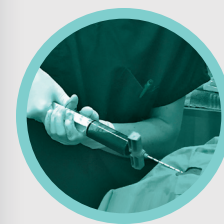


REGENERATIVE MEDICINE FOR SOFT TISSUE INJURY & OSTEOARTHRITIS

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This article reviews information from the session, **Canine Sports Medicine & Rehabilitation**, presented at the NAVC Institute 2015. The NAVC Institute 2017 takes place in Orlando, Florida, May 21 to 26; visit navc.com/institute for further information.



Regenerative medicine therapy has become increasingly popular in both human and veterinary medicine for treatment of multiple disease processes, and recent studies have demonstrated its efficacy in managing numerous orthopedic conditions in humans, dogs, and horses, including osteoarthritis and soft tissue injuries.¹⁻¹³

While some tissues can heal to their original or near-original strength and stiffness, other tissues, such as cartilage, heal poorly. Regenerative medicine has been used to stimulate healing in areas that have not responded to more traditional approaches, helping injured tissues heal to their original or near-original condition.

Regenerative medicine is often used as an adjunct to surgical, medical, and/or rehabilitation therapy in a multimodal approach to treat a condition or injury. As with any other treatment modality, it is important to obtain a definitive diagnosis and tailor an appropriate treatment plan for the patient.

PLATELET-RICH PLASMA THERAPY

Platelet-rich plasma (PRP) is an autogenous fluid concentrate composed primarily of platelets and growth factors. Recent studies indicate that PRP mediates healing by supplying growth factors, cytokines, chemokines, and other bioactive compounds.^{14,15}

PRP is used in both humans and animals to aid healing in numerous tissues. Recent studies have shown PRP to be efficacious in managing many different orthopedic conditions, including osteoarthritis and soft tissue injuries.^{1,2,16-36} One recent study in dogs with partially transected cranial cruciate ligaments and meniscal release demonstrated improved range of motion, decreased pain, and improved limb function for up to 6 months—after treatment with 5 intra-articular injections of leukoreduced PRP—compared with the control group.³⁷

Role in Tissue Healing

Platelets play roles in both hemostasis and wound healing, and PRP has been used as a regenerative medicine therapy to aid in tissue healing.

Platelets contain alpha granules that release growth factors to stimulate other cells of the body to migrate to the area of trauma, facilitating tissue healing. The growth factors—including platelet-derived, vascular endothelial, basic fibroblastic, and epidermal growth factors and transforming growth factor beta1 and beta2—contained within the platelets are important for tissue healing.^{1,2,14-16}

Many growth factors act individually or synergistically to enhance cellular migration and proliferation, angiogenesis, and matrix deposition, which promotes tendon and wound



FIGURE 1. Blood collected for platelet-rich plasma (PRP) processing using an 18-gauge butterfly needle and syringe. Most systems require 10 to 60 mL of blood for PRP processing.

healing, aids in cartilage health, and counteracts the cartilage breakdown associated with osteoarthritis.^{1-3,5,6,15-20,25-28,31,34,36} Platelets also recruit, stimulate, and provide a scaffold for stem cells.^{32,36,38-45}

Components of PRP

Multiple formulations of PRP have been developed and studied. Previous studies in humans suggest that the ideal PRP product should lead to a 4- to 7-fold increase in platelets.^{1,2,14,15,17}

However, platelet concentration is not the only important component of a PRP product. Inclusion or exclusion of mononuclear cells, neutrophils, and red blood cells not only defines an autologous platelet product but also affects the clinical efficacy of the product and the inflammatory responses after PRP injection.^{15,16,19,25-28,46-50} In general, red blood cells and neutrophils should be reduced because they have an inflammatory effect, while the effect of mononuclear cells remains largely unknown.^{42,46,47,51-54}

Recent studies compared key parameters of the PRP product from the commonly used commercial canine PRP systems in healthy, adult canines and found variations in product composition.^{50,55,56}

Performing PRP Therapy

PRP therapy is a minimally invasive procedure that typically can be performed on an outpatient basis. It is often performed as a series of 1 to 3 injections, with 2 weeks between each injection. If



FIGURE 2. Both centrifugation and filtration systems are available for PRP processing. This centrifugation PRP system used for processing produces a leukocyte- and erythrocyte-poor PRP sample.

PRP is being used to manage moderate to severe osteoarthritis, in my experience, about 50% of dogs require more than 1 injection for significant improvement.

To perform PRP therapy:

- Approximately 30 to 60 mL of blood is obtained using an 18-gauge needle or butterfly needle, processed, and prepared for injection (**Figures 1 and 2**).
- Once the PRP is processed, the area to be treated is clipped and aseptically prepared.
- Sedation or general anesthesia may be required for injection, depending on the location of the injection.

For osteoarthritis, PRP joint injections are usually performed without sedation; however, some joints, such as the hip, require sedation and may also require advanced imaging (fluoroscopy) for guidance. If one is not familiar with joint injections, it is wise to sedate patients until comfort with the procedure is obtained.

PRP has been used for tendon and ligament injuries, and is most commonly used for low grade strains or sprains. For soft tissue injuries, ultrasonography guidance is used to ensure accuracy of the injection because PRP is most

effective when administered directly into the lesion. If musculoskeletal ultrasound is not available for obtaining a definitive diagnosis or guidance for treatment administration, referral should be considered. Ultrasound-guided injections also require sedation.

Pain Management & Rehabilitation

The most common side effect is discomfort associated with the injection, which can be managed with pain medications, if needed, and typically resolves within 12 to 24 hours of the injection. However, nonsteroidal anti-inflammatory medication and steroids need to be avoided 2 weeks *before* and *after* PRP therapy because they have been shown to alter platelet function.⁵⁷

A dedicated rehabilitation therapy program is often recommended in conjunction with PRP therapy to achieve and maintain the fullest musculoskeletal potential and performance level. Since the effects of certain modalities on PRP have not been well documented, therapeutic ultrasound, electrostimulation, and hydrotherapy are not recommended during the 4 weeks *following* PRP therapy.

STEM CELL THERAPY

Stem cells are the body's progenitor cells, from which all other cells are derived. Recent studies have shown that stem cells can regenerate and heal injured tissue, decrease inflammation, stimulate new blood supply to support healing, activate resident stem cells, create a scaffold for healing tissue, protect cells from death, and break down scar tissue.^{9,10,58-61}

Mechanisms of Stem Cells

The mechanisms by which stem cells initiate healing within the body are complex. Mesenchymal stem cells (MSCs) become immunosuppressive after activation by soluble factors; then secrete factors that inhibit T-lymphocyte activation and proliferation.^{9,10,58-61} They:

- Use their diverse plasticity to help numerous types of injured tissues regenerate and heal
- Decrease proinflammatory, while increasing anti-inflammatory, mediators
- Secrete bioactive levels of cytokines and growth factors that support angiogenesis, tissue remodeling, differentiation, antiapoptotic events, and neovascularization.^{9,10,58-61}

Orthopedic Treatment Indications

Recent studies have demonstrated the efficacy of stem cell therapy for canine osteoarthritis.⁶²⁻⁶⁴ While many factors play a role in the decision to choose stem cell therapy for a patient, in our experience, patients with severe to end-stage osteoarthritis typically respond better to a combination of stem cell and PRP therapy rather than PRP therapy alone.

A recent study in dogs with elbow osteoarthritis caused by spontaneous fragmented coronoid process demonstrated that those that underwent arthroscopic fragment removal and a proximal ulnar ostectomy and received stromal vascular fraction (SVF; see **Adipose-Derived Stem Cells**, page 56) or allogeneic stem cells had a more favorable outcome than those treated with surgery alone.⁶²

In another recent study, dogs with hip osteoarthritis that received a single intra-articular injection of adipose-derived cultured stem cells had a better outcome than control patients and those that received plasma rich in growth factors (PRGF).¹²

Similarly, other recent studies also demonstrated superiority in osteoarthritic dogs treated with adipose-derived cultured stem cells over control patients and those treated with PRGF on controlled blinded force platform analysis.^{13,63} A recent study in a dog with a gastrocnemius strain concluded that stem cell therapy with a custom, progressive, dynamic orthosis may be a viable, minimally invasive treatment option.⁶⁴

Sources of Stem Cells

Stem cells that can be obtained from the patient's own body are called *autologous adult-derived MSCs*. The most common places from which to harvest adult-derived MSCs are the patient's bone marrow or adipose tissue (**Figures 3 and 4**, page 56). Both bone marrow-derived and adipose-derived stem cells can differentiate into cartilage, bone, tendons, and ligaments. To date, no evidence supports superiority of one over the other in terms of viability or efficacy of the derived stem cells. However, adipose tissue may be a preferred source in dogs (see **Adipose-Derived Stem Cells**).

Stem Cell Procedure

Once the sample is obtained, it is processed and prepared for injection. Both bone marrow-derived stem cells and adipose-derived stem cells can be processed onsite or shipped to a university or private company for processing, culturing, and banking for future use.⁶⁵

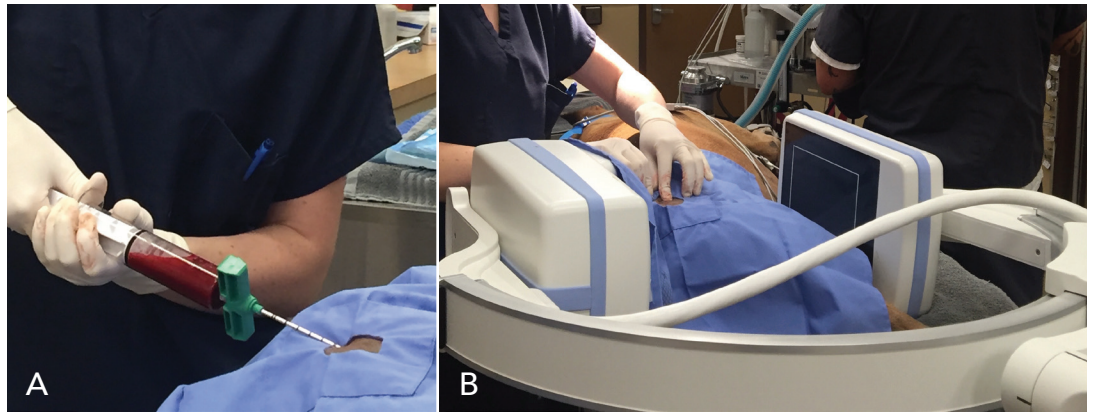


FIGURE 3. Bone marrow collection (A) from the proximal femur with fluoroscopic guidance (B) for processing of bone marrow aspirate concentrate (BMAC).

As with other forms of regenerative medicine, stem cell therapy is a minimally invasive procedure that typically can be performed on an outpatient basis with or without sedation, depending on the location of the injection. In addition, because recent studies have shown that PRP recruits and stimulates stem cells, PRP is often combined with stem cells before injection to both activate and act as a scaffold for the stem cells.^{43,66-71}

Adipose-Derived Stem Cells

Almost all veterinary research has focused on adult stem cells, specifically MSCs, derived from bone marrow (BM-MSCs) or adipose tissue. Adipose tissue may be a preferred source in dogs for several reasons, including ease of access, low morbidity and pain associated with collection, and high-yielding MSC count (especially falciform) (Figure 4).

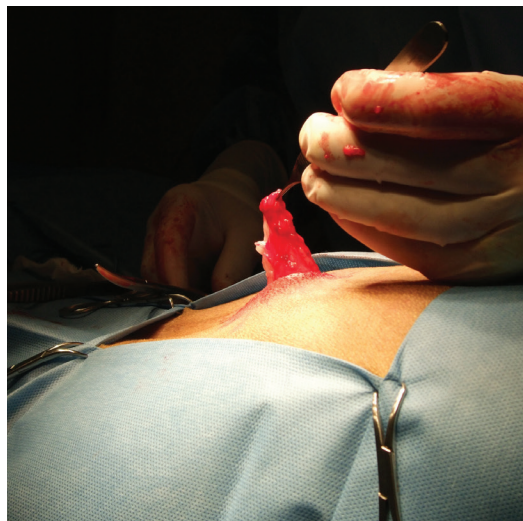


FIGURE 4. Collection of adipose tissue from the falciform ligament for processing of stromal vascular fraction (SVF).

The cells isolated from the adipose tissue include not only the MSCs but also endothelial progenitor cells, pericytes, immune cells, fibroblasts, and other growth factor-secreting bioactive cells. The use of this combination of stem cells and other regenerative cells is known as SVF therapy, and this mixture can be injected directly into the injured tissue or joint or can be administered by IV route. However, recent studies have shown that stem cells given by IV do not actually reach joints or injured tissues⁷²; thus, for orthopedic applications, we currently do not recommend administering stem cells IV.

Alternatively, stem cells can be isolated from adipose tissue, cultured, and expanded. This technique, which yields a more homogenous population with a larger quantity of cells for injection, is known as *adipose-derived cultured progenitor cell*, or *ADPC, therapy*. To date, no studies show superiority of adipose-derived SVF versus culture-expanded adipose-derived MSCs for treatment of canine orthopedic conditions.

Bone Marrow-Derived Stem Cells

BM-MSCs are most commonly used in equine regenerative medicine but can also be used in dogs. There are 2 primary techniques for canine BM-MSC therapy: bone marrow aspirate concentrate (BMAC) and cultured-expanded.

Only 2% to 4% of the mononuclear cell population of bone marrow is considered an MSC. The BMAC technique evolved such that the nucleated cellular portion of tissue aspirates obtained from bone marrow was concentrated and then applied to the injured tissue. This therapy is appealing for several reasons:

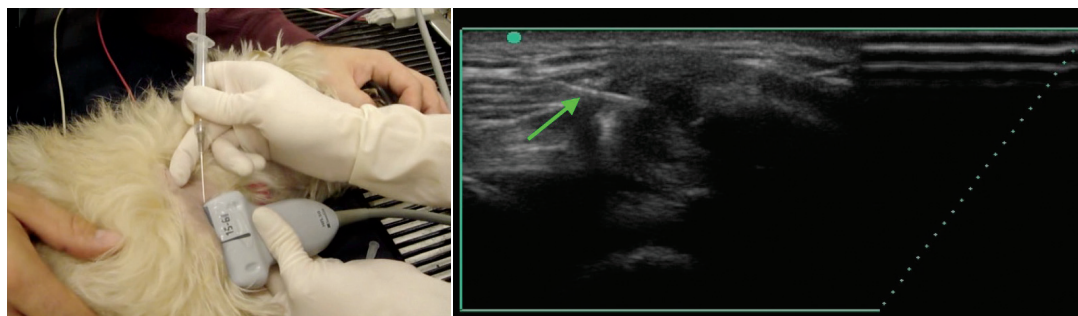


FIGURE 5. Ultrasonography-guided injection of BMAC and PRP into the supraspinatus tendon. The **green arrow** points to the needle being inserted into the supraspinatus tendon.

- The cells can be processed quickly for faster therapeutic application. Processing takes only 1 to 2 hours if it can be performed in-house by using a commercially available kit, which allows the practitioner to initiate therapy 3 to 4 weeks earlier than can be done with culture-expanded cells.
- These cells are not manipulated in culture to the extent that culture-expanded cells are, meaning that they do not undergo adherence, expansion, or trypsinization through multiple passages, which can alter cellular phenotype.
- This cellular therapy also delivers portions of the bone marrow cell pool that could potentially participate in tissue regeneration.

Alternatively, BM-MSCs can be isolated, cultured, and expanded. This yields a more homogenous population with a larger quantity of cells for injection. To date, no studies show superiority of BMAC over culture-expanded BM-MSCs in the treatment of canine orthopedic conditions. In addition, no studies have documented the superiority of BM-MSCs over adipose-derived stem cells or identified the number of stem cells needed for treating soft tissue injuries or osteoarthritis.

REHABILITATION AFTER THERAPY

A dedicated rehabilitation therapy program guided by trained and certified individuals in canine rehabilitation is often recommended for 12 weeks after regenerative medicine therapy, depending on the diagnosed condition. Rehabilitation therapy should be performed weekly in conjunction with an at-home exercise program.

During PRP/Stem Cell Therapy

Rehabilitation therapy helps speed healing by decreasing inflammation and swelling, building muscle mass, increasing range of motion, and

Procedural Pearls

Injection of PRP or stem cells is a minimally invasive procedure that typically can be performed on an outpatient basis.

- Sedation or general anesthesia may be required, depending on the location of the injection; sedation is required for injections administered under ultrasound guidance.
- Joint injections are usually performed without sedation; however, some joints, such as the hip, require sedation and may also require advanced imaging (fluoroscopy) for guidance. If one is not familiar with joint injections, it is wise to sedate patients until comfort with the procedure is obtained.
- For soft tissue injuries, ultrasonography guidance ensures accuracy of the injection because both PRP and stem cells are most effective when administered directly into the site of injury (**Figure 5**).
- The most common side effect is mild discomfort associated with the injection, which typically resolves within 12 to 24 hours.

improving overall comfort. Therapy sessions often include manual therapies, standard isometric exercises, and class IIIb low-level laser therapy, which is recommended because recent studies have shown that this laser therapy can stimulate stem cell differentiation, proliferation, and viability.⁷³

Certain therapies are contraindicated within the first 8 weeks of regenerative medicine therapy because their effects on stem cells and PRP have not been fully studied; these therapies include class IV low-level laser therapy, therapeutic ultrasound, shockwave therapy, neuromuscular electrical stimulation/transcutaneous electrical neurostimulation, and nonsteroidal anti-inflammatory drugs.

After PRP/Stem Cell Therapy

Underwater treadmill therapy can usually be initiated 8 weeks after the start of rehabilitation therapy.

Once the tissue has healed, as confirmed via orthopedic examination, gait analysis, and diagnostic ultrasonography or needle arthroscopy,

the rehabilitation program focuses on strengthening and conditioning.

After appropriate muscle mass has been attained, dogs are cleared for retraining and return to sport. On average, patients treated with regenerative medicine therapy typically return to competition or normal activity within 4 to 6 months of treatment.

IN SUMMARY

Regenerative medicine has been used to stimulate healing and help restore injured tissues to their original or near-original condition. Canine regenerative medicine therapy can be used to help treat medial shoulder syndrome, shoulder tendinopathies (eg, supraspinatus tendinopathy or biceps tendinopathy), iliopsoas strain, Achilles tendon injury, early partial cranial cruciate ligament tear, carpal and tarsal ligament injuries, and osteoarthritis.

It is important to obtain a definitive diagnosis and ensure that the patient is an appropriate candidate for regenerative medicine. It is equally important to incorporate a dedicated rehabilitation therapy plan into the recovery process to optimize results from regenerative medicine therapy.

ADPC = adipose-derived cultured progenitor cell; BMAC = bone marrow aspirate concentrate; BM-MSC = bone marrow-derived mesenchymal stem cell; MSC = mesenchymal stem cell; PRGF = plasma rich in growth factors; PRP = platelet-rich plasma; SVF = stromal vascular fraction

References

1. Filardo G, Kon E, Roffi A, et al. Platelet rich plasma: Why intra-articular? A systematic review of preclinical studies and clinical evidence on PRP for joint degeneration. *Knee Surg Sports Traumatol Arthrosc* 2015; 23(9):2459-2474.
2. Hsu WK, Mishra A, Rodeo SR, et al. Platelet-rich plasma in orthopaedic applications: Evidence-based recommendations for treatment. *J Am Acad Orthop Surg* 2013; 21:739-748.
3. Abrams GD, Frank RM, Fortier LA, Cole BJ. Platelet-rich plasma for articular cartilage repair. *Sports Med Arthrosc Rev* 2013; 21:213-219.
4. Cho K, Kim JM, Kim MH, et al. Scintigraphic evaluation of osseointegrative response around calcium phosphate-coated titanium implants in tibia bone: Effect of platelet-rich plasma on bone healing in dogs. *Eur Surg Res* 2013; 51:138-145.
5. Dragoo JL, Wasterlain AS, Braun HJ, Nead KT. Platelet-rich plasma as a treatment for patellar tendinopathy: A double-blind, randomized controlled trial. *Am J Sports Med* 2014; 42(3):610-618.
6. Filardo G, Kon E, Di Martino A, et al. Platelet-rich plasma vs hyaluronic acid to treat knee degenerative pathology: Study design and preliminary results of a randomized controlled trial. *BMC Musculoskelet Disord* 2012; 13(229):1-8.
7. Khoshbin A, Leroux T, Wasserstein D, et al. The efficacy of platelet-rich plasma in the treatment of symptomatic knee osteoarthritis: A systematic review with quantitative synthesis. *Arthroscopy* 2013; 29(12):2037-2048.
8. Randelli P, Arrigoni P, Ragone V, et al. Platelet rich plasma in arthroscopic rotator cuff repair: A prospective RCT study, 2-year follow-up. *J Shoulder Elbow Surg* 2011; 20:518-528.
9. Kristjansson B, Honsawek S. Current perspectives in mesenchymal stem cell therapies for osteoarthritis. *Stem Cells Int* 2014:1-13.
10. Mazor M, Lespessailles E, Coursier R, et al. Mesenchymal stem-cell potential in cartilage repair: An update. *J Cell Mol Med* 2014; 18(12):2340-2350.
11. Sampson S, Batto-van Bemden A, Aufiero D. Stem cell therapies for treatment of cartilage and bone disorders: Osteoarthritis, avascular necrosis, and non-union fractures. *PMR* 2015; 7:S26-S32.
12. Cuervo B, Rubio M, Sopena J, et al. Hip osteoarthritis in dogs: A randomized study using mesenchymal stem cells from adipose tissue and plasma rich in growth factors. *Int J Mol Sci* 2014; 15:13437-13460.
13. Vilar JM, Batista M, Morales M, et al. Assessment of the effect of intraarticular injection of autologous adipose derived mesenchymal stem cells in osteoarthritic dogs using a double blinded force platform analysis. *BMC Vet Res* 2014; 10:143.
14. Boswell SG, Cole BJ, Sundman EA, et al. Platelet-rich plasma: A milieu of bioactive factors. *Arthroscopy* 2012; 28(3):429-439.
15. Dohan Ehrenfest DM, Doglioli P, de Peppo GM, et al. Choukroun's platelet-rich fibrin (PRF) stimulates in vitro proliferation and differentiation of human oral bone mesenchymal stem cell in a dose-dependent way. *Arch Oral Biol* 2010; 55:185-194.
16. McLellan J, Plevin S. Does it matter which platelet-rich plasma we use? *Equine Vet Educ* 2011; 23(2):101-104.
17. Pelletier MH, Malhotra A, Brighton T, et al. Platelet function and constituents of platelet rich plasma. *Int J Sports Med*. 2013; 34:74-80.
18. Sundman EA, Cole BJ, Karas V, et al. The anti-inflammatory and matrix restorative mechanisms of platelet-rich plasma in osteoarthritis. *Am J Sports Med* 2013; 42(1):35-41.
19. Dragoo JL, Braun HJ, Durham JL, et al. Comparison of the acute inflammatory response of two commercial platelet-rich plasma systems in healthy rabbit tendons. *Am J Sports Med* 2012; 40(6):1274-1281.



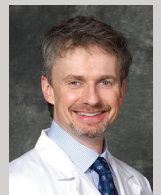
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20. Filardo G, Kon E, Buda R, et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. *Knee Surg Sports Traumatol Arthrosc* 2011; 19:528-535.
21. Franklin S, Cook J. Prospective trial of autologous conditioned plasma versus hyaluronan plus corticosteroid for elbow osteoarthritis in dogs. *Can Vet J* 2013; 54:881-884.
22. Jang SJ, Kim JD, Cha SS. Platelet-rich plasma (PRP) injections as an effective treatment for early osteoarthritis. *Eur J Orthop Surg Traumatol* 2013; 23:573-580.
23. Kon E, Buda R, Filardo G, et al. Platelet-rich plasma: Intra-articular knee injections produced favorable results on degenerative cartilage lesions. *Knee Surg Sports Traumatol Arthrosc* 2010; 18:474-479.
24. Kon E, Mandelbaum B, Buda R, et al. Platelet-rich plasma intra-articular injection versus hyaluronic acid viscosupplementation as treatments for cartilage pathology: From early degeneration to osteoarthritis. *Arthroscopy* 2011; 27(11):1490-1501.
25. McCarrel T, Fortier L. Temporal growth factor release from platelet-rich plasma, trehalose lyophilized platelets, and bone marrow aspirate and their effect on tendon and ligament gene expression. *J Orthop Res* 2009; 27(8):1033-1042.
26. McCarrel TM, Minas T, Fortier LA. Optimization of leukocyte concentration in platelet-rich plasma for the treatment of tendinopathy. *J Bone Joint Surg Am* 2012; 94:e143(1-8).
27. Mishra A, Pavelko T. Treatment of chronic elbow tendinosis with buffered platelet-rich plasma. *Am J Sports Med* 2006; 34(11):1774-1778.
28. Patel S, Shillon MS, Aggarwal S, et al. Treatment with platelet-rich plasma is more effective than placebo for knee osteoarthritis: A prospective, double-blinded, randomized trial. *Am J Sports Med* 2013; 41(2):356-364.
29. Raicissadat SA, Rayegani SM, Babae M, Ghorbani E. The effect of platelet-rich plasma on pain, function, and quality of life of patients with knee osteoarthritis. *Pain Res Treat* 2013; 1:1-7.
30. Sampson S, Gerhardt M, Mandelbaum B. Platelet rich plasma injection grafts for musculoskeletal injuries: A review. *Curr Rev Musculoskelet Med* 2008; 1:165-174.
31. Silva RE, Carmona JU, Rezende CME. Intra-articular injections of autologous platelet concentrates in dogs with surgical reparation of cranial cruciate ligament rupture. *Vet Comp Orthop Traumatol* 2013; 26:122-125.
32. Smith JJ, Ross MW, Smith RKW. Anabolic effects of acellular bone marrow, platelet rich plasma, and serum on equine suspensory ligament fibroblasts in vitro. *Vet Comp Orthop Traumatol* 2006; 19:43-47.
33. Souza TFB, Andrade AL, Ferreira GTNM, et al. Healing and expression of growth factors (TGF- β and PDGF) in canine radial osteotomy gap containing platelet-rich plasma. *Vet Comp Orthop Traumatol* 2012; 25:445-452.
34. Van Buul GM, Koevoet WLM, Kops N, et al. Platelet-rich plasma releasate inhibits inflammatory processes in osteoarthritic chondrocytes. *Am J Sports Med* 2011; 39(11):2362-2370.
35. Xie X, Hua W, Zhao S, et al. The effect of platelet-rich plasma on patterns of gene expression in a dog model of anterior cruciate ligament reconstruction. *J Surg Res* 2013; 180:80-88.
36. Xie X, Wang Y, Zhao C, et al. Comparative evaluation of MSCs from bone marrow and adipose tissue seeded in PRP-derived scaffold for cartilage regeneration. *Biomaterials* 2012; 33:7008-7018.
37. Cook JL, Smith PA, Bozynski CC, et al. Multiple injections of leukoreduced platelet rich plasma reduce pain and functional impairment in a canine model of ACL and meniscal deficiency. *J Orthop Res* 2015; 1-9.
38. Broeckx S, Zimmerman M, Crocetti S, et al. Regenerative therapies for equine degenerative joint disease: A preliminary study. *PLoS One* 2014; 9(1):e85917.
39. Cho HS, Song IH, Park SY, et al. Individual variation in growth factor concentrations in platelet-rich plasma and its influence on human mesenchymal stem cells. *Korean J Lab Med* 2011; 31:212-218.
40. Del Bue M, Riccò S, Ramoni R, et al. Equine adipose-tissue derived mesenchymal stem cells and platelet concentrates: Their association in vitro and in vivo. *Vet Res Commun* 2008; 32(S1):S51- S55.
41. Drengk A, Zapf A, Stürmer EK, et al. Influence of platelet-rich plasma on chondrogenic differentiation and proliferation of chondrocytes and mesenchymal stem cells. *Cells Tissues Organs* 2009; 189:317-326.
42. Dohan Ehrenfest DM, Rasmussen L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leukocyte- and platelet-rich fibrin (L-PRF). *Trends Biotech* 2008; 27(3):158-167.
43. Mishra A, Tummala P, King A, et al. Buffered platelet-rich plasma enhances mesenchymal stem cell proliferation and chondrogenic differentiation. *Tissue Eng Part C Methods* 2009; 15:431-435.
44. Schnabel LV, Lynch ME, Van der Meulen MC, et al. Mesenchymal stem cells and insulin-like growth factor-I gene-enhanced mesenchymal stem cells improve structural aspects of healing in equine flexor digitorum superficialis tendons. *J Orthop Res* 2009; 27(10):1392-1398.
45. Torricelli P, Fini M, Filardo G, et al. Regenerative medicine for the treatment of musculoskeletal overuse injuries in competition horses. *Internat Orthop* 2011; 35:1569-1576.
46. Braun HJ, Kim HJ, Chu CR, Dragoo JL. The effect of platelet-rich plasma formulations and blood products on human synoviocytes. *Am J Sports Med* 2014; 42(5):1204-1210.
47. Sundman EA, Cole BJ, Fortier LA. Growth factor and catabolic cytokine concentrations are influenced by the cellular composition of platelet-rich plasma. *Am J Sports Med* 2013; 39(10):2135-2140.
48. Sundman EA, Boswell SG, Schnabel LV, et al. Increasing platelet concentrations in leukocyte-reduced platelet-rich plasma decrease collagen gene synthesis in tendons. *Am J Sports Med* 2013; 42(1):35-41.
49. Castillo TN, Pouliot MA, Kim HJ, Dragoo JL. Comparison of growth factor and platelet concentrations from commercial platelet-rich plasma separation systems. *Am J Sports Med* 2011; 39(2):266-271.
50. Stief M, Gottschalk J, Ionita JC, et al. Concentration of platelets and growth factors in canine autologous conditioned plasma. *Vet Comp Orthop Traumatol* 2011; 24:285-290.
51. Boswell SG, Schnabel LV, Mohammed HO, et al. Increasing platelet concentrations in leukocyte-reduced platelet-rich plasma decrease collagen gene synthesis in tendons. *Am J Sports Med* 2013; 42(1):42-49.
52. Cavallo C, Filardo G, Mariani E, et al. Comparison of platelet-rich plasma formulations for cartilage healing. *J Bone Joint Surg Am* 2014; 96:423-429.
53. Naldini A, Morena E, Fimiani M, et al. The effects of autologous platelet gel on inflammatory cytokine response in human peripheral blood mononuclear cells. *Platelets* 2008; 19(4):268-274.
54. Yoshida R, Murray MM. Peripheral blood mononuclear cells enhance the anabolic effects of platelet-rich plasma on anterior cruciate ligament fibroblasts. *J Orthop Res* 2013; 31(1):29-34.
55. Franklin SP, Garner BC, Cook JL. Characteristics of canine platelet-rich plasma prepared with five commercially available systems. *Am J Vet Res* 2015; 76(9):822-827.
56. Carr BJ, Canapp SO, Mason DM, et al. Canine platelet rich plasma systems: A multicenter, prospective analysis. *Front Vet Sci* 2015; 2:73.
57. Schippinger G, Pruller F, Divjak M, et al. Autologous platelet-rich plasma preparations: Influence of nonsteroidal anti-inflammatory drugs on platelet function. *Orthop J of Sports Med* 2015; 3(6):1-6.
58. Grassel S, Lorenz J. Tissue engineering strategies to repair chondral and osteochondral tissue in osteoarthritis: Use of mesenchymal stem cells. *Curr Rheumatol Rep* 2014; 16:452.
59. Ham O, Lee CY, Kim R, et al. Therapeutic potential of differentiated mesenchymal stem cells for treatment of osteoarthritis. *Int J Mol Sci* 2015; 16:14961-14978.
60. Wang W, Cao W. Treatment of osteoarthritis with mesenchymal stem cells. *Sci China Life Sci* 2014; 57(6):586-595.
61. Wolfstätt JI, Cole BJ, Ogilvie-Harris DJ, et al. Current concepts:

- The role of mesenchymal stem cells in the management of knee osteoarthritis. *Sports Health* 2015; 7(1):38-44.
62. Kiefer K, Wucherer KL, Pluhar GE, Conzemius MG. Autologous and allogeneic stem cells as adjuvant therapy for osteoarthritis caused by spontaneous fragmented coronoid process in dogs. *VOS Symposium Proc*, 2013.
 63. Vilar JM, Morales M, Santana A, et al. Controlled, blinded force platform analysis of the effect of intraarticular injection of autologous adipose-derived mesenchymal stem cells associated to PRGF-Endoret in osteoarthritic dogs. *BMC Vet Res* 2013; 9:131.
 64. Case JB, Palmer R, Valdes-Martinez A, et al. Gastrocnemius tendon strain in a dog treated with autologous mesenchymal stem cells and a custom orthosis. *Vet Surg* 2013; 42:355-360.
 65. Martinello T, Bronzini I, Maccatrozzo L, et al. Canine adipose-derived-mesenchymal stem cells do not lose stem features after a long-term cryopreservation. *Res Vet Sci* 2011; 91:18-24.
 66. Carvalho AM, Badial PR, Alvarez LE, et al. Equine tendonitis therapy using mesenchymal stem cells and platelet concentrations: A randomized controlled trial. *Stem Cell Res Ther* 2013; 22(4):85.
 67. Chen L, Dong SW, Liu JP, et al. Synergy of tendon stem cells and platelet-rich-plasma in tendon healing. *J Orthop Res* 2012; 30(6):991-997.
 68. Uysal CA, Tobita M, Hyakusoku H, Mizuno H. Adipose-derived stem cells enhance primary tendon repair: Biomechanical and immunohistochemical evaluation. *J Plast Reconstr Aesthet Surg* 2012; 65(12):1712-1719.
 69. Manning CN, Schwartz AG, Liu W, et al. Controlled delivery of mesenchymal stem cells and growth factors using a nanofiber scaffold for tendon repair. *Acta Biomater* 2013; 9(6):6905-6914.
 70. Yun JH, Han SH, Choi SH, et al. Effects of bone marrow-derived mesenchymal stem cells and platelet-rich plasma on bone regeneration for osseointegration of dental implants: Preliminary study in canine three-wall intrabony defects. *J Biomed Mater Res B Appl Biomater* 2014; 102(5):1021-1030.
 71. Tobita M, Uysal CA, Guo X, et al. Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model. *Cryotherapy* 2013; 15(12):1517-1526.
 72. Harting MT, Jimenez F, Xue H, et al. Intravenous mesenchymal stem cell therapy for traumatic brain injury. *J Neurosurg* 2009; 110(6):1189-1197.
 73. Ginani F, Soares DM, Barreto MP, Barboza CA. Effect of low-level laser therapy on mesenchymal stem cell proliferation: A systemic review. *Lasers Med Sci* 2015; 30(8):2189-2194.