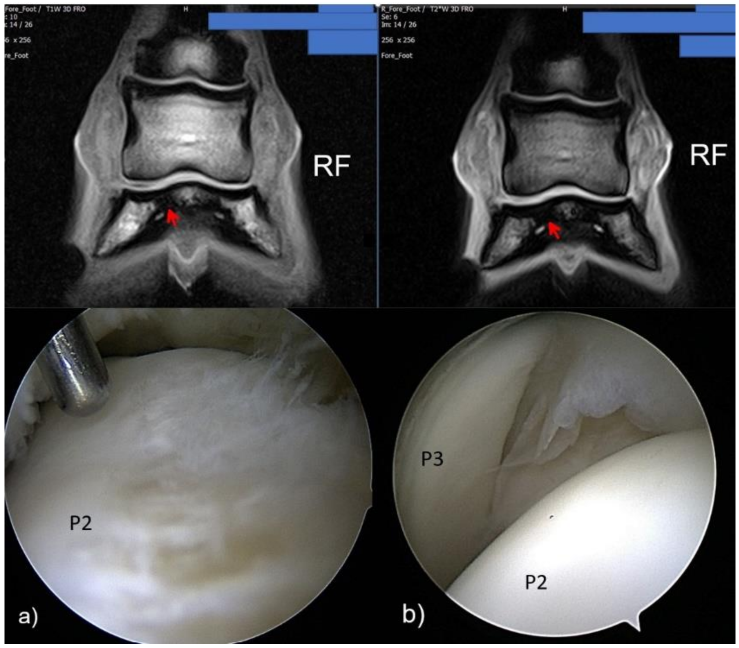

Figure 4. Top: images of T1 and T2* MRI. Lateral is to the right. There is a lesion in the medial aspect of the distal phalanx, which has moderate T1 and T2* hypo intensity. Below: The arthroscopic findings of the same joint. a) The pastern area has a focal, full-thickness articular cartilage damage. The arthroscopic probe showed chondromalacia in that area. b) In medial aspect of the extensor process (right is medial) a large depression of the cartilage is emerged. This horse remained sound. P2= middle phalanx, pastern bone. P3= distal phalanx, pedal bone.

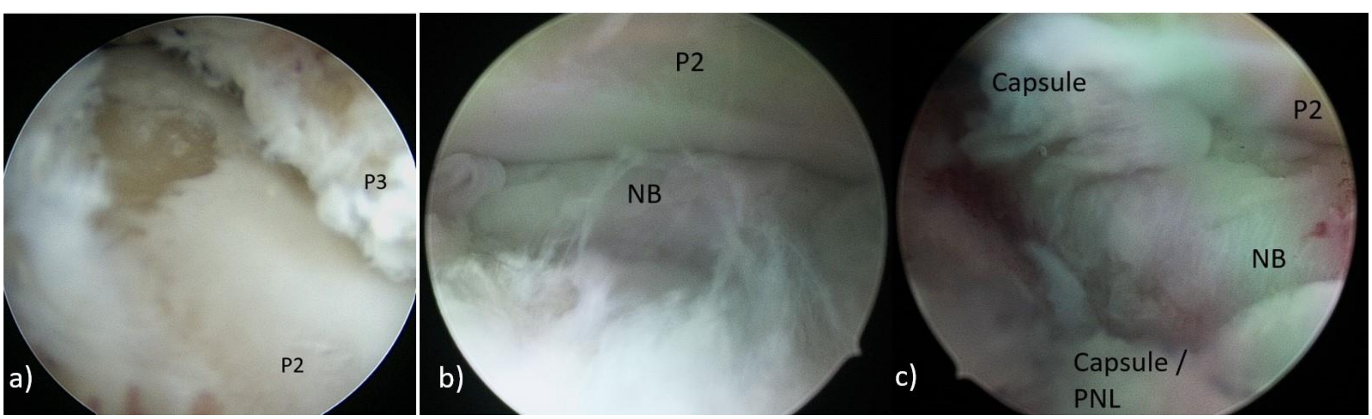

Figure 5. Dorsal a) and palmar b), c) appearance of a coffin joint with full-thickness cartilage erosions and torn capsular tissue. This horse returned to previous performance. NB=navicular bone, P2= middle phalanx, pastern bone. P3= distal phalanx, pedal bone, PNL= proximal ligament of the navicular bone (proximal navicular ligament).

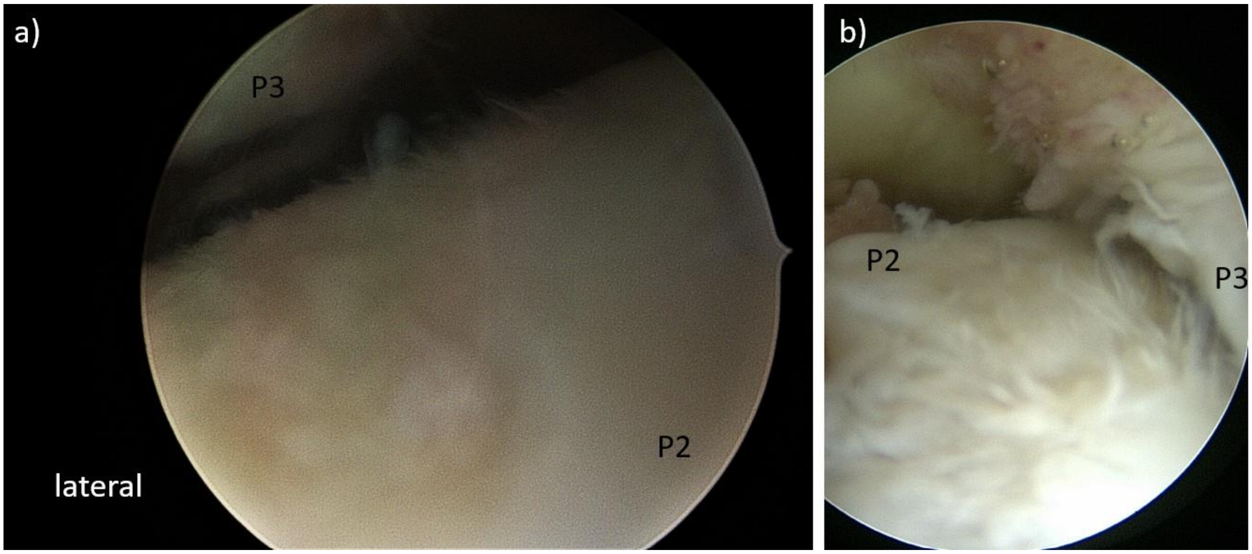

Figure 6. Arthroscopy images of the dorsal pouch of the coffin joint of the figure 2. A diffuse full- and partial-thickness of articular cartilage defects extended nearly to the whole articular surface of the pastern bone. P2= middle phalanx, pastern bone. P3= distal phalanx, pedal bone.

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ACKNOWLEDGEMENTS: There was no proprietary interested, or funding provided for this project.