How to Incorporate Stem Cell Therapy in Your Practice

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  - Jennifer Barrett, DVM, PhD, DACVS
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• Arthrex
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• PulseVet
• CRT

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Objectives

• Stem cell therapy as a treatment for orthopedic – sports medicine conditions in the canine

• Overview of the technology

• Indications & applications

• Current canine literature & studies

• Combination stem cell – PRP therapy

• Retrospective stem cell - PRP study analysis

University at Buffalo
School of Medicine
Regenerative Medicine
“Soap-box”

• Must have a definitive diagnosis and treat the underlying condition to get the best response
• Not a “Silver bullet”
• Combination therapy is the key
  • Medical Management
  • Surgical treatment
  • Stem cell therapy / Platelet Rich Plasma
  • Rehabilitation therapy
How the Coming Revolution in STem Cells Could Save Your Life

Diabetes, Heart Disease, Parkinson's.
Stem Cell Therapy
Controversy

Is 'stem cell therapy' becoming 21st century snake oil?
Jeffery ND, Granger N.
Click Here to Visit Official Site of Stem Cell Therapy Cream

Better walk away with a wrinkle free despite uptight years loaded behind your back! skin than go fighting the odds of passing time. It is akin to the myth of Sisyphus - a pursuit utterly useless and futile. But the signs and symptoms of age can be defeated only if you know the right key to unlock the mystery. Just rely on Stem Cell to attain a smooth glow and a shimmery appeal on your skin. It is a perfect way to get rid of the rigid and uptight signs and symptoms of aging and reveal a super gorgeous glow on your skin.

Each bottle of this wrinkle lift solution is packed with a miracle solution to roll up your rumpled skin and fill the fissures making the criss-crossing lines, wrinkles and folds on your skin. This solution is equipped with vital ingredients and anti-oxidants which can make the skin regain its charming freshness and rejuvenate the skin.

To get a flawless countenance with a wrinkle free skin, just smear down some drops of this anti-aging cream and be ready to dazzle like a diva. To verify the claims made by the composers of Stem Cell, just check the list highlighted below:

Click Here to Visit Official Site of Stem Cell Therapy Cream

- Boosts the production of new skin cells
- Increases collagen production
- Revives the skin repair system naturally
- Fills in lines and wrinkles
- Softens and soothes your skin
- Increases elasticity of the skin
- Improves texture
- Steals away years from the skin
Be 100% Confident in the Safety of Your Stem Cell Treatment!

When it comes to the safety of a stem cell transplant, it is completely safe. Here are some facts you should understand, to be more confident of our stem cell treatment procedures:

- None of our performed procedures has ever resulted in a negative outcome.
- Our practices have been carefully designed in order to avoid complications of Graft-Versus-Host disease as well as other side effects. The stem cells are also acquired directly from the patient to avoid risk of infection.
- We make sure that all stem cells obtained, until they are transplanted, are kept in a closed sterile environment. There is absolutely no culture and manipulation of the stem cells before activation.
- Our goal is to improve our patients’ overall medical condition through our stem cell treatment practice and in return, also greatly improve the quality of life for our patients.
Germany

Why Did Kobe Go to Germany?
An aging star and the new procedure that could revolutionize sports medicine
Biologic approaches to enhance rotator cuff healing after injury.

Isaac C, Gharabeh B, Witt M, Wright VJ, Huard J.

Stem Cell Research Center, University of Pittsburgh, Pittsburgh, PA, USA.

The combination of stem cells and growth factors resulted in enhanced repair that emulated uninjured tissue.
By definition, a stem cell is characterized by its ability to self-renew and its ability to differentiate along multiple lineage pathways.

Ideally, a stem cell for regenerative medicinal applications should meet the following criteria:

- Can be found in abundant quantities (millions to billions of cells)
- Can be harvested by a minimally invasive procedure
- Can be differentiated along multiple cell lineage pathways in a regulatable and reproducible manner.
- Can be safely and effectively transplanted to either an autologous or allogeneic host
- Can be manufactured in accordance with current Good Manufacturing Practice guidelines
Regenerative Medicine - Canine Stem Cell Terminology

Autologous stem cell therapy

• Tissue is harvested, cells are isolated and expanded, and then returned to the patient

• Canine sources of autologous stem cells:
  • Bone marrow
  • Adipose tissue
Regenerative Medicine - Canine

Stem Cell Terminology

Bone marrow derived

- Contains hematopoietic stem cells (forms all the types of blood cells in the body)
- Bone marrow stromal stem cells (mesenchymal stem cells)
  - Can generate bone, cartilage, fat, connective tissue
- Culture expanded
- Bone Marrow Aspirate Concentrate (BMAC)
- Bone marrow derived more commonly clinically used in horses
Regenerative Medicine
Stem Cell Terminology

Adipose derived

- Adipose-derived stem/stromal cells (ASCs)
  - International Fat Applied Technology Society

- Adipose stromal stem cells (mesenchymal stem cells)
  - Can generate bone, cartilage, fat, connective tissue

- Culture expanded

- Stromal vascular fraction (SVF)

- Adipose derived more commonly used/reported in dogs and humans
Regenerative Medicine
Stem Cell Terminology

Allogeneic stem cell therapy

• Tissue is harvested from a donor, cells are isolated and expanded, and then returned to a different person/animal

• When cultured changes the cell markers which “disables the immune response”

• Cells are available for use more rapidly, as they must proliferate only for a few days rather than weeks after harvest.

• "off the shelf"

• Some people/animals produce more and "better" stem cells than others (meaning the cells' characteristics are more predictable and consistent), so using allogeneic cells from a donor can mean higher-quality cells

• Stems cells age as the individual ages, therefore we want “our kids stem cells”

• According to the FDA allogeneic cells are considered a “drug”
Regenerative Medicine – Canine Stem Cell Therapy

**Stem Cells**
- Contribute to generating new tissue
- Chemotactic for progenitor cells
- Supply growth factors
- Make extracellular matrix
- Angiogenesis
- Anti-apoptosis
- Anti-inflammatory
- Anti-fibrotic

**Regeneration and replacement of injured / diseased tissue**
- via cell differentiation
- modulation of signaling pathways via cytokines to decrease progression of disease
- resident stem cell activation and recruitment

Suresh et al., Tissue Eng, 2003
Regenerative Medicine - Canine Stem Cell Therapy

• May be used clinically in the canine to promote tissue regeneration in areas of limited healing capability

• Indications:
  • Osteoarthritis
  • Tendon injury
  • Ligament injury
  • Spinal cord injury

• Contra-indications:
  • Neoplasia
Stem Cell Therapy
Evidence Based Medicine

Canine???
Stem Cell Therapy - Canine Evidence Based Medicine

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.

Vilar JM¹, Morales M, Santana A, Spinella G, Rubio M, Cuervo B, Cugat R, Carrillo JM.

Use of autologous mesenchymal stem cells derived from bone marrow for the treatment of naturally injured spinal cord in dogs.

Penha EM¹, Meira CS², Guimarães ET³, Mendonça MV⁴, Gravely FA⁵, Pinheiro CM⁶, Pinheiro TM⁶, Barroquín-Melo SM⁷, Ribeiro-Dos-Santos R⁸, Soares MB⁹.

Gastrocnemius tendon strain in a dog treated with autologous mesenchymal stem cells and a custom orthosis.

Case JB¹, Palmer R, Valdes-Martinez A, Egger EL, Haussler KK.

Comparing the osteogenic potential of canine mesenchymal stem cells derived from adipose tissues, bone marrow, umbilical cord blood, and Wharton's jelly for treating bone defects.

Kang BJ¹, Ryu HH, Park SS, Kovama Y, Kikuchi M, Woo HM, Kim WH, Kweon OK.

Homing and efficacy of intra-articular injection of autologous mesenchymal stem cells in experimental chondral defects in dogs.

Mokbel A¹, El-Tookhy O, Shamaa AA, Sabry D, Rashed L, Mostafa A.
Stem Cell Therapy - Canine Evidence Based Medicine

Production of canine mesenchymal stem cells from adipose tissue and their application in dogs with chronic osteoarthritis of the humeroradial joints.
Guercio A¹, Di Marco P, Casella S, Cannella V, Russotto L, Purpari G, Di Bella S, Piccione G.

Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: a randomized, double-blinded, multicenter, controlled trial.
Black LL¹, Gaynor J, Gahring D, Adams C, Aron D, Harman S, Gingerich DA, Harman R.

Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs.

A comparison of autologous and allogenic bone marrow-derived mesenchymal stem cell transplantation in canine spinal cord injury.
Jung DJ¹, Ha J, Kang BT, Kim JW, Quan FS, Lee JH, Woo EJ, Park HM.

Functional recovery and neural differentiation after transplantation of allogenic adipose-derived stem cells in a canine model of acute spinal cord injury.
Ryu HH¹, Lim JH, Byeon YE, Park JR, Seo MS, Lee YW, Kim WH, Kang KS, Kweon OK.
Canine: Cartilage
• Purpose was to verify the likelihood of homing of intra-articularly injected mesenchymal stem cells (MSCs) and its involvement in the healing process of experimentally induced, acute and chronic, partial chondral defects in dogs.

• Partial thickness chondral defects were created on the lateral femoral condyle of stifle joint in dogs.

• MSCs were harvested in a separate procedure, labelled with green fluorescent protein (GFP) using monster GFP vector and suspended in buffer phosphate solution for intra-articular (IA) injection.

• Dogs were divided into three groups.
  • Group I - control
  • Group II- single IA injection of MSCs one day
  • Group III- one month after creating the defect

• Morphological, histological, and fluorescence analysis was performed at 2 and 8 weeks post-surgery for group I, and 2 and 8 weeks post-treatment, for the cell-treated groups

**RESULTS:** Recovery was significant both clinically and histologically in the two cell-treated groups (Group II and III) compared to the control (Group I), (p<0.001). In the meantime, Group-II showed better results at 8 weeks than Group III (p=0.01). Homing was confirmed by the incorporation of injected GFP-labelled MSCs within the newly formed cartilage.

**CONCLUSIONS:** The obtained results prove that the use of IA injection of autologous MSCs is a viable option for treating partial cartilage defects. Cell labelling gave evidence to the certainty of cell homing within the neocartilage of all treated cases and the participation in the reparative process.
Stem Cell Therapy
Evidence Based Medicine

Canine:

Osteoarthritis
• Autologous and Allogeneic Stromal Cells as Adjuvant Therapy for Osteoarthritis Caused by Spontaneous Fragmented Coronoid Process in Dogs

• Kiefer K, Wucherer K, Fitzpatrick N, Pluhar E, Conzemius M.
Elbow OA Study
Kiefer K, et al.

- Forty dogs diagnosed with FCP and secondary OA via radiographs and MRI were randomized into four groups (n = 10/group)
- All dogs underwent arthroscopy and fragment removal.
  - Control Group: no further treatment
  - Group one: PUO
  - Group two: PUO + autologous stromal vascular fraction
  - Group three: PUO + allogeneic fat derived stromal cells
- Each dog had force platform gait analysis, Canine Brief Pain Inventory (CBPI) questionnaires, and a dGEMRIC intensity score before and six months after intervention.
Elbow OA Study
Kiefer K, et al.

- Successful outcomes were found in 30% of cases that received arthroscopy alone, arthroscopy + PUO, and arthroscopy + PUO + SVF.

- Successful outcomes were found in 70% of cases that received arthroscopy + PUO + allogeneic cells.

<table>
<thead>
<tr>
<th>Group</th>
<th>% Improvement</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOC</td>
<td>28.7</td>
</tr>
<tr>
<td>SOC + PUO</td>
<td>-1</td>
</tr>
<tr>
<td>SOC + PUO + SVF</td>
<td>14.9</td>
</tr>
<tr>
<td>SOC + PUO + ALLO</td>
<td>15.0</td>
</tr>
</tbody>
</table>

Standard of Care (SOC) 30%
SOC+PUO 30%
SOC + PUO + SVF 30%
SOC + PUO + ALLO 70%
Elbow OA Study
Kiefer K, et al

- This pilot data suggests that intra-articular autologous and allogeneic injections are safe and well tolerated.

- Allogeneic stromal cell therapy may improve clinical outcome in dogs with OA secondary to a fragmented coronoid process.
Stem Cell Therapy
Evidence Based Medicine

Canine:

Tendon
4-year-old spayed female Border Collie

Bone-marrow derived, cultured autologous mesenchymal stem cells

Injected into the tendon core lesion.

A custom, progressive, dynamic orthosis was fit to the tarsus.

Serial orthopedic examinations and ultrasonography as well as long-term force-plate gait analysis were utilized for follow up.

**RESULTS:** Lameness subjectively resolved and peak vertical force increased from 43% to 92% of the contralateral pelvic limb. Serial ultrasonographic examinations revealed improved but incomplete restoration of normal linear fiber pattern of the gastrocnemius tendon.

Findings suggest that autologous mesenchymal stem cell transplantation with custom, progressive, dynamic orthosis may be a viable, minimally invasive treatment option.
Stem Cell Therapy
Evidence Based Medicine

Canine:
Ligament

Partial ACL tears were created (medial band)

Group 1- intra-articular injection of fresh whole bone marrow cells (BMCs)

Group 2 - cultured mesenchymal stem cells (MSCs)

Group 3 – saline

IA injections performed 1 week after transection
Biomechanically, the tensile strength in the BMC group reached near normal levels at 4 weeks.

TGF-β1 in the BMC group were increased significantly compared with that of the saline group.
Donor cells expressing green fluorescent protein were detected in ACLs of BMC and MSC groups at 4 weeks.

Fluorescence photo-micrograph showing green fluorescent protein–positive cells.
• ACLs appeared almost normal histologically

• Significantly more mature spindle cells in the BMC group than in the saline group at 4 weeks
• In conclusion, intra-articular bone marrow transplantation using fresh whole BMCs is an effective treatment for ACL partial rupture.

• This therapy is easy to apply in a clinical setting because no culture system is required for collecting MSCs.
Partial CCL Tear
Stem Cell – PRP Therapy

- Early partial tears
  - arthroscopic assessment
  - <25-50% rupture of craniomedial band
  - Intact caudolateral band

- Culture expanded ADPC with PRP combination or BMAC with PRP combination

- Used in combination with rehabilitation therapy

- Used in combination with a functional stifle brace (based on severity)

- Pre and post treatment object gait analysis (baseline, 30, 60, 90 days)

- 90 day second look arthroscopy
CCL Pre-SCT

CCL 90 – day post-SCT
CCL Partial Tear
Stem Cell – PRP Therapy

“Cool” Seattle, Washington Pre SCT-PRP tx
CCL Partial Tear
Stem Cell – PRP Therapy

“Cool” Seattle, Washington

90-day second look
Objective Gait Analysis

Temporal-spatial gait analysis
Light V, et al. AJVR. 2010

Pre-injection
Objective Gait Analysis

90 day post-injection

Temporal-spatial gait analysis
Light V, et al. AJVR. 2010
Stem Cell Therapy
Evidence Based Medicine

Canine:
Spinal cord injury
Thirty adult Beagle dogs with experimentally-induced spinal cord injury (SCI)
- control group = 10,
- autologous group = 10
- allogenic group = 10

Prelabeled bone-marrow derived MSCs were intrathecally transplanted through the lumbar spinal cord into the injured lesion at a density of $1 \times 10^7$ cells 7 days after SCI.

Neurological examination, behavior analysis (based on Olby scores), MRI at 1, 2, and 5 weeks post SCI, histopathological examination and immunofluorescence analysis.

Results: Immunofluorescence analysis revealed that prelabeled autologous and allogenic MSCs were detected in the injured lesions both at 1 and 4 weeks after transplantation.

Neurological signs of dogs, size of injured spinal cord on MRI, and histopathological findings in both autologous and allogenic groups were improved compared with those in control group.

This study demonstrates that both autologous and allogenic MSC transplantation could be clinically useful therapeutic approaches for treating SCI.
Four dogs with chronic unfavorable follow-up evaluations (no neurological gain for 6 months after surgery) were included in this study.

Bone marrow was collected and stem cells culture expanded.

MSC were surgically administered into the spinal cord. The animals were clinically evaluated and examined by nuclear magnetic resonance.

Results:

Ten days after the surgical procedure and MSC transplantation progressive recovery of the panniculus reflex and diminished superficial and deep pain response were observed.

By the 18th month a remarkable clinical amelioration accompanied by improved movement was observed in three of the four dogs.

No clinical gain was associated with alterations in magnetic resonance imaging.

Results indicate that MSC are potential candidates for the stem cell therapy following spinal cord injury.
Stem Cell Therapy - Canine

How to incorporate in your practice:

Adipose derived
Regenerative Medicine - Canine Adipose Stem/Stromal Cell Therapy

- Procedure: Stromal Vascular Fraction (SVF)
- 20-50g of adipose tissue-subcutaneous tissue
- Falciform my recommended location (key hole incision)
  - Adequate sample even in lean dogs
  - Less complications and morbidity then other locations (seroma; large incisional pain; cosmetics)
- In-house processing or mail-out to commercial laboratory
- ASCs are enzymatically and/or mechanically isolated
- Residual cells are banked for future use (product dependent)
Regenerative Medicine
Adipose Stem/Stromal Cell Therapy

Adipose tissue as a source of stem cells:

- Readily available
- Yields a high volume of tissue
- Low morbidity
- Able to differentiate into all cell lines
Patient factors influencing the concentration of stromal vascular fraction (SVF) for adipose-derived stromal cell (ASC) therapy in dogs.

Astor DE, Hoelzler MG, Harman R, Bastian RP.

Table II

<table>
<thead>
<tr>
<th>Collection site</th>
<th>Number of dogs</th>
<th>Median</th>
<th>Range</th>
<th>Mean +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falciform</td>
<td>687</td>
<td>3.38 × 10⁵</td>
<td>4.19 × 10⁴ to 2.09 × 10⁶</td>
<td>3.94 × 10⁵ +/- 2.43 × 10⁵</td>
</tr>
<tr>
<td>Thoracic wall</td>
<td>477</td>
<td>4.00 × 10⁵</td>
<td>5.59 × 10⁴ to 1.34 × 10⁶</td>
<td>4.49 × 10⁵ +/- 2.02 × 10⁵</td>
</tr>
<tr>
<td>Inguinal</td>
<td>101</td>
<td>4.56 × 10⁵</td>
<td>8.39 × 10⁴ to 3.41 × 10⁵</td>
<td>6.23 × 10⁵ +/- 5.36 × 10⁵</td>
</tr>
</tbody>
</table>

The median VCPG of tissue at the falciform location was significantly lower than tissue and the inguinal locations (P < 0.001). There was no significant difference between the thoracic wall and the inguinal locations (P = 0.022). (Bonferroni adjusted alpha = 0.01).

Table V

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Number of dogs</th>
<th>Age range</th>
<th>Median</th>
<th>Range</th>
<th>Mean +/- SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>316</td>
<td>1 to 5 y</td>
<td>3.94 × 10⁵</td>
<td>4.19 × 10⁴ to 3.41 × 10⁵</td>
<td>4.81 × 10⁵ +/- 1.10 × 10⁵</td>
</tr>
<tr>
<td>2</td>
<td>317</td>
<td>5 to 8 y</td>
<td>3.51 × 10⁵</td>
<td>4.23 × 10⁴ to 1.86 × 10⁵</td>
<td>4.04 × 10⁵ +/- 1.09 × 10⁵</td>
</tr>
<tr>
<td>3</td>
<td>316</td>
<td>8 to 11 y</td>
<td>3.75 × 10⁵</td>
<td>5.59 × 10⁴ to 1.61 × 10⁵</td>
<td>4.25 × 10⁵ +/- 1.08 × 10⁵</td>
</tr>
<tr>
<td>4</td>
<td>316</td>
<td>11 to 18 y</td>
<td>3.49 × 10⁵</td>
<td>6.77 × 10⁴ to 2.01 × 10⁵</td>
<td>4.11 × 10⁵ +/- 1.07 × 10⁵</td>
</tr>
</tbody>
</table>

Tissue from dogs in age quartile 1 had a significantly higher median VCPG than quartile 2 (P = 0.003) and age quartile 4 (P = 0.002). SD — standard deviation.
• 1265 dogs underwent adipose collection surgeries by veterinarians for processing by the Vet-Stem laboratory

• Data on cell counts and patient factors were collected.

• Age significantly affected the Viable cells per gram (VCPG), younger dogs having a significantly higher VCPG than older dogs.

• Adipose tissue collected at the falciform location had significantly fewer VCPG than tissue collected at the thoracic wall and inguinal locations.

• Altered dogs had significantly fewer VCPG in tissue collected at the falciform location than at the thoracic wall and inguinal locations when compared to intact dogs.

• Specific patient factors should be taken into consideration in order to obtain the maximal yield of VCPG from an adipose collection procedure.
Automated Extraction of Stem Cells from Canine Falciform Adipose Tissue

Point of care Extraction of Stromal Vascular Fraction
Automated Characterization of Cells:

- Fully automated
- Closed system
- Rapid processing (<60min)
- Easy to use

Cell Recovery & Viability

Mesenchymal cell enumeration

ADAM Automated Cell Counter

Accuri C6 Flow Cytometer
~7X More Cells Recovered than Previously Known

Initial results ($n=2$):

<table>
<thead>
<tr>
<th></th>
<th>Dog 1</th>
<th>Dog 2</th>
<th>Literature*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Falciform Tissue Size</td>
<td>21 g</td>
<td>23 g</td>
<td>91 ± 49 g</td>
</tr>
<tr>
<td>Viable Cell Count</td>
<td>2.4 M/g</td>
<td>3.5 M/g</td>
<td>0.39 ± 0.24 M/g</td>
</tr>
<tr>
<td>Total Viable Cells Recovered</td>
<td>51 M</td>
<td>81 M</td>
<td>36 M</td>
</tr>
</tbody>
</table>

*Donniel E Astor et al., Patient Factors Influencing the Concentration of Stromal Vascular Fraction for Adipose Derived Stromal Cell Therapy in Dogs. The Canadian Journal of Veterinary Research (77): 177-182, 2013*
Rich in Mesenchymal Cells Compared to Bone Marrow

- ~20% Canine SVF cells are mesenchymal cells according to flow cytometry, compared to <0.5% in bone marrow. **40X more concentrated!**
- ~10M MSC are extracted from 21g of falciform fat vs. ~2M from 30ml of bone marrow.
- Anti-human antibodies used in flow cytometry. Cross reaction with Canine to be evaluated. Ongoing cell culture experiments will determine the adequacy of such antibodies against Canine.

**Mageed AS et al., Isolation of Large Numbers of Mesenchymal Stem Cells from the Washings of Bone Marrow Collection Bags: Characterization of Fresh Mesenchymal Stem Cells. Transplantation (8): 1019-26, 2007
Regenerative Medicine
Adipose Stem/Stromal Cell Therapy

Stromal Vascular Fraction (SVF) systems/companies

• VetStem - Commercial mail-out product

• MediVet; StemLogix; InGeneron; CRT in-house countertop systems
  • Mechanical separation
  • Enzymatic and mechanical separation
Regenerative Medicine - Canine Adipose Stem Cell Therapy

**Procedure: Culture expanded ASCs**

- 10-20g adipose tissue

- Falciform is recommended location (key hole incision)
  - Adequate sample even in lean dogs
  - Less complications and morbidity than other locations (seroma)

- Shipped Fed-x in premade packaging on ice

- Mesenchymal cells are isolated, cultured and returned in 10-14 days

- Residual cells are banked for future use
Regenerative Medicine
Adipose Stem/Stromal Cell Therapy

- Adipose samples sent fed-x to local university for culture expansion
- More homogeneous population
- 10-14 day turn around for injection

Cell Biology International 33 (2009) 100-105
• Fully characterize canine adipose derived-MSC (cA-MSC) and examine the effects of cryopreservation on their stemness features

• Each sample of cA-MSC was analyzed immediately and then again after being frozen in liquid nitrogen for one year

• After the cryopreservation
  • cells conserved their fibroblast-like morphology &
  • alkaline phosphatase positivity and CD expression
  • showed a lower proliferation ratio and a lower telomerase activity in comparison with fresh cells

• Cryopreservation protocol did not change the cA-MSC adipogenic, osteogenic and myogenic differentiative potential.
# Regenerative Medicine

## Adipose Stem/Stromal Cell Therapy

- **Cultured expanded ASCs**
  - **Advantages**
    - Validated techniques
    - Homogeneous population
    - 5-10 million cells
    - More objective data
    - Safety and controlled processing standards
    - Banked Cells
  - **Disadvantages**
    - Turn around time
    - Shipping issues
    - Cost (more expensive)
    - Some believe cultured cells have less “horsepower”
    - Cells are manipulated and may considered a “drug” by the FDA in the future

- **Stromal Vascular Fraction (SVF)**
  - **Advantages**
    - Same day treatment
    - No shipping issues
    - Cost (less expensive)
    - Some believe fresh cells have greater “horsepower”
    - No cell manipulation
    - Scaffold
  - **Disadvantages**
    - Minimal validated systems
    - Heterogeneous population
    - Lower cell count
    - Safety and controlled standards in a clinical practice
    - Less objective data
    - Typically no banked cells
Stem Cell Therapy - Canine

How to incorporate in your practice:

Bone marrow derived
Regenerative Medicine - Canine Bone Marrow Stem Cell Therapy

Procedure: Culture expanded BMSCs

- 5-10 mls of bone marrow
- 16-18 gauge bone marrow needle
- Femur, Ilium, or humerus
- Shipped FedEx in premade packaging on ice
- Mesenchymal cells are isolated, cultured and returned in 21 days
- Residual cells are banked for future use
Regenerative Medicine - Canine Bone Marrow Stem Cell Therapy

Procedure: Bone Marrow Aspirate Concentrate (BMAC)

- 30-60 mls of bone marrow
- 16-18 gauge bone marrow needle
- Femur, Ilium, or humerus
- In-house processing (15 minutes) or mail-out to commercial laboratory
- BMSCs are mechanically isolated
Regenerative Medicine - Canine Bone Marrow Stem Cell Therapy

Bone Marrow Aspirate Concentrate (BMAC)

- Harvest and CRT in-house countertop systems
- Mechanical separation
Regenerative Medicine - Canine Bone Marrow Stem Cell Therapy

• Cultured expanded BMSCs

• Advantages
  • Validated techniques
  • Homogeneous population
  • 5-10 million cells
  • More objective data
  • Safety and controlled processing standards
  • Banked Cells

• Disadvantages
  • Turn around time
  • Shipping issues
  • Cost (more expensive)
  • Some believe cultured cells have less “horsepower”
  • Cells are manipulated and may considered a “drug” by the FDA in the future

• Bone Marrow Aspirate Concentrate (BMAC)

• Advantages
  • Same day treatment
  • No shipping issues
  • Cost (less expensive)
  • Some believe fresh cells have greater “horsepower”
  • No cell manipulation

• Disadvantages
  • Minimal validated systems
  • Heterogeneous population
  • Lower cell count
  • Safety and controlled standards in a clinical practice
  • Less objective data
  • Typically no banked cells
Regenerative Medicine
ADSC and BMSC injections

Intra-articular treatments:

• Cells administered via routine intra-articular injection

• Aseptic technique

• 22 gauge needle

• Awake or sedation

• Landmark guided “blind injection”

• Fluoroscopic guided

• Digital radiography guided
Regenerative Medicine - Canine ADSC and BMSC injections

Soft tissue injections (tendons and ligaments):

• Cells are administered ultrasound guided

• Sedation or brief anesthesia

• Aseptic technique

• 22 gauge needle

• Fenestration technique
Stem cell IV administration
Adverse reaction

- Nine healthy beagle dogs distributed equally (n = 3 per group) and randomly into three groups
  - Group A: allogeneic BM-derived MSCs intravenously: $2 \times 10^6$ once
  - Group B: $2 \times 10^7$ once
  - Group C: $2 \times 10^6$ for three consecutive days

- Various laboratory examinations, CT features, and histopathology were evaluated prior to receiving MSCs and on days 1, 3, and 7 post transplantation.

- Results:
  - Only one dog had clinical signs (vomiting) during and after MSCs transplantation.
  - CT revealed pulmonary parenchymal changes in one dog and histopathologic examination revealed pulmonary parenchymal edema and hemorrhage in four dogs
  - The presence of pulmonary thromboembolism was not detected in either examination.

- Conclusion: The presence of pulmonary edema and hemorrhage is a possible adverse reactions after intravenous MSCs transplantation; however these results should be cautiously interpreted.
Combination Therapy: Regenerative Medicine

- **Stem Cells (cultured adipose derived progenitor cells)**
  - Contribute to generating new tissue
  - Chemotactic for progenitor cells
  - Supply growth factors
  - Make extracellular matrix
  - Angiogenesis
  - Anti-apoptosis
  - Anti-inflammatory
  - Anti-fibrotic

- **Platelet Rich Plasma (PRP)**
  - Positive effects on angiogenesis and extracellular matrix remodeling
  - Stem cell recruitment and chemotaxis
  - Cell proliferation and differentiation
  - Potent source of growth factors important in regenerative process
  - Fibrin for matrix / scaffold
Optimizing Tendon Regeneration

Adipose Progenitor cells plus PRP

- Cells for regeneration:
  - tendon matrix
  - inhibit scarring (trophic effects)
  - improve healing

- Growth factors:
  - Cell proliferation
  - Trophic factors

- Scaffold to provide template for cell attachment
Stem Cell – PRP Combination
Evidence Based Medicine


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.

Yun JH, Han SH, Choi SH, Lee MH, Lee SJ, Song SU, Oh N.

Periodontal tissue regeneration by combined implantation of adipose tissue-derived stem cells and platelet-rich plasma in a canine model.

Tobita M, Uysal CA, Guo X, Hyakusoku H, Mizuno H.

CONCLUSIONS: These findings suggest that a combination of autologous ASCs and PRP promotes periodontal tissue regeneration that develops the appropriate architecture for this complex tissue.
Conclusions

• Stem cell therapy may be considered as a treatment option for orthopedic conditions in the canine

• Not a “silver bullet”
  • Rehabilitation therapy

• Increased need for training/proficiency in canine musculoskeletal ultrasound and intra-articular injections

• More questions than answers:
  • Bone marrow vs adipose derived
  • Cultured vs non-cultured
  • Optimal cell counts
  • Combination therapy (APC & PRP)
  • Placebo controlled???
Questions?
Thank You!!!
Adipose Progenitor Cell Therapy & Platelet Rich Plasma Combination

• Supraspinatus Tendinopathy

• Retrospective Data:

• 327 supraspinatus tendiopathy cases

• 116 were treated with ADPC – PRP combination therapy

• 66 unilateral; 50 bilateral

• 55 unilateral cases without concurrent elbow or shoulder pathology with follow-up msk ultrasound

• 25 unilateral cases without concurrent elbow or shoulder pathology with follow-up objective gait analysis

Jennifer Barrett, DVM, PhD, DACVS, DACVSMR
Debra Canapp, DVM, CCRT, CVA, DACVSMR
Victor Ibrahim, MD
Signalment

Age Range: 1 to 14 years
Average Age – 6.4 years (median - 6 years)

Female: Male = 55:55

Intact: Altered = 2:2

n=55
<table>
<thead>
<tr>
<th>Breed</th>
<th>Population</th>
<th>Percent</th>
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<tr>
<td>Basenji</td>
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<tr>
<td>Bernese Mountain Dog</td>
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<tr>
<td>Border Collie</td>
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<td>23.6%</td>
</tr>
<tr>
<td>Boxer</td>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>Corgi</td>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>German Shepherd Dog</td>
<td>7</td>
<td>12.7%</td>
</tr>
<tr>
<td>Golden Retriever</td>
<td>3</td>
<td>5.5%</td>
</tr>
<tr>
<td>Greater Swiss Mountain Dog</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Labrador Retriever</td>
<td>13</td>
<td>23.6%</td>
</tr>
<tr>
<td>Mixed</td>
<td>5</td>
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</tr>
<tr>
<td>Poodle – Standard</td>
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<tr>
<td>Rhodesian Ridgeback</td>
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<td>1.8%</td>
</tr>
<tr>
<td>Rottweiler</td>
<td>1</td>
<td>1.8%</td>
</tr>
<tr>
<td>Wheaten Terrier</td>
<td>1</td>
<td>1.8%</td>
</tr>
</tbody>
</table>

n=55
Occupation

- Sport/Performance: 49.1%
- Companion: 50.9%

- Agility: 37.0%
- Field Trial: 7.4%
- Flyball: 7.4%
- Herding: 7.4%
- Hunting: 3.7%
- Obedience: 18.5%
- Rally: 7.4%
- Show: 11.1%

n=55
History

n=55

Population

40%  23.6%  16.4%  9%
History
Previous Treatment

<table>
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<tr>
<th>Population</th>
<th>61.8%</th>
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<tbody>
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<td>45.5%</td>
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n=55
ADPC / PRP Combo
Supraspinatus Tendinopathy

- Definitive Diagnosis:
  - Radiographs (shoulder and elbow)
  - Musculoskeletal ultrasound
  - +/-MRI
  - +/-Arthroscopy (elbow and shoulder)

- Treatment:
  - Ultrasound guided injections (fenestration technique)
  - Standardized post treatment rehabilitation therapy

- Objective Follow Up:
  - Ultrasound every 30 days
  - Objective gait analysis every 30 days
  - Goniometric measurements and limb circumference every 30 days

- Pre Treatment Objective Measures:
  - Objective gait analysis (GAIT4)
  - Goniometric measurements and limb circumference
  - Pre-treatment ultrasound measurements (objective scoring system)

- Tissue Collection:
  - Adipose collection (8-12 grams) from falciform and culture expanded
  - Blood collections from jugular for PRP
Supraspinatus Tendinopathy
Diagnostics - Radiographs

Mineralization 12.4%
Supraspinatus Tendinopathy

Diagnostics - Ultrasound

- Greater tubercle of humerus
- Mixed Echogenicity representing tendinopathy
Supraspinatus Tendinopathy
Pre-treatment Ultrasound

L supra enlarged

Mixed echogenicity

R SUPRA = 0.50CM²
Musculoskeletal Ultrasound
Objective Grading Scale

- Quantitative Ultrasound Shoulder Pathology Rating Scale (USPRS)

- Modified (USPRS)

- ST Cross-Sectional Area at a Standardized Level
  Canapp D, Barrett J, Ibrahim V

0 = Normal fibrillar pattern and echogenicity
1 = Mild loss of fibrillar pattern and/or echogenicity
2 = Moderate loss of fibrillar pattern and/or echogenicity
3 = Calcified area of tendon
4 = Clear tear partial thickness
5 = Clear tear full thickness
Objective Gait Analysis

Temporal-spatial gait analysis
Light V, et al. AJVR. 2010

Velocity: 120.6 **
Reach (LR): 25.4 / 20.9

<table>
<thead>
<tr>
<th></th>
<th>Stance Time (sec)</th>
<th>%GC (%)</th>
<th>Total Pressure Index</th>
<th>TPI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Front</td>
<td>38.3 (0.01)</td>
<td>54.8 (17.7)</td>
<td>24.1 (0.02)</td>
<td>13.1 %</td>
</tr>
<tr>
<td>Right Front</td>
<td>42.0 (0.04)</td>
<td>63.3 (20.5)</td>
<td>54.9 (0.01)</td>
<td>41.7 %</td>
</tr>
<tr>
<td>Left Hind</td>
<td>37.0 (0.05)</td>
<td>55.1 (14.6)</td>
<td>26.3 (0.02)</td>
<td>21.9 %</td>
</tr>
<tr>
<td>Right Hind</td>
<td>37.1 (0.04)</td>
<td>55.3 (2.76)</td>
<td>26.5 (1.86)</td>
<td>19.2 %</td>
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Symmetry Ratio
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<thead>
<tr>
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<th>Front/Hind</th>
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<th>Left Front/Right Front</th>
<th>Left Hind/Right Hind</th>
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<tbody>
<tr>
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<td>1.08</td>
<td>1.49</td>
<td>1.21</td>
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<td>0.95</td>
<td>0.95</td>
<td>0.64</td>
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<td>0.90</td>
<td>0.90</td>
<td>0.43</td>
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<td>1.10</td>
<td>1.10</td>
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<td></td>
<td>1.03</td>
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<td>0.86</td>
<td>0.80</td>
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<td>1.13</td>
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<tr>
<td></td>
<td>1.13</td>
<td>1.13</td>
<td>1.99</td>
<td>1.54</td>
</tr>
</tbody>
</table>

Total Pressure Index
<table>
<thead>
<tr>
<th></th>
<th>TPI %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left Front</td>
<td>18.1 %</td>
</tr>
<tr>
<td>Right Front</td>
<td>41.7 %</td>
</tr>
<tr>
<td>Left Hind</td>
<td>21.0 %</td>
</tr>
<tr>
<td>Right Hind</td>
<td>19.2 %</td>
</tr>
</tbody>
</table>
Regenerative Medicine
Adipose Progenitor Cell Therapy

- **Procedure:** Cultured expanded cells

  - 8-12g adipose tissue (falciform) suspended in adipose cell media

  - Shipped Fed-Ex in premade packaging on ice

  - Mesenchymal cells are isolated, cultured and returned in 10-14 days

  - Residual cells are banked for future use
Regenerative Medicine Tissue Processing

• VA Tech Regenerative Medicine Services Proprietary Techniques

• ADPC Processing:
  • 8-12g of adipose suspended in adipose cell media
  • Adipose was mechanically and enzymatically separated to release ADPCs
  • Nucleated cells counted and cultured in stem cell media
  • Daily monitoring for adherence, growth and phenotype
  • Once 70% confluent cells detached, washed and suspended in autologous PRP

• PRP Processing:
  30 mls of blood (>30 lb dog) in CPDA
  Prepared from anti-coagulated blood
  Centrifugation to obtain a four-fold increase in platelets
  80% reduction in white blood cells
  Platelet and WBC counts performed to verify concentrations
Regenerative Medicine

Adipose & Blood Collection

Culture & Expansion of Adipose Progenitor Cells

Resuspension for Injection in PRP or ACS

Rehabilitation Program

Ultrasound-guided Injection into Lesion

Rehabilitation Program
Ultrasound Guided Injection
Supraspinatus Fenestration Injection Technique


Post-injection Standardized Rehab Program

- Manual therapy
- Modalities
- Therapeutic home exercise program
- Hydrotherapy
- Strengthening techniques
  - Isometric exercises
- End stage eccentric exercises
  - Trotting and waling down hills

PhyS Ther, 2004
J Orthop Sports Phys Ther, 1994
Lasers Surg Med, 2005
Statistical Analysis

- Objective gait analysis: Total pressure index (TPI) of treated forelimb was compared to the contra-lateral (non-affected) forelimb at baseline and at each follow-up evaluation.
- Repeated measures ANOVA (significance $p<0.05$)

- Dogs returned at ~30, 60, and 90 days for objective follow-up
  - musculoskeletal ultrasound ($n=55$)
  - objective gait analysis ($n=25$)

- Ultrasound: Cross-sectional area of the treated (ADPC-PRP injected) tendon was compared to the contralateral (non-affected) tendon at baseline and at each follow-up evaluation.
APDC – PRP Combination
Results - Ultrasound

• Of the 55 unilateral supraspinatus cases treated with ADPC – PRP combination therapy:
  • Reduction in SST size was noted in all cases
  • 82% reached the contralateral “normal” size
  • All cases showed improvement in fiber pattern
Individual Results of Unaffected and Affected Supraspinatus Measurements

n=55
Response to Regenerative Medicine
Musculoskeletal Ultrasound

Unilateral Supraspinatus Tendinopathy

<table>
<thead>
<tr>
<th>Cross Sectional Area (cm²)</th>
<th>Injured</th>
<th>Contralateral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Exam</td>
<td>0.45</td>
<td>0.40</td>
</tr>
<tr>
<td>4-6 weeks</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>6-12 weeks</td>
<td>0.40</td>
<td>0.40</td>
</tr>
<tr>
<td>&gt;12 weeks</td>
<td>0.40</td>
<td>0.40</td>
</tr>
</tbody>
</table>

*Significant difference
Supraspinatus Tendinopathy Over Time

Response to Regenerative Medicine
Musculoskeletal Ultrasound

Cross Sectional Area (cm²)

Initial | 4-6 weeks | 6-12 weeks

0.00 | 0.18 | 0.35 | 0.53 | 0.70

* indicates significant difference.
Of 55 unilateral supraspinatus cases treated with ADPC – PRP, we had follow-up gait analysis on 25 cases at 90 days post-treatment:

- 88% (22 cases) were sound on G4D compared to baseline
- 12% (3 cases) improved but not sound
Response to Regenerative Medicine

Objective Gait Analysis

Day 0
Day 90

n=25

Individual Results of Affected Forelimb

TPI %
Response to Regenerative Medicine
Objective Gait Analysis

Unilateral Supraspinatus Tendinopathy

<table>
<thead>
<tr>
<th></th>
<th>Pre-Tx</th>
<th>Post-Tx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injured</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>Contralateral</td>
<td>31</td>
<td>32</td>
</tr>
</tbody>
</table>

Bar chart showing Total Pressure Index before (Pre-Tx) and after (Post-Tx) treatment.
Clinical Impressions

- Results of combination ADPC-PRP treatments are promising for dogs with supraspinatus tendinopathies
- Appears to offer an option for cases that have failed to respond to conservative management and rehab therapy
- Treatment option for tendons with disrupted fiber patterns and core lesions
- Treatment requires proficiency in diagnostic musculoskeletal ultrasound
Clinical Impressions.....

• Unexpected findings:
  • mineralization “resorption”
  • nodule/bulge reduction

• In addition to favorable follow-up US findings and gait analysis, ability for return to sport is encouraging

• Sporting and performance dogs that are > 4 months post-tx have returned to competition (96.4% dogs returned to sport)
Unexpected Findings
Unexpected Findings

• Similar results in gait analysis and ultrasound with BMAC and PRP combination therapy for the past 12 months
  • 17 cases

• Used as an alternative to culture expanded ADPC due to client convenience

• ~65% of caseload is from out-of-state or international
Limitations

- Nature of retrospective studies
  - loss of follow-up
  - missing data points
  - no randomization

- No placebo group although 45.5% of cases had failed previous rehabilitation therapy
Conclusions

• ADPC-PRP combination may be considered as a treatment option for SST in the canine

• Not a “silver bullet”
  • Rehabilitation therapy

• Increased need for training/proficiency in canine musculoskeletal ultrasound

More questions than answers:

• Bone marrow vs adipose derived
• Cultured vs non-cultured
• Optimal cell counts
• Combination therapy (APC & PRP)
• Placebo controlled???
Conclusions
Future Directions

• Randomized, blinded, placebo controlled

• Phase I Study
  • SVF/PRP combination

  - VS -
  • Cultured ADPC/PRP combination
    • -VS-
    • Plasma control/Rehab

• Phase II Study
  • Adipose –vs- bone marrow

AKC CHF Prospective Supraspinatus Study
2014-2016; Currently enrolling cases.....please send!!!
Questions?
Thank You!!!