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

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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 intra-venous administration. PRP was injected intraarticular. 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

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BACKGROUND

Hip dysplasia commonly leads to osteoarthrosis 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 osteoarthrits. (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 succes in horses, from 2007 veterinaries begun to use regenerative medicine to treat similar conditions in dogs, including osteoarthritis. (collecting of adipose tissue, sent for processing at Vet Stem Laboratories and administrated 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 ramollissment 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
- 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
Matrase 2.5ml and centrifuged according to the Ingenieron 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 lombosacrat 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)

DISCUSSION 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 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-
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