Ospedale Veterinario San Michele

Skalpellen

SKALPELLEN MEETING
8-10 april 2016 – Wisby Gotland

STEMCELL TREATMENT IN JOINT DISEASES

Offer Zeira
San Michele Veterinary Hospital, Italy

San Michele Veterinary Hospital
who are we ?

San Michele Veterinary Hospital

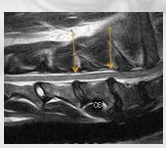
We named our Veterinary Hospital «San Michele» in honor and memory of a Swedish gentleman who dedicated all his life to people and animals – Dr. AXEL MUNTHE, who passed most of his time in Villa San Michele, Island of Capri, where he wrote the famous book «the story of San Michele».

Furthermore, we dedicate much of our activities to advanced diagnostics, reasearch and therapies for neurological diseases which were his main interests.

Finaly, our hospital is committed to take care of abandoned and homeless animals. Also this represents the spirit of Axel Munthe.

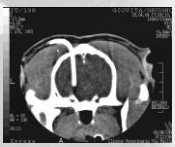
San Michele Veterinary Hospital

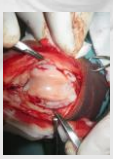
Neurology & imaging diagnostics

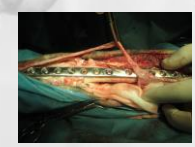


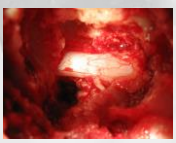
San Michele Veterinary Hospital

Neurosurgery & orthopedics



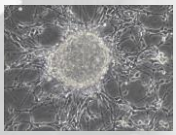


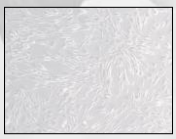




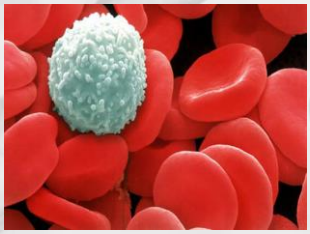
San Michele Veterinary Hospital

Regenerative medicine & fisiotherapy





Rigenerative Medicine



We are alive thanks to our innate regenerative capacity

Every second, millions of blood cells expire and are replaced in the human body.

Rigenerative Medicine

In nature there are planty of examples where damaged tissues are replaced with similar ones

Rigenerative Medicine

DRUGS ≠ REGENERATION:

- **Insulin** is used to treat DIABETICS but does not cure the disease. Need to regenerate islet cells or a pancreas.
- **Nitroglycerin** is used to treat cardiac *angina* (since 1879 for symptomatic relief) but does not cure the problem. Need to regenerate damaged or blocked cardiac tissue.
- *No single drug* can regenerate cardiac or other tissue.

Rigenerative Medicine

Repairing, replacing, maintaining, or enhancing organ function that has been lost due to **congenital abnormalities, injury, disease, or aging, is possible only by:**

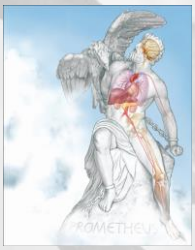
- Engineering of tissue *in vitro* for subsequent implantation *in vivo*

or

- Regeneration of tissue directly *in vivo.*

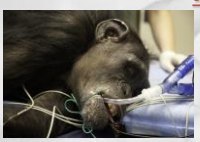
Rigenerative Medicine

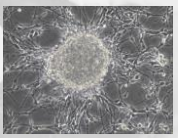
So what do we aim for ?



To regenerate any tissue, but we concentrate in arthropaties

Rigenerative Medicine – regulations





What we can and what we can't do depends NOT ONLY on science

Regulation in human and veterinary medicine (USA)

- Procedure should constitute a tissue transfer in the eyes of the US Food and Drug Administration (FDA), as **opposed** to the administration of a drug.
- The treatment must be autologous (the donor and patient must be the same person or animal); the cells can only be minimally manipulated, with no alteration of the cells' relative biological characteristics.
- The transfer must take place in the same day, with no storage of the cells overnight.

Regulation in human and veterinary medicine (Europe)

- Mainly similar to the USA.
- In Sweden - The Swedish National Council of Bioethics (Statens Medinsk-Etiska Råd) has published recommendations on biomedical issues including stem cell research.
- In Italy - the first regulation of stem cell-based therapies in veterinary medicine was pubblised in 17.10.2013 and **only autologous stem cells are authorized.**

just to have an idea of the approved clinical trials in human medicine

CLINICALTRIALS.GOV

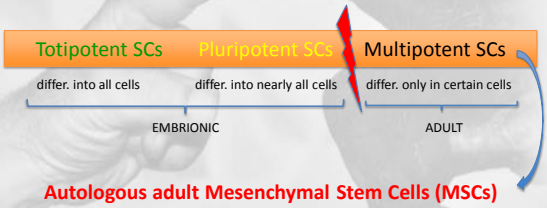
10-2013. Found **354** studies with search of: **Mesenchymal Stem Cells:**

Clinical Conditions for MSC-therapy: ~25% autologous.

Ulcerative Colitis, Diabetes Mellitus, Type 1, Liver Cirrhosis,Nonunion Fractures, Diabetic Foot, Critical Limb Ischemia, Dilated Cardiomyopathy, Autoimmune Diseases; Immune System Diseases; Demyelinating Diseases; Nervous System Diseases; Demyelinating Autoimmune Diseases, CNS; Autoimmune Diseases of the Nervous System(MS), Sjogren's Syndrome, Graft Versus Host Disease; Chronic and Expanded Graft Versus Host Disease, Middle Cerebral Artery Infarction, Osteoarthritis, Aplastic Anemia, Maxillary Cyst; Bone Loss of Substance, Spinal Cord Injury, Parkinson's Disease, Crohn's Disease, Acute Myocardial Infarction, Multiple Sclerosis, Hematological Malignancies, Organ Transplantation, Ischemia; Stroke, Systemic Sclerosis, Hereditary Ataxia, Liver Failure, Retinitis Pigmentosa, Kidney Transplant; Rheumatoid Arthritis, Lumbar Spondylolisthesis Involving L4-L5, Chronic Allograft Nephropathy, Degenerative Arthritis; Chondral Defects; Osteochondral Defects, Progressive Multiple Sclerosis; Neuromyelitis Optica, Primary Biliary Cirrhosis, Osteonecrosis of the Femoral Head, Pened Chest Surgery for Programmes Coronary Bypass, Lupus Nephritis, Wilson's Disease, Multiple System Atrophy, Burns, Intervertebral Disc Disease, Chronic Myocardial Ischemia; Left Ventricular Dysfunction, Relapsing-Remitting Multiple Sclerosis; Secondary Progressive Multiple Sclerosis; Progressive Relapsing Multiple Sclerosis, Tibial Fracture, Bone Cyst,Buerger's Disease, Amyotrophic Lateral Sclerosis, Allogeneic Stem Cell Transplantation, Idiopathic Pulmonary Fibrosis, Type 2 Diabetes Mellitus, Refractory Systemic Lupus Erythematosus, Leukemia, Myeloid, Acute; Leukemia, Lymphoblastic, Acute; Leukemia, Myelocytic, Chronic; Myeloproliferative Disorders; Myelodysplastic Syndromes; Multiple Myeloma; Leukemia, Lymphocytic, Chronic; Hodgkin's Disease; Lymphoma, Non-Hodgkin, Degenerative Arthritis, Myelodysplastic Syndrome, ST-Elevation Myocardial Infarction, Pulmonary Disease, Chronic Obstructive; Pulmonary Emphysema; Chronic Bronchitis, Lower Back Pain; Disc Degeneration, Articular Cartilage Lesion of the Femoral Condyle, Osteoporotic Fractures, Bone Neoplasms, Solid Tumors; Acute Kidney Injury, Hereditary Cerebellar Ataxia, Primary Disease, Autism, Limbus Cornease Insufficiency Syndrome, Wound Healing, Dementia of the Alzheimer's Type, Non-ischemic Dilated Cardiomyopathy, Stroke, Epidermolysis Bullosa, Tibia or Femur Pseudo-arthrosis, Recovery Following Partial Medial Meniscectomy, Human Immunodeficiency Virus, Stable Angina; Heart Failure; Atherosclerosis; Multivessel Coronary Artery Disease, Osteogenesis Imperfecta, Emphysema, Progressive Hemifacial Atrophy; Romberg's Disease, Complex Perianal Fistula, Multiple Trauma, Osteodysplasia, Tibiotalar Arthrodesis; Subtalar Arthrodesis; Calcaneocuboid Arthrodesis; Talonavicular Arthrodesis; Double Arthrodesis (i.e. Calcaneocuboid and Talonavicular); Triple Arthrodesis (i.e. Subtalar, Calcaneocuboid, and Talonavicular), Recto-vaginal Fistula, Peripheral Vascular Diseases, Prostate Cancer; Erectile Dysfunction, Diabetic Wounds; Venous Stasis Wounds,

Rigenerative Medicine

Stem cells potency



Autologous adult Mesenchymal Stem Cells (MSCs)

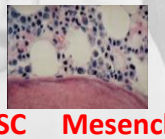
Rigenerative Medicine in Domestic Animals: background

Mesenchimal Stem Cells (MSCs) properties

- Relatively **easy to obtain** and culture (but can be used without it)
- **High plasticity** - they cross lineage barriers adopting functions of other cells (Forbes et al.2002)
- Release **growth factors** (Paumier et al.2006)
- **Modulate immune system** (Uccelli et al. 2011)
- Create an environment that stimulates **repair** by resident cells (Bai et al. 2007)
- Migration capacity (**homing**) due to specific molecular signals found in the microenvironment as a result of damaged tissue (Kotaro et al.2004)

Back to regulations – only autologous

Stem Cells (SC) from adult BONE MARROW



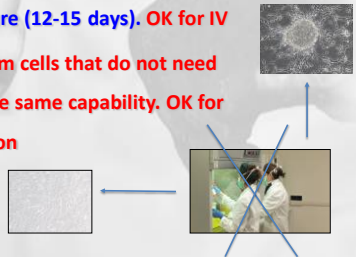
Hemopoietic SC → **all blood cells**

Mesenchimal SC → **bone, cartilage, muscle, fat**

The same for ADIPOSE-derived Stem Cells (SC) Only adult and outologous

Rigenerative Medicine – modification of our approach

- Stem cells from bone marrow or adipose tissue needs colture (12-15 days). OK for IV
- We looked for stem cells that do not need colture but has the same capability. OK for local administration



Rational

- It has been shown that an elaborated fat tissue is harboring a preserved stromal vascular fraction rich in perycytes and mesenchymal stem cells
- The product is obtained by mild mechanical forces and with no enzymatic process by a very simple, friendly, novel device

Canine arthropaties - safety and efficacy of the lipogems device

Aim

- To verify clinical safety and efficacy of the device for advanced therapy
- Quality and quantitative evaluation of the device's outcomes

Patient Selection

- 19 dogs
- From 2 to 13 years old
- Single or multiple arthropaties, otherwise healthy

Canine arthropaties - safety and efficacy of the lipogems device

Diseases treated by Mesenchimal Stem Cells

- Vascular (Legg-Calve-Perthes disease)
- Immune mediated arthritis (OA)
- Traumatic injury (crCr ligament and meniscus rapture, joint fractures)
- Hereditary/developmental (UAP, FCP, OCD, IOHC)
- DJD

Canine Osteoarthrosis: safety and efficacy of lipogems device

Project Design

Orthopedic examination

- Complete check up (CBC + biochemistry)
- RX (3/4 projections)

If the dignostic suspect is of a severe DJD/OA, the owner will be asked to proceed with:

- MRI
- Synovial fluid analisys
- Treatment with Lipogems (where possible contralateral joint will be the control)

The outcomes of this treatment will be evaluated with:

- control at 3 and 6 months: clinical examination, RX e MRI
- post mortem histologiacal joint's exam

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

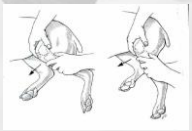
DIAGNOSTIC WORKUP IN ARTHROPATIC PATIENT – OUR ROUTINE PROTOCOL

- Medical history and physical exam
- Radiographs
- Arthroscopy
- CT / MRI
- Ultrasound
- Synovial fluid cytology
- Histopathology

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

1. medical history and a physical exam (film)

- "noises" when you move the joints
- swelling of the joints
- loss of range of motion (ROM)
- tenderness of the joints
- pain during movement



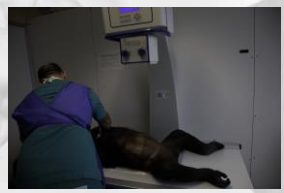
DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

2. radiographs

- Excess fluid in the joint
- Bone damage
- Bone spurs
- Anesthesia
- Patient positioning
- Low cost

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

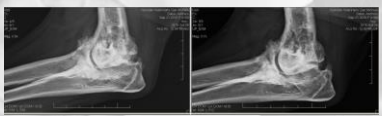
2. radiographs

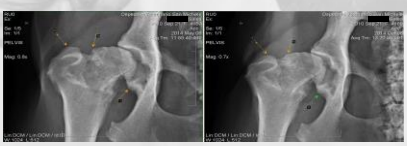


Attention to patient positioning

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

2. Radiographs – what to expect ?





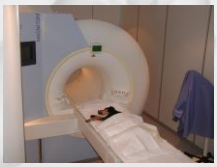
DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

3. MRI

- Unique soft tissue info
- Also bone !
- No internal metals
- Anesthesia
- Patient positioning
- High cost
- Trained radiologist

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

3. MRI




High field / low field ?

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

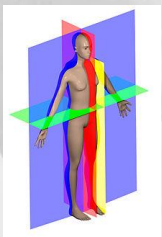
3. MRI


Positioning is critical

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

3. MRI – what to expect ?

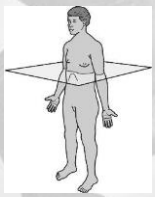
3 planes – sagittal, dorsal, transversal



DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

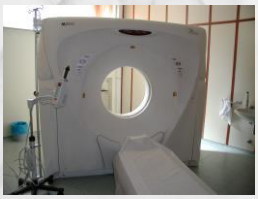
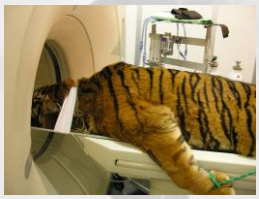
4. CT

- Good bone images
- Fast exam
- One plane – transverse
- 3D riconstructions
- Anesthesia
- Patient positioning
- High cost
- Trained radiologist



DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

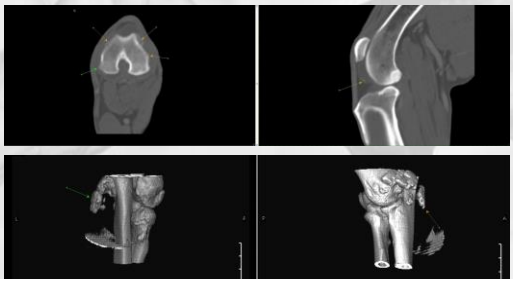
4. CT

Multi-slice = fast exam

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

4. CT - what to expect ?



DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

5. Ultrasound

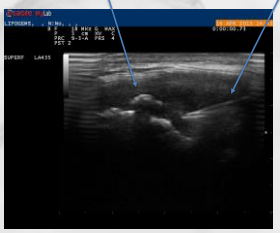
- No high definition
- Mainly periarticular structures (tendons)
- No anesthesia needed
- Low cost
- Fast

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

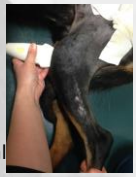
5. Ultrasound - what to expect ?

OCD fragment

fine needle aspiration



sagittal

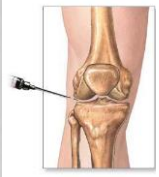


transverse

DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

6. Synovial fluid cytology

- DD of joint diseases (PCR, culture)
- Asses therapy outcome
- Easy to obtain
- Low cost

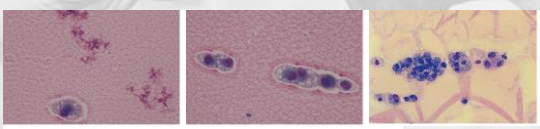


DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

6. Synovial fluid cytology - what to expect ?

- Degenerative Joint Disease (DJD)
- Chronic, visiable in x-rays
- Many synoviocytes, large cytoplasm (often vaculized, with round nucleus), abundant proteinic fondus

Canine and feline cytology" 2nd edition, Rose E.

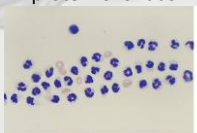


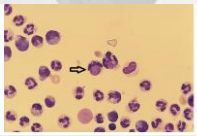
DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

6. Synovial fluid cytology - what to expect ?

- Inflammatory arthropaties (immune mediated / infectious)
- Less degenerated, less chronic, less visiable in x-rays
- Synoviocytes + many neutrophilic granulocytes, macrophages, less abundant proteinic fondus

Canine and feline cytology" 2nd edition, Rose E.

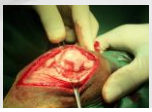


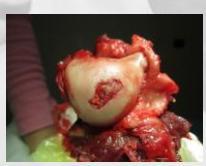


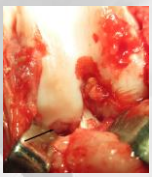
DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

7. Histopathology

- Definitive diagnosis
- Global view of the joint disease
- Critical for **assessment of post therapy regenerative process**

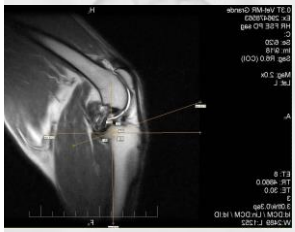


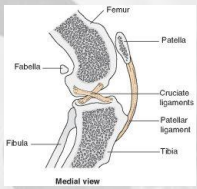


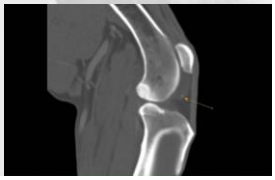


DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

EXAMPLES: STIFLE JOINT

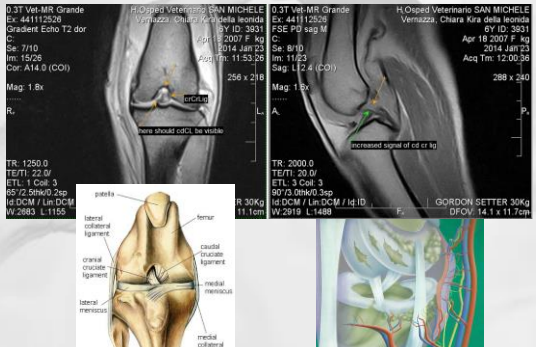






DIAGNOSTIC IMAGING IN DOG'S ARTHROPATIES

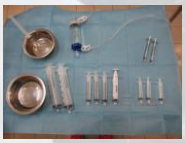
EXAMPLES: STIFLE JOINT – crCr ligament rapture



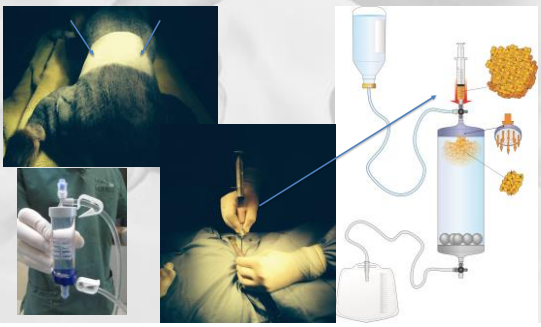
Device and procedure

- A disposable device for liposuction, processing and reinjection of adipose tissue
- The entire procedure is done in one session
- It progressively reduces the size of adipose tissue clusters, completely eliminating pro-inflammatory oily and blood residue through minimal "enzyme-free" manipulation in a closed aseptic system
- The entire process occurs in a system immersed in saline solution which minimizes any trauma caused by the cellular products.

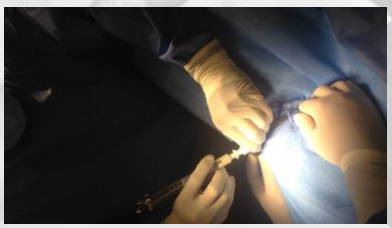
Device and procedure

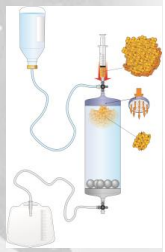


Device and procedure



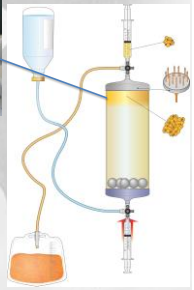
Device and procedure





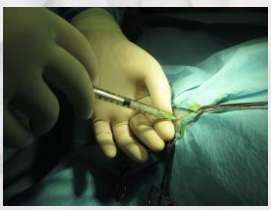
Device and procedure

Device and procedure



Device and procedure

IA administration and cryoconservation



Clinical cases

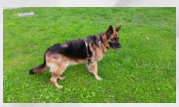
Gilda, GSD, F, 8Y, UAP

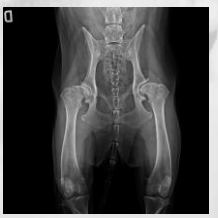
10.5.15 **3 days** pre treatment

18.5.15 **5 days** post treatment

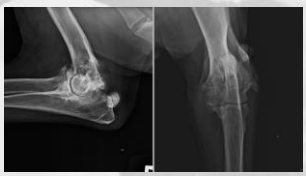
23.5.15 **10 days** post treatment

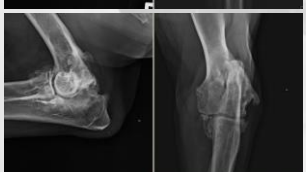
2.4.16 **11 months** post treatment



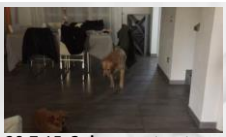


Gilda, GSD, F, 8Y, UAP





Lucky, LR, M, 9Y, OA ELBOW

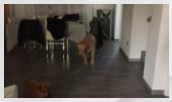


20.7.15, **3 days** pre treatment

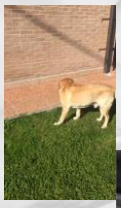
24.7.15, **4 days** post treatment

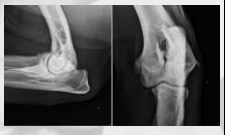
21.2.16, **7 months** post treatment

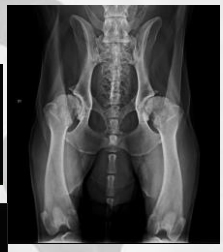
12.8.15, **22 days** post treatment

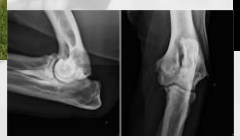


Lucky, LR, M, 9A, OA gomito



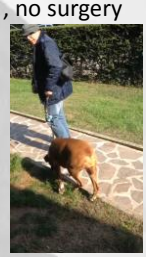






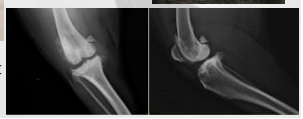
Eva, Boxer, F, 6Y, Lt CrCrL , no surgery

3.10.14 pre treatment



19.10.15, 1 year post treatment

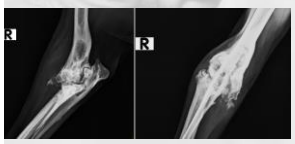
16.12.14, 50 days post treatment

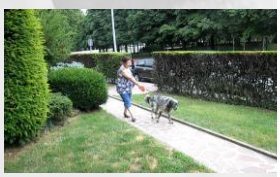


English Setter, M, 2Y, intercondylar fracture

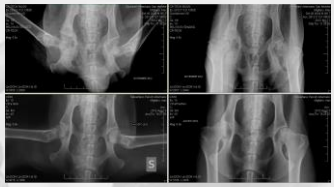
4.6.15, pre treatment

16.6.15, 16 days post treatment





GSD, M, 4Y, post infectious DJD



First feed-back: the owners point of view

Salsa – German Shepard: LipoGems and our side of the story

Salsa is of course an important part of our family and when we discovered in March 2014 that she had dysplasia in all 4 joints, at the early age of 3, which had already started to affect her mobility and quality of life, of course we were very worried. In a very short space of time, Salsa was unable to walk very far at all, her front legs unable to support her, and any steps taken were made with great difficulty.

We gave Salsa various FAN treatments which gave no relief and When 3 consultations with recommended orthopedic vets delivered the same verdict which was to either try and operate Salsa by implanting artificial joints (very painful and costly) or put Salsa out of her misery and end her short life, we were heartbroken. Then we had the immense luck to find a local vet, Piermario Bagalini who, having already referred many of his four legged patients to Dr. Offer Zeira, encouraged us not to give up hope and to pay a visit to Lodi to see if there was a chance to save Salsa.

From the moment we set foot in the clinic we realized that if anyone could help us it would be Dr. Zeira. He and his colleague Marina explained to us a procedure that they had developed called "Lipogems" which substituted Salsa's missing cartilage to alleviate the discomfort and pain, if not completely, enough to offer her a better quality of life.

On 14th May, Salsa underwent the therapy which took approximately 3 hours, while we waited anxiously to find out if it had been successful.

Operation completed we went home and followed Dr. Zeira's instructions to give painkillers and keep Salsa from moving around. The first few days were not encouraging. Salsa couldn't move at all. She whined all day. We had to pick her up and take her into the garden and she was very uncomfortable. We began to think that we had made a terrible mistake by making Salsa endure such pain for our own selfishness.

Although we had been told that the treatment would take a week or so to take effect, Salsa was not responding at all and after day 5 we went back to the clinic with heavy hearts, convinced that the only option was to say goodbye to our best friend. Dr. Zeira took some more x-rays to analyze what was going on and we were so very surprised to see that her joints had already started to change form showing signs that her "cauliflower" joints were looking smoother and rounder. The evidence gave us hope, so with new painkillers, we returned home, in the hope that her progress would continue.

We shouldn't have doubted. After day 11 we awoke to find Salsa bounding round the kitchen, searching for a slipper to bring, looking as mischievous and as full of life as the puppy we had brought home 4 years earlier.
Of course our joy was immense.

Safety and Feasibility of autologous MSCs implantation in 100 dogs with arthropaties

Conclusions

Safety?

- hematology and imaging diagnostics results after MSCs administration showed no worsening of previous clinical status and **no onset of new pathologies**
- intra-articular injection of MSCs showed no difficulty and no adverse reaction

Orthopedic assessment

- **Condroprotection ?**
- **Rigeneration – in some cases x-rays and MRI presented a better situation**
- **Anti-inflammatory – in all cases synovial fluid analysis shown normalization**
- **All of them – most probably, the response to the therapy is due to all factors**
- **Maximum of 24 months of fully documented follow-up (65 dogs are alive at present time)**

Safety and Feasibility of autologous MSCs implantation in 100 dogs with arthropaties

Conclusions

Limits?

1. Small **number of patients – not a limit any more. Above 100 dogs and more than 350 articulations treated in different clinics in 5 countries**
2. **Long term follow-up – not a limit any more. More than 2 years of follow-up**

What Next?

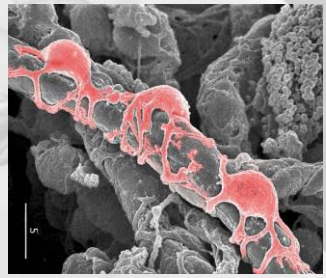
- greater number of dogs, greater number of clinics, more countries
- Better evaluation of therapeutic effect (3D gait analysis)
- Histological evidence of therapy

Conclusions

- lipogems is a minimally manipulated tissue product
- it encompassess a stromal vascular niche rich in pericytes and mesenchymal stem cells
- its properties are not altered by cryoconservation
- it can be easily expanded in culture
- it has multilineage potential
- it exibits spontaneous and modulable vasculogenic potential
- it can be easily transferred into multifaceted clinical settings

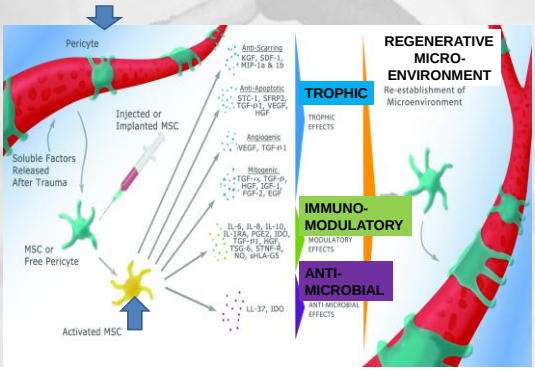
TAKE HOME:

PERICYTES are the key element

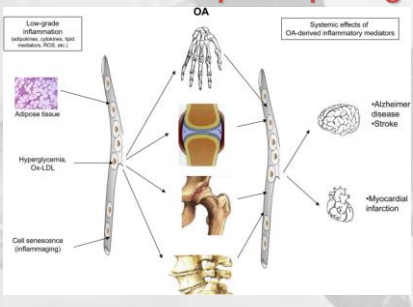


modified by *BRUNO PEAULT* from http://www.geocities.co.jp/HeartLand-Suzuran/9389/kekkan

TAKE HOME: innate MSCs functions



TAKE HOME: Arthropatie's pathogenesis



Systemic effects and potential consequences of OA-derived inflammatory mediators. A proposed novel paradigm for the role of low-grade inflammation in OA (characterized by the release of inflammatory mediators into the blood)

F. Berenbaum, Osteoarthritis and Cartilage, Volume 21, Issue 1, 2013, 16 - 21

So, we belive that what is true for reptiles since millions of years, will soon be true in other animals and man. THANK YOU

Many thanks to:

STEM CELL LAB
Dr. Aralla Marina, DVM, Phd
Dr. Letizia Pettinari , MSC, Phd
Dr. Erica Ghezzi, BSC

RADIOLOGY
Dr. Martin Konar, DVM, Phd, dipl ECVN
Dr. Laura Martinelli, DVM, MA imag.diag.
Dr. Davide Moscarello , RT

SURGERY
Dr. Daniele Zahirpour, DVM
Dr Nimrod Asiag, DMV
Dr. Simone Scaccia, DMV

ANIMALS CARE AND REHAB
Dr. Michela Lionello, VT
Dr. Lorenza Sironi, DVM

ADMINISTRATION AND PSYCHOLOGICAL SUPPORT
Dr. Michela Cesana

FACS ANALISYS
Dr. Stefano Comazzi, DVM, PhD, dipl ECVCP

HYSTOLOGICAL ANALISYS
Dr. Carlo Cantile, DMV, Phd