

## Case Report

# Protocol for Fat Tissue Manipulation in Regenerative Treatment for Bilateral Coxarthrosis in Dogs

Dana Jianu<sup>1</sup>, Gabriel Predoi<sup>2</sup>, Iuliana Ionascu<sup>2</sup>, Oltjon Cobani<sup>1</sup>, Stefan Jianu<sup>1</sup>, Andreea Nita<sup>1</sup>, Mircea Ifrim<sup>3</sup>

<sup>1</sup>ProEstetica Hospital, Bucharest, Romania

<sup>2</sup>Faculty of Veterinary Medicine, Bucharest, Romania

<sup>3</sup>Romanian Academy of Medical Sciences, Bucharest, Romania

### REZUMAT

#### *Protocolul de manipulare a țesutului adipos în tratamentul coxartrozei la câini*

Protocolul de manipulare a țesutului adipos este important în tratamentul coxartrozei, în care se utilizează celule stem autologe din țesutul adipos și PRP. Din 2014, împreună cu Facultatea de Medicină Veterinară București, am început un studiu prospectiv referitor la terapii regenerative în bolile osteoarticulare degenerative. Relatăm cazul unui câine metis de 9 ani cu coxartroză bilaterală, secundară displaziei coxo-femorale de grad III- IV, diagnosticată clinic și radiologic, în tratament cu antiinflamatoare de 3 ani. Tehnica chirurgicală: 120 ml țesut adipos a fost prelevat și centrifugat, parțial procesat (80 ml), folosind echipamentul Ingeneron (USA) pentru obținerea CSDA în scopul injectării intra, periarticulară și intravenoasă. PRP s-a injectat intra și periarticular. 6 ml de țesut adipos centrifugat au fost utilizați pentru a se obține "nanofat" (o emulsie foarte fin triturată) în scopul injectării intra și periarticulare. Câinele a tolerat bine procedura efectuată sub anestezie generală cu IOT. Terapia antiinflamatoare a fost întreruptă imediat după. Evaluarea radiologică la 2 luni după intervenție a evidențiat îmbunătățiri notabile, câinele nu a mai prezentat durere și dificultate la mers. Rezultatele preliminare sunt promițătoare, mai multe date sunt necesare pentru concluzii relevante.

**Cuvinte cheie:** coxartroza, țesut adipos, celule stem ADSCS, PRP

### ABSTRACT

The manipulation protocol of fat tissue that uses adipose derived stem cells (ADSCs), fat tissue and PRP, is important in the treatment of coxarthrosis in dogs. From 2014, in association with Faculty of Veterinary Medicine of Bucharest, we started a prospective study regarding regenerative therapies in degenerative osteoarticular diseases. We like to present the case of a 9 years old Crossbreed which presented osteoarthicular degenerative conditions, diagnosed by clinical and radiographic examination and treated with anti-inflammatory for 3 years. Surgical technique: A quantity of 120 ml of adipose tissue was harvested and processed, using the Ingeneron equipment(USA), for intrarticular, periarticular and intravenous administration. PRP was injected intrarticular. 6cc of adipose tissue were transformed in "nanofat" (a very fine emulsion) in order to be injected intra and periarticular. The dog tolerated very well the treatment. The anti-inflammatory therapy was interrupted. The radiographic aspect of the coxofemoral joints, 2 months after the administration of ADSC, fat graft and PRP, showed improvement, the dog no longer presented pain and lameness in conjunction with fat soft. Preliminary results suggest that ADSC therapy seems to be a novel and effective treatment, nevertheless more data are necessary.

**Key words:** coxarthrosis, adipose tissue, stem cell

**Corresponding author:**

Dana Jianu, MD  
ProEstetica Hospital, Bucharest, Romania

e-mail: djianu02@gmail.com

## BACKGROUND

Hip dysplasia commonly leads to osteoarthritis of the hip joint (coxarthrosis) and these two diseases are two of the most frequently encountered problems in veterinary practice. The available treatment modalities are varied and range from drugs to alleviate pain and inflammation to surgical techniques, such as hip replacement, and various physical therapy measures.

The research regarding therapy using adipose derived stem cells (ADSC) in veterinary medicine, started in 2002. Advances in understanding of the biology of adult stem cells have attracted the attention of the biomedical research community, including those studying osteoarthritis. (1) Autologous adult stem cells are immunologically compatible, can be harvested from a variety of sources, including bone marrow and adipose tissue (1), and have no ethical issues related to their use.

Veterinarians have used ADSC therapy to treat tendon and ligament injuries and joint disease in horses since 2004. (2-4) Studies and anecdotal clinical experience with more than 2,000 horses till 2007 and more than 4000 horses till 2010 demonstrated that ADSC therapy helps horses with ligament and tendon injuries. (2-5) Based on scientific evidence and therapeutic success in horses, from 2007 veterinarians began to use regenerative medicine to treat similar conditions in dogs, including osteoarthritis. (collecting of adipose tissue, sent for processing at Vet Stem Laboratories and administered strictly intra-articular). (3)

All these data encouraged us to start a prospective study, from 2014, in association with Faculty of Veterinary Medicine of Bucharest, for bilateral coxarthrosis treated with ADSC therapy.

## MATERIAL AND METHODS

Bibi, a nine years old, female Crossbreed, was diagnosed at 5 years old with bilateral coxarthrosis secondary to hip dysplasia of stage III-IV, which received multiple treatments with anti-inflammatory (Fig. 1). Clinically it accused for the last 2 years pain during walking and when adopting and leaving decubitus position, difficulty when climbing and descending the stairs, persistent lameness mostly on the left hind limb. X-RAY exam showed bilateral coxarthrosis with degenerative phenomena (severe ramollissement of the articular cartilages of femoral head) and thickened femoral neck with exaggerated development of the acetabular roof for the correction of the capsular ligament. (Fig. 2) It received Cosequin for all this symptoms.

The process consists in:

- Choosing and marking of the donor area for the harvesting of adipose tissue (lumbosacral region) (Fig. 3)
- Infiltration of the donor site with Kleine solution



Figure 1. Bibi 9 years crossbreed



Figure 2. Bilateral coxarthrosis X-ray image



Figure 3. Elected area for the harvesting

- Collecting the adipose tissue using Khouri cannulas (2 mm, 12 holes). 120 ml of adipose tissue was collected and centrifuged, resulting 80 ml adipose suspension. (Fig. 4)
- Extraction of ADSC using Ingeneron equipment (USA). The adipose tissue suspension is mixed with Ringer's Lactate and collagenase enzyme



Figure 4. Collecting the adipose tissue

(Matrase 2.5ml) and centrifuged according to the Ingeneron protocol (warming, processing, concentration 3 time) (Fig. 5) 6 ml ADSC were extracted from 80 ml adipose suspension

- 5 ml ADSC were mixed with 1 ml „nanofat” (adipose tissue after 30 passages through 3 ml syringes)(Fig. 6)
- PRP processing: 8.5 ml blood collected in a tube with 1.5 anticoagulant, Glofinn Kit (Finland). Double centrifugation 1200G (RCF)/2600RPM for 5 and 10 minutes. 2 ml PRP result

ADSC administration:

1. 1 ml intra-articular and 2.5 ml peri-articular (coxo-femoral joint);
2. 1 ml ADSC in 59 ml Ringer’s lactate slowly intravenous 60 ml/30 min;
3. 2 ml PRP periarticular left hind limb (most affected).

### RESULTS

The patient tolerated very well the harvesting of adipose tissue from the lumbosacrat region and the administration of ADSC, no special care was needed. (Fig. 7).

Macroscopically, we observed that dog adipose tissue is of white-grey color compared with human adipose tissue which is yellow. (Fig. 8)

Postoperatively, the anti-inflammatory therapy was interrupted and on clinical examination the dog no longer presented pain and lameness. (Fig. 9) The radiographic aspect of the coxofemoral joints, 2 months after the administration of ADSC, showed the line shaping of the articular surfaces of the femoral heads. (Fig. 10)

### DISSCUTION AND CONCLUSION

Osteoarthritis is the most common cause of chronic pain in dogs, with more than 20%, or 10 to 12 million dogs, afflicted in the USA at any time. (6-8) In osteoarthritis, there is an overproduction of destructive



Figure 5. Aspect of ADSC sediment at the end of the processing, using Ingeneron equipment



Figure 6. 6 ml of ADSC after mixing with “nanofat”



Figure 7. Bibi, 3 days post-op



Figure 8. Human (left) / canine (right) adipose tissue comparison

and pro-inflammatory mediators relative to the inhibitors, resulting in a balance in favor of catabolism rather than anabolism, which in turn leads to the progressive destruc-



**Figure 9.** Bibi, 53 days post-op



**Figure 10.** Bibi, 53 days post-op X-ray image

tion of articular cartilage. (9)

Clinical experience with osteoarthritis therapy in dogs suggest that NSAIDS, the current therapy, often do not provide complete pain relief. (10-14) Cellular therapy does not rely on a single target receptor or pathway for their action, it functions trophically by secreting cytokines and growth factors and by recruiting endogenous cells to the injured site to enhance healing.

The preliminary results in our patient, regarding pain relief and lameness are in concordance with Linda L. Black, James Gaynor, et al randomized, double-blinded, multicenter, controlled trial from 2007 (15), except that we also injected PRP periarticular and ADSC intravenous and also with Marx C., Silveira MD, Beyer Nardi N retrospective study from 2015 (16). We hope that with this method to achieve rapid and long term results.

Further data and patient need to be observed to establish the real potential of ADSC.

#### REFERENCES

1. Luyten FP: Mesenchymal stem cells in osteoarthritis. *Curr Opin Rheumatol* 16(5):599-603, 2004.
2. Harman R, Cowles B, Orava C, et al: Retrospective review of 62 cases of suspensory ligament injury in sport horses treated with adipose-derived stem and regenerative cell therapy. *Proc Vet Orthop Soc*, 2006.
3. Vet-Stem Inc. Data on file, 2005.
4. Dahlgren LA. Use of adipose derived stem cells in tendon and ligament injuries. *Am Coll Vet Surg Symp Equine Small Anim Proc*: 150-151, 2006 .
5. Nixon A, Dahlgren LA, Haupt J, et al: Effect of adipose-derived nucleated cell fractions on tendon repair in a collagenase-induced tendinitis model. *Am J Vet Res*, 2007
6. Hedhammar A, Olsson SE, et al: Canine hip dysplasia: Study of heritability in 401 litters of GERMANSHEPHERD dogs. *JAVMA* 174:1012-1016,1979
7. Johnson JA, Austin C, Breur GJ: Incidence of canine appendicular musculoskeletal disorders in 16 veterinary teaching hospitals from 1980 to 1989. *Vet Comp Orthop Traumatol* 7:56-69,1994
8. Moore GE, Brukman KD, Carter MN, et al: Causes of death or reasons for euthanasia in military working dogs: 927 cases (1993-1996). *JAVMA* 219:209-214, 2001
9. Mortellaro CM: Pathophysiology of osteoarthritis. *Vet Res Comm* 27(suppl1):75-78,2003
10. Lascelles BD, Main DC: Surgical trauma and chronically painful conditions – within our comfort level but beyond theirs? *JAVMA* 221:215-222, 2002.
11. Budsberg SC, Johnston SA, Schvarz PD, et al: Efficacy of etodolac for treatment of OA of the hip joints in dogs. *JAVMA* 214: 206-210, 1999.
12. Holsinger RH, Parker RB, Beale BS, et al: The therapeutic efficacy of carprofen (Rimadyl-V) in 209 clinical cases of canine degenerative joint disease. *Vet Comp Orthop Traumatol* 5: 140-144, 1992
13. Vasseur P, Johnson A, Budsberg S, et al: Randomized, controlled trial of the efficacy of carprofen, NSAIDS in the treatment of OA in dogs. *JAVMA* 206(6): 807-811, 1995
14. Johnson SA, Budsberg SC: NSAIDS and corticosteroids for the management of canine OA. *Vet Clin North Am Small Anim Pract* 27:841-862,1997.
15. Linda L, James G, et al: Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dog with chronic OA of the coxofemoral joints. *Veterinary Therapeutics*, vol8, 272-282, 2007
16. Marx C, Silveira MD, Beyer Nardi N: Adipose-derived stem cells in veterinary medicine: characterization and therapeutic applications. *Stem Cells Dev*,24(7), 800-813, 2015