AMNIOTIC DERIVED PROGENITOR CELLS IN DIFFERENT ANIMAL SPECIES IN VIEW OF CELL THERAPY APPLICATIONS

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Therapeutic application of adult MSCs in veterinary medicine

VETERINARY MEDICINE	
Indication	References
Myocardial infarction	Orlic et al., 2001; Saito et al., 2002
Muscular dystrophy	Gussoni et al., 1999
Pulmonary fibrosis	Ortiz et al., 2003
Spinal fusion	Mushler et al., 2003
Segmental bone defects	Bruther and kurt 1998,
Craniotomy defect	Krebsbach et al., 1998
Tendon injury (equine)	Yiung et al., 1998
Meniscus	Murphy et al., 2003

- A [illegible] study the properties and potential of stem cells

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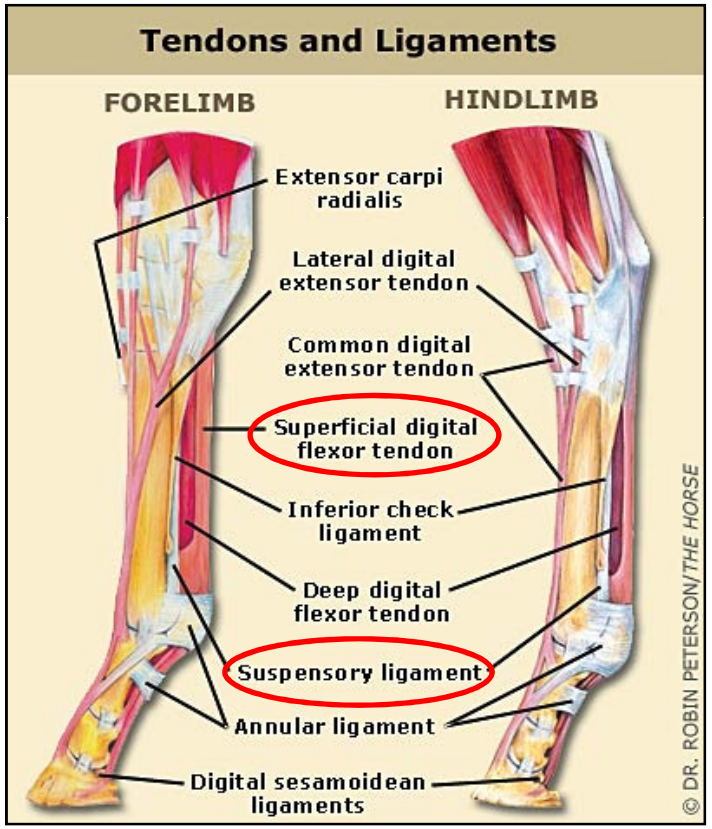
Mesenchymal stem cell in tendon repair

- **Emphasis on veterinary experimental and clinical studies and possible translation into human medicine**
- **SDFT has many similarities to the human Achilles tendon in both its structure and matrix composition**
  - ❖Energy-Storing Tendons
  - ❖Essential for efficiency of high-speed locomotion
  - ❖Large size of both species



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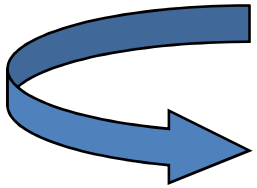
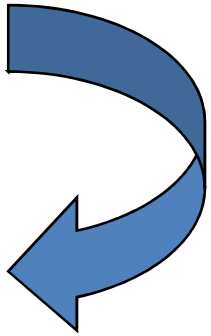
Main sources of adult MSCs



Bone marrow



Fat tissue

Positive clinical outcome of cellular therapy, with a lower re-injury rate (18-25%) with respect to conventional conservative therapies (23% up to 80%)

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Characteristics of adult MSCs

Bone marrow

↓

Invasive procedure
Low density

potential
- proliferation
- differenziation

decrease

Donor's age

In vitro passage number (6-10)

Fat tissue

↓

Low density

→ Invasive procedure

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Limit of adult autologous MSCs

The **delay of 2-4 weeks** because of the expansion of MSCs before reaching the sufficient amount of cells needed for the treatment **represents a limiting factor** in the context of the use of autologous BM- SCs

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Different sources of MSCs

Nowadays, it is possible to choose among **several sources of cells** to use in regenerative medicine,

BUT

it is still not clear **which one can be considered therapeutically optimal**

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What are the conditions necessary in stem cells therapy?

- To collect a **large number** of cells **inexpensively** and **non invasively**
- To have cells with high target of **proliferation** and **differentiation** to regenerate organ damages
- To have cells with characteristics of **homing**
- To have cells without immunogenic properties and with **immunomodulatory properties**

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Alternative source of MSCs

Extra-fetal tissues

- **Tissues discarded at birth (no ethics)**
- **No impact on the health of mother and child**

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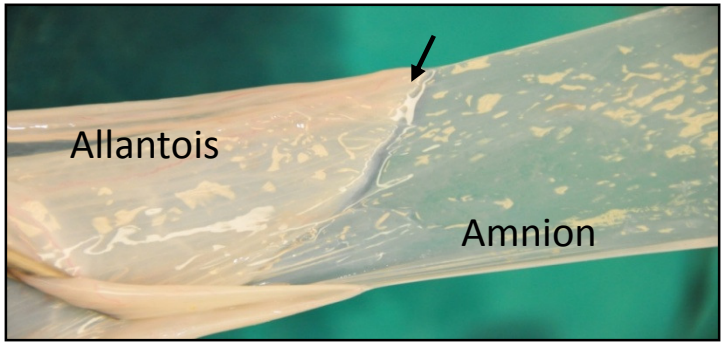
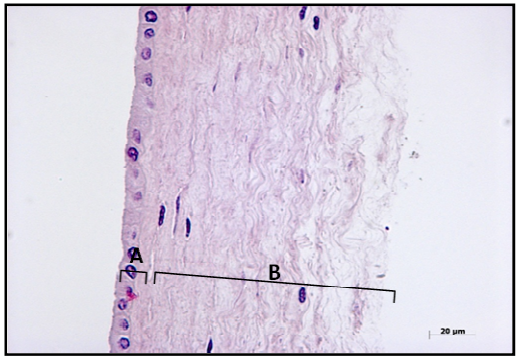
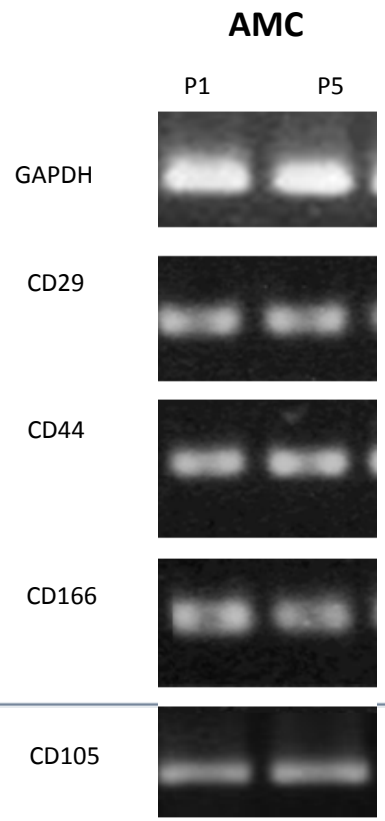
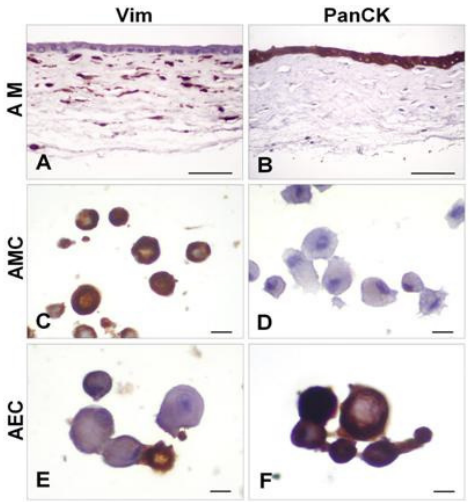
...To collect a large number of cells inexpensively and non invasively









Enzymatic digestion (25 gr)

***100*10⁶* AMCs** **(1 CFU each 240 cells)**

***250*10⁶* AECs**

***25 ml di BM = 8-25*10⁶* MSCs**
(1 CFU each 600 cells)

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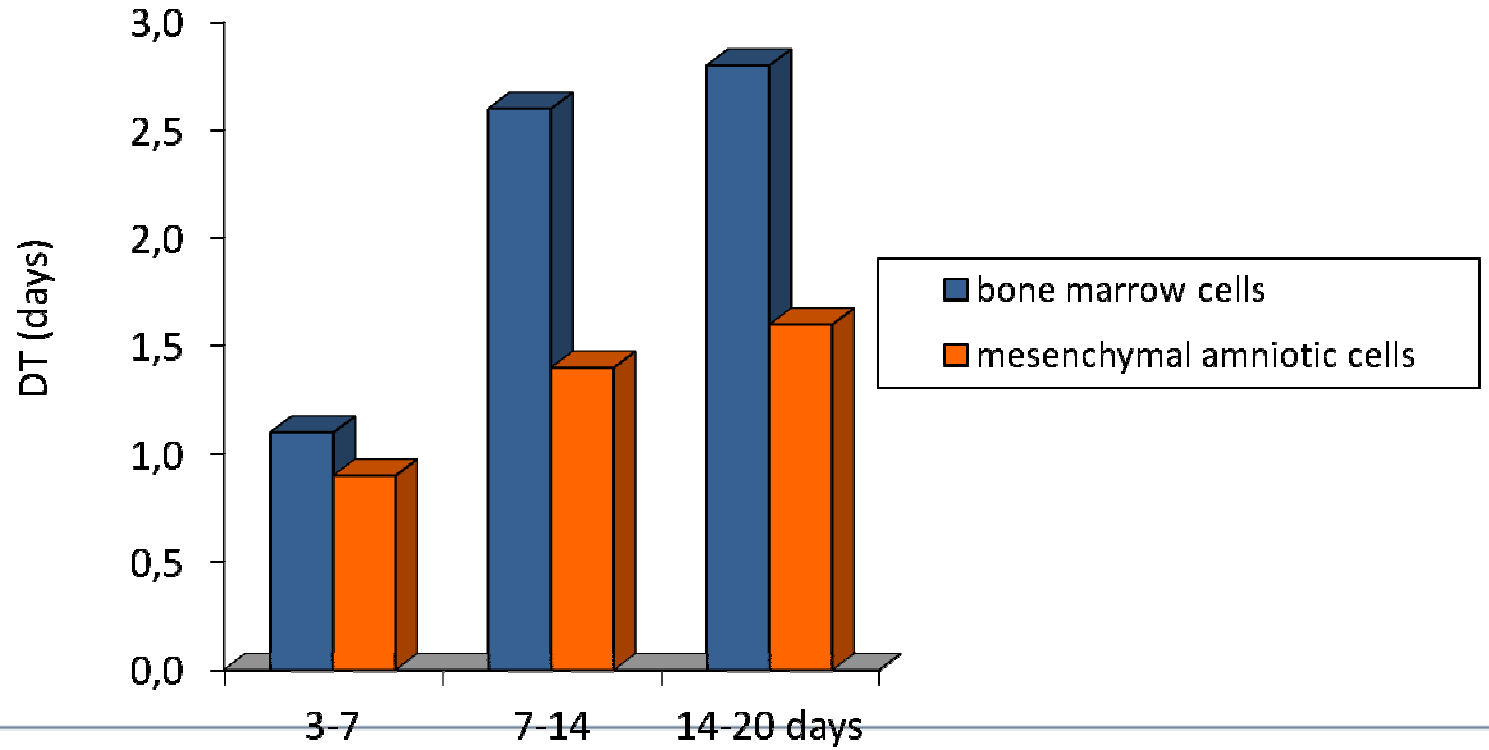
Lange-Consiglio et al., J Tissue Eng and Reg Med, 2012
Lange-Consiglio et al., Equine Veterinary Journal, 2013
Rutigliano & Lange Consiglio, Stem Cell Research, 2013
Corradetti & Lange Consiglio Reproduction, 2013

...To have cells with high target of proliferation



After 14 days mesenchymal amniotic-derived cells show higher proliferative capacity (3 replicates)

Lange-Consiglio et al., Equine Veterinary Journal, 2013

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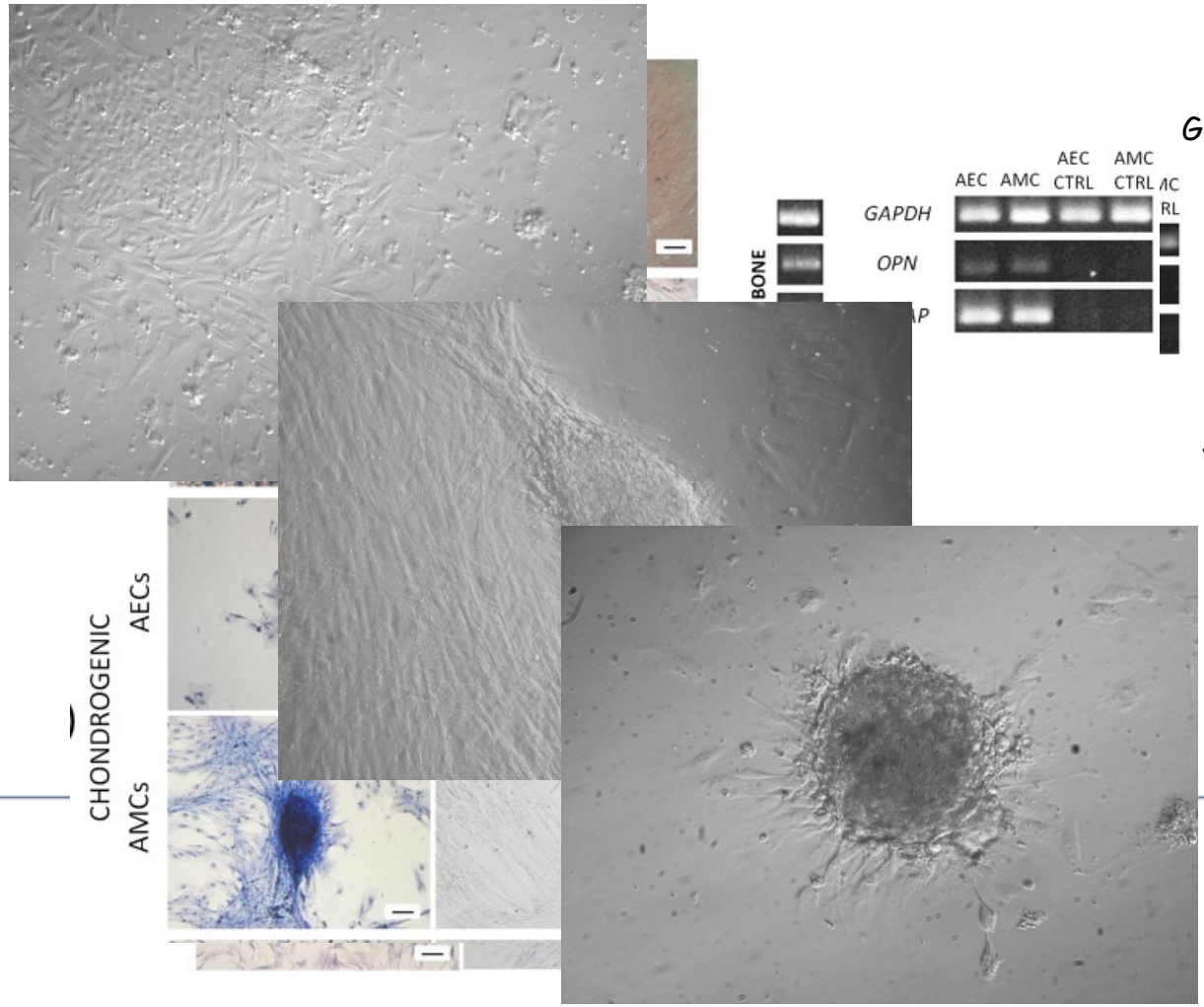
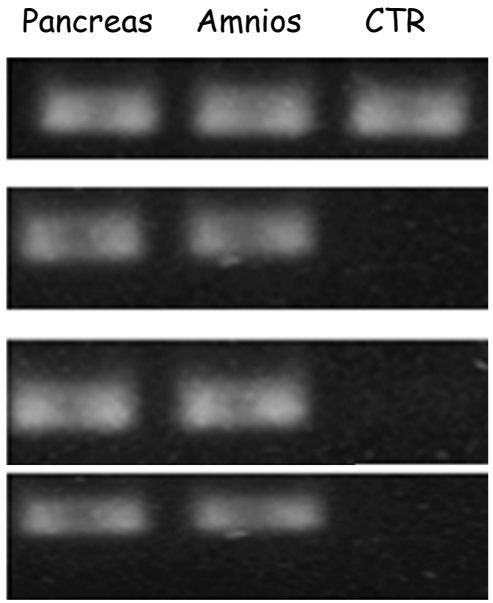
…To have cells with high target of differentiation (pluripotent cells?)





Pancreatic differentiation
in dog and cat

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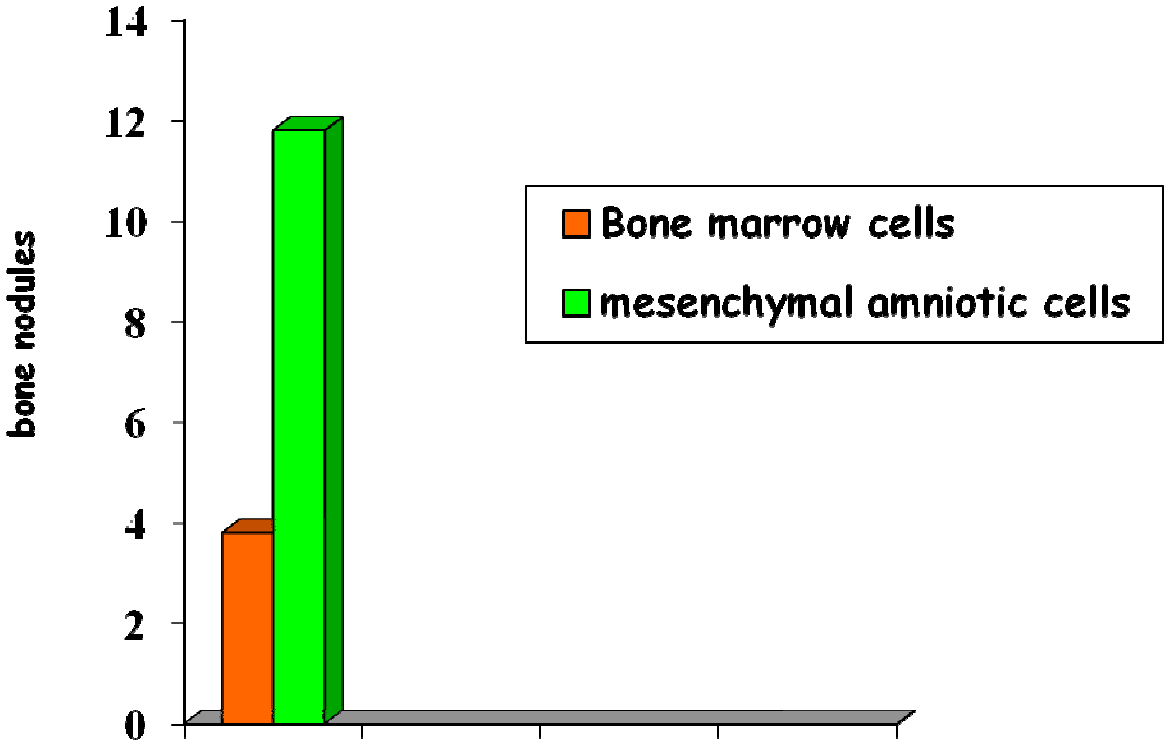
Lange-Consiglio et al., J Tissue Eng and Reg Med, 2012
Lange-Consiglio et al., Equine Veterinary Journal, 2013
Rutigliano & Lange Consiglio, Stem Cell Research, 2013
Corradetti & Lange Consiglio Reproduction, 2013

Speed of differentiation

Nodules observed

- 3ª week in mesenchymal amniotic cells
- 5ª week in bone marrow cells



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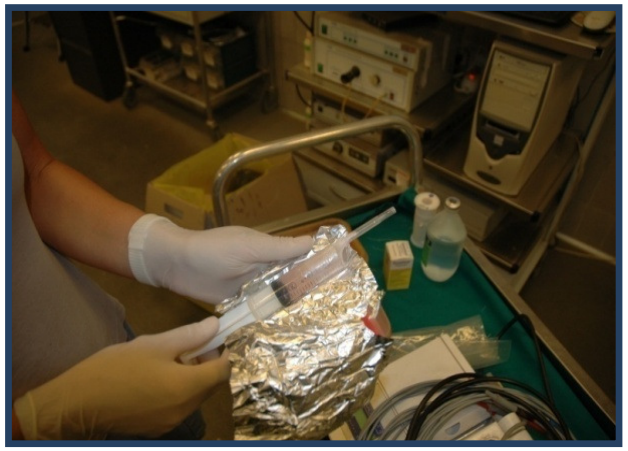
Lange-Consiglio et al., Equine Veterinary Journal, 2013

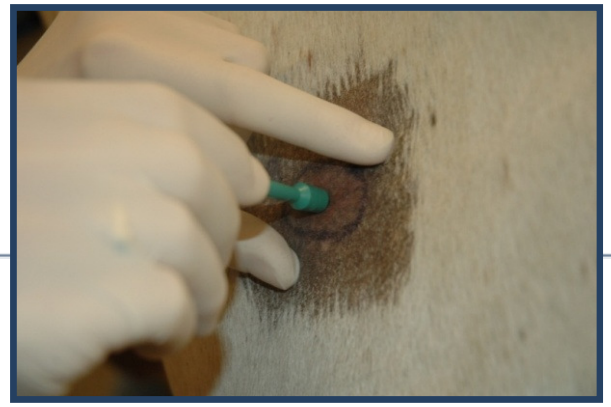
…To have cells with characteristics of homing to the site of injury or disease

10 µl of Candida albicans antigen



After 48h, $1*10^8$ AMCs labeled by PKH26 were administrated

by the intravenus or by endobronchial route



Punch biopsies were taken on 48 h, 1 week and 2 weeks after the administration of AMCs cells

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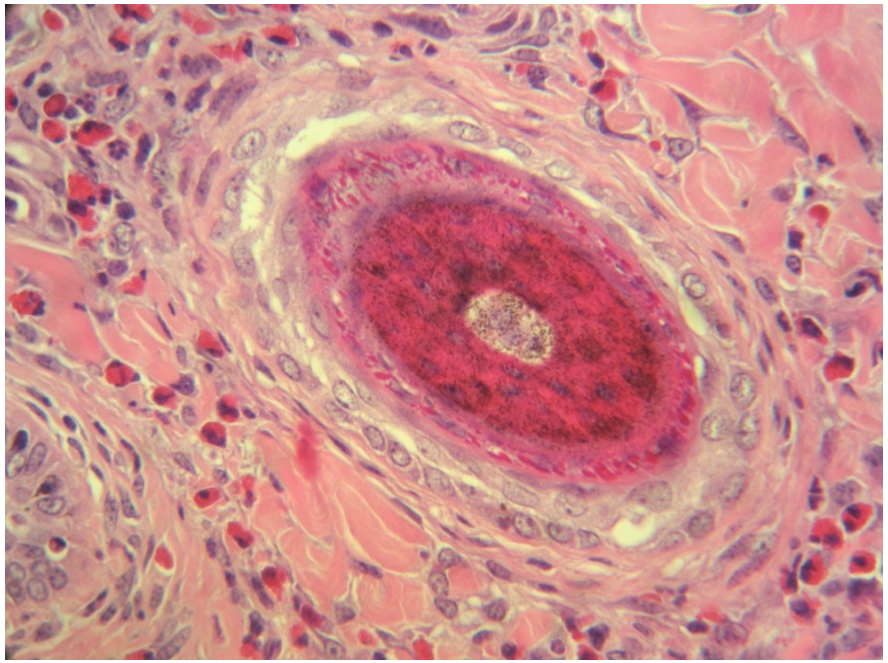
…To have cells with characteristics of homing to the site of injury or disease



by intravenous route, cells were present after 48h

by endobronchial route amniotic cells arrived in site after 7-14 days

Lange-Consiglio et al., ***TILL & MCCULLOCH MEETING 2012***

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…To have cells with immunogenic properties

Pregnancy is a unique event in which a genetically and immunologically foreign fetus survives to full term whitout rejection by the mother's immune system

Poole and Claman, Clin Rev Allergy Immunol, 2004

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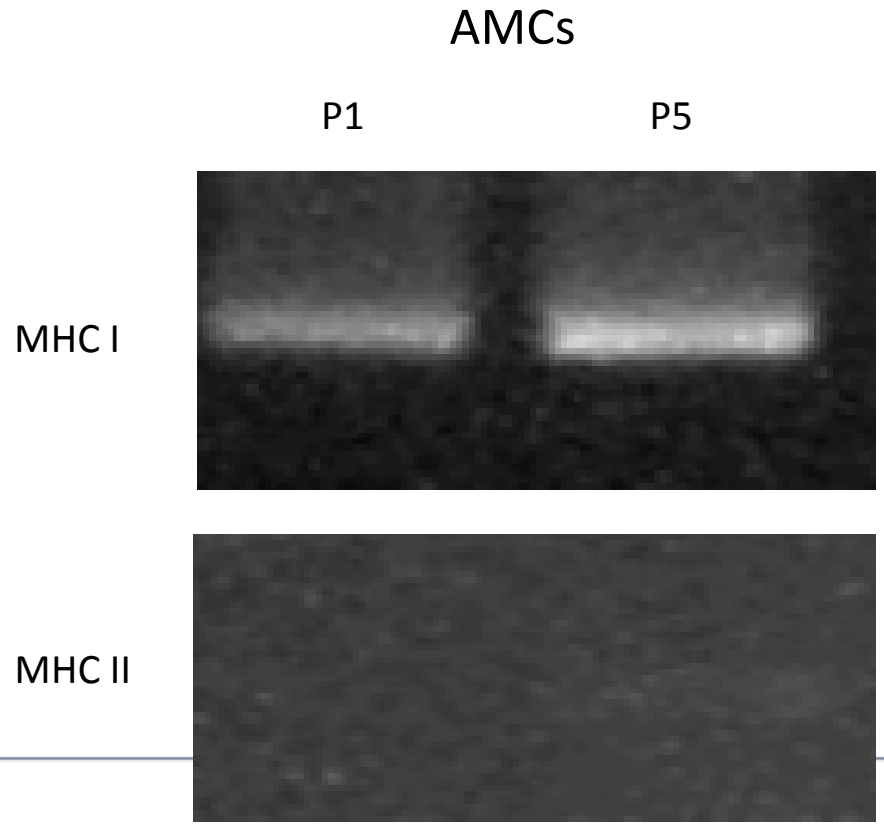
Immunogenic properties



Lange Consiglio et al. Tissue Eng Reg Med, 2012

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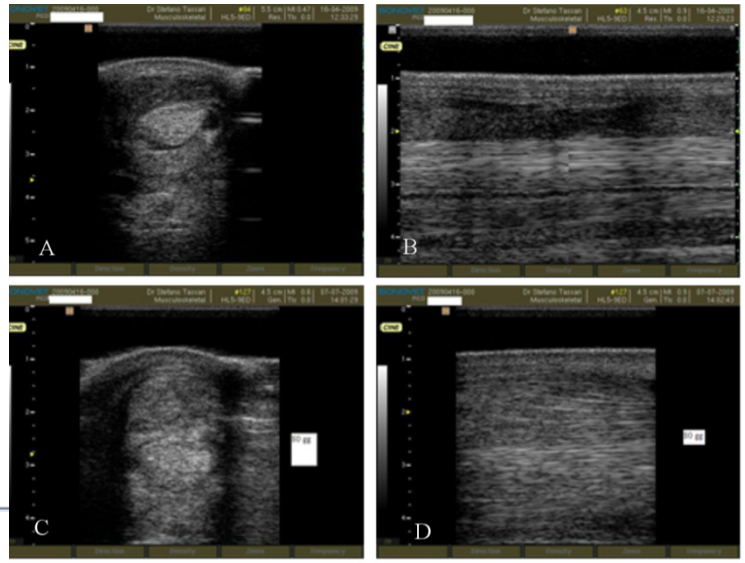
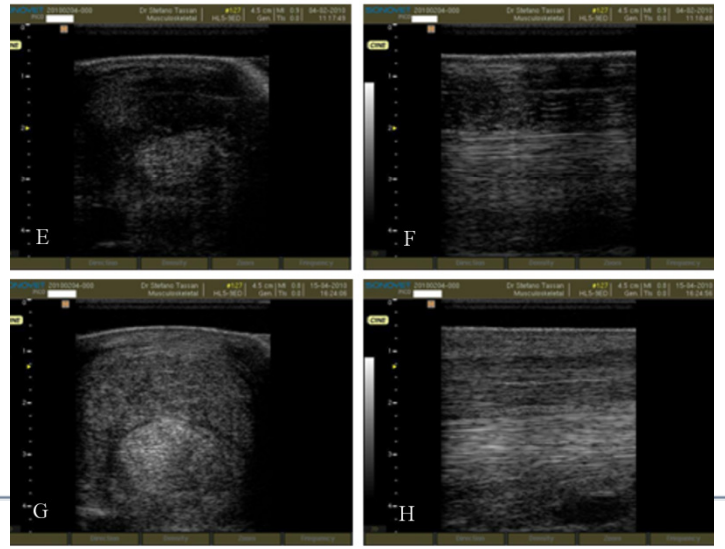
Amniotic mesenchymal stem cells (AMCs)

When allogeneically transplanted in vivo AMCs are well tolerated and exert beneficial effects on tendon regeneration after ***spontaneous lesions*** *better than adult bone marrow-derived MSCs*



BM-MSCs
(autologous and fresh cells)

23.5% re-injuries



AMCs
(eterologous and cryopreserved cells)

4% re-injuries

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Lange-Consiglio et al., Cytotherapy 2013

Stem cell biology

The facility of isolation and expansion and the *in vivo* results have made AMCs of great interest in **tendon regenerative application**

Whether MSCs differentiate into tenocytes, supply immunomodulatory and trophic factors or if a combination of the two mechanisms occurs, is **still debated**

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Regeneration mechanism?

- Differentiation
- Cell fusion with already-differentiated cell
- Stimulate differentiation of MSCs in tissue niches
- Anti-apoptosis (stop cell death)
- Anti-fibrotic (inhibit scarring)
- Angiogenis (new blood supply)
- Anti-inflammatory (inhibit degeneration)

- **Supply growth factors (paracrine action)**

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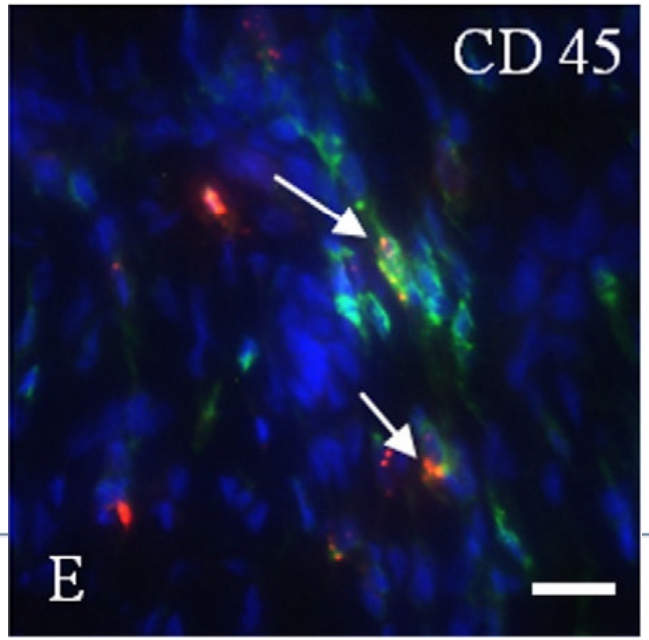
Supply growth-factors

…Because of the inhospitable microenvironment of the injured or degenerating tissues, a large proportion of the implanted MSCs may die or undergo apoptosis in a short period post-transplantation *(Leung et al., Eur Spine J, 2006)*



Leukocytes labeled with GF

AECs labeled with PKH-26

Muttini et al., 2013 Research Vet Sci

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CAN CELLS BE CONSIDERED AS A BIOLOGICAL LABORATORY FOR THE PRODUCTION OF THERAPEUTIC SUBSTANCES?

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To test this hypothesis, we examined:

1. The immunomodulatory characteristics of AMCs and of their conditioned medium (AMCs-CM) ***in vitro***

2. The therapeutic effect of AMCs-CM in horse spontaneous tendon and ligaments injuries ***in vivo***

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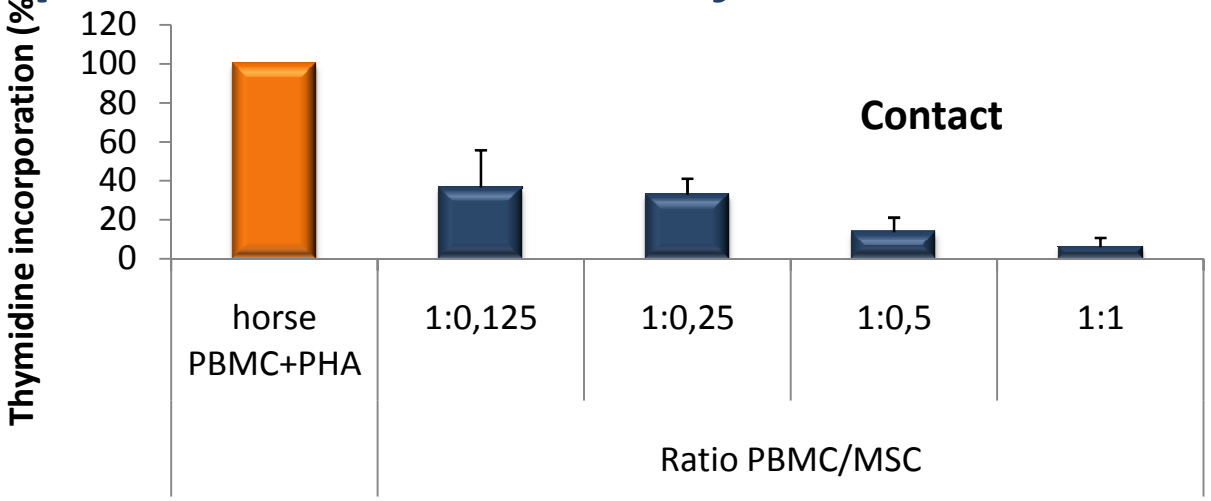
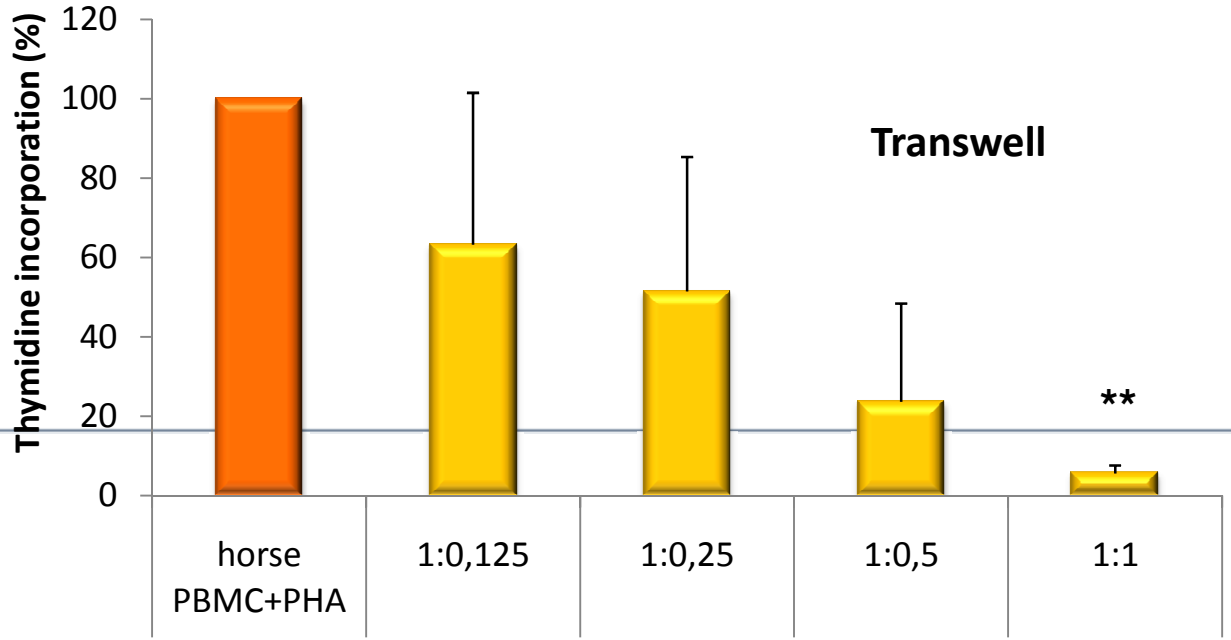
Experiment 1: *RESULTS of immunomodulatory characteristics*





Inhibition of PBMC proliferation in a dose-dependent manner reaching a 90% ($P \leq 0.05$) at a ratio of 1:1.

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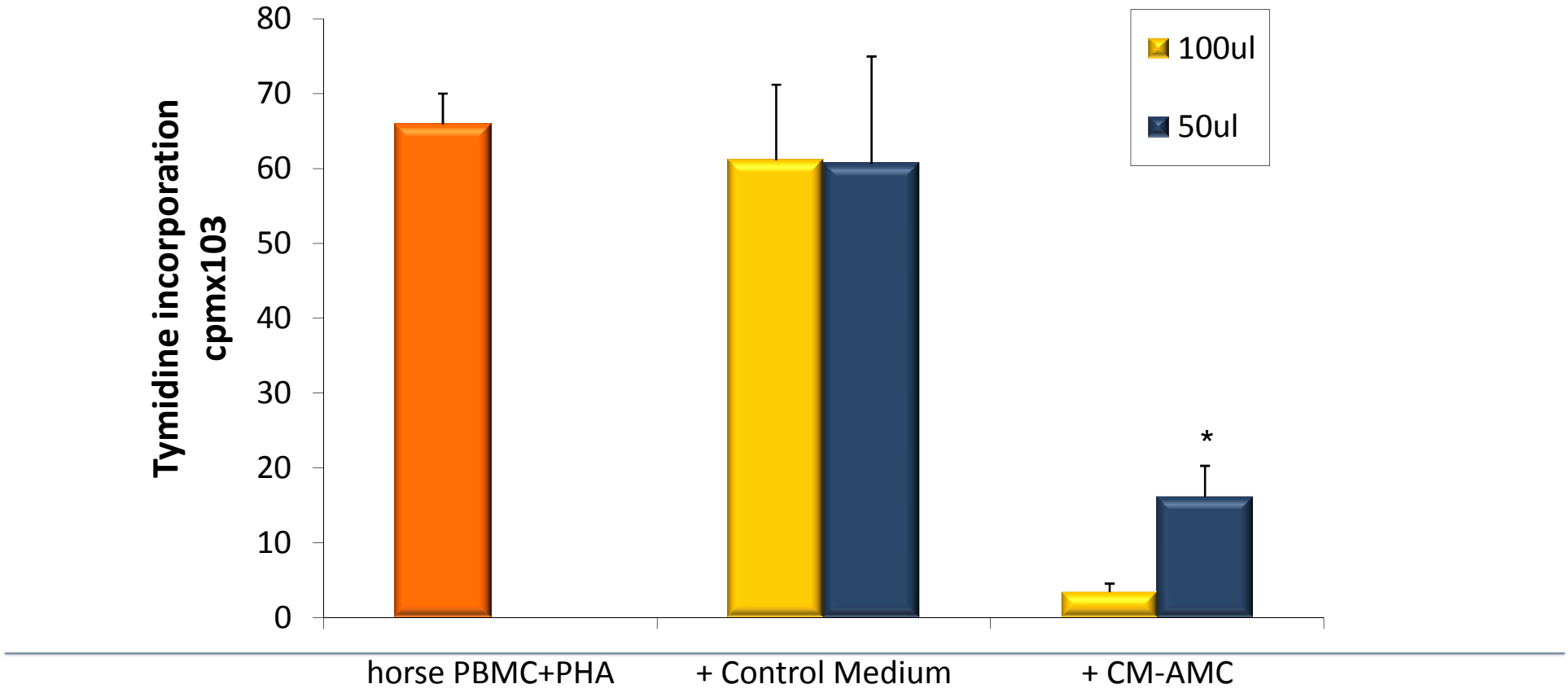
Lange-Consiglio et al., Stem Cell Development, 2013

Experiment 1: *RESULTS with AMCs-CM*



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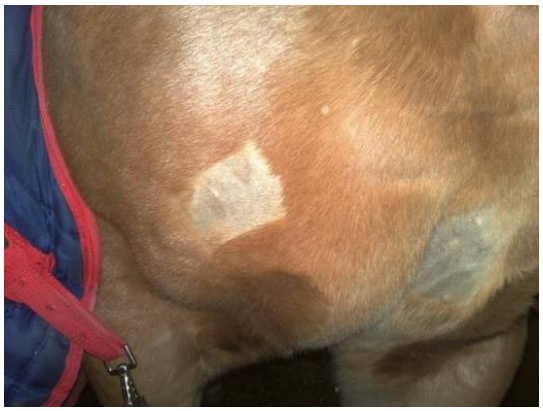
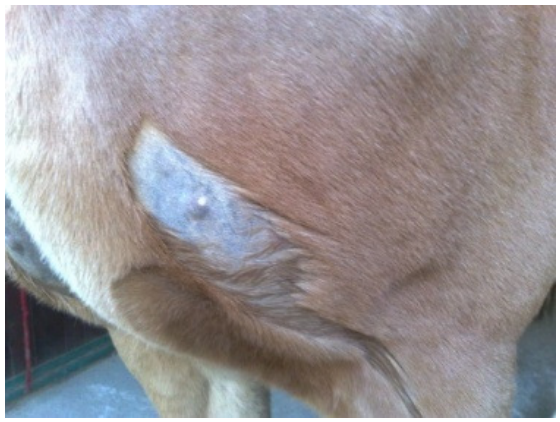
Lange-Consiglio et al., Stem Cell Development, 2013

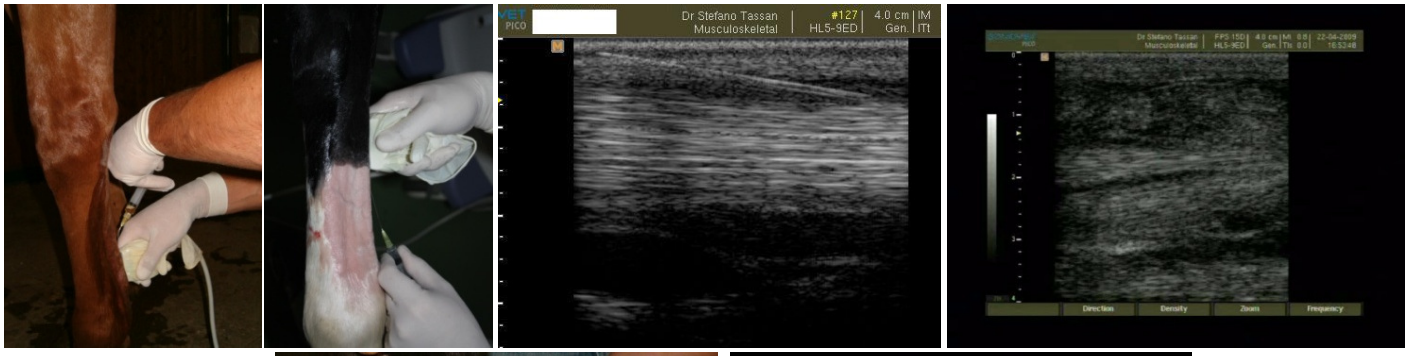
Experiment 2: Results of therapeutic effects

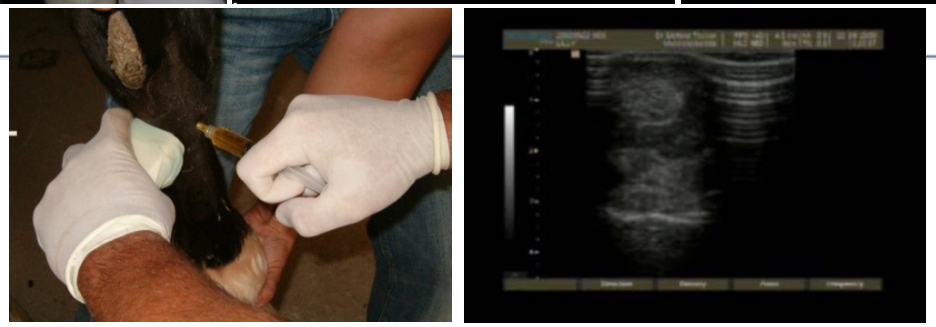


Preliminary phase
Subcutaneous injections of CM were well tolerated



In vivo treatment by AMCs-CM





Injection of **2 ml of AMCs-CM** by ultrasonographic guidance in **spontaneous acutely damaged tendons** and ligaments of 13 private sport horses.

Patients were clinically and ultrasonographically **monitored** monthly.

Success criteria were: ecographic evolution, return to former athletic function, and absence of relapses.

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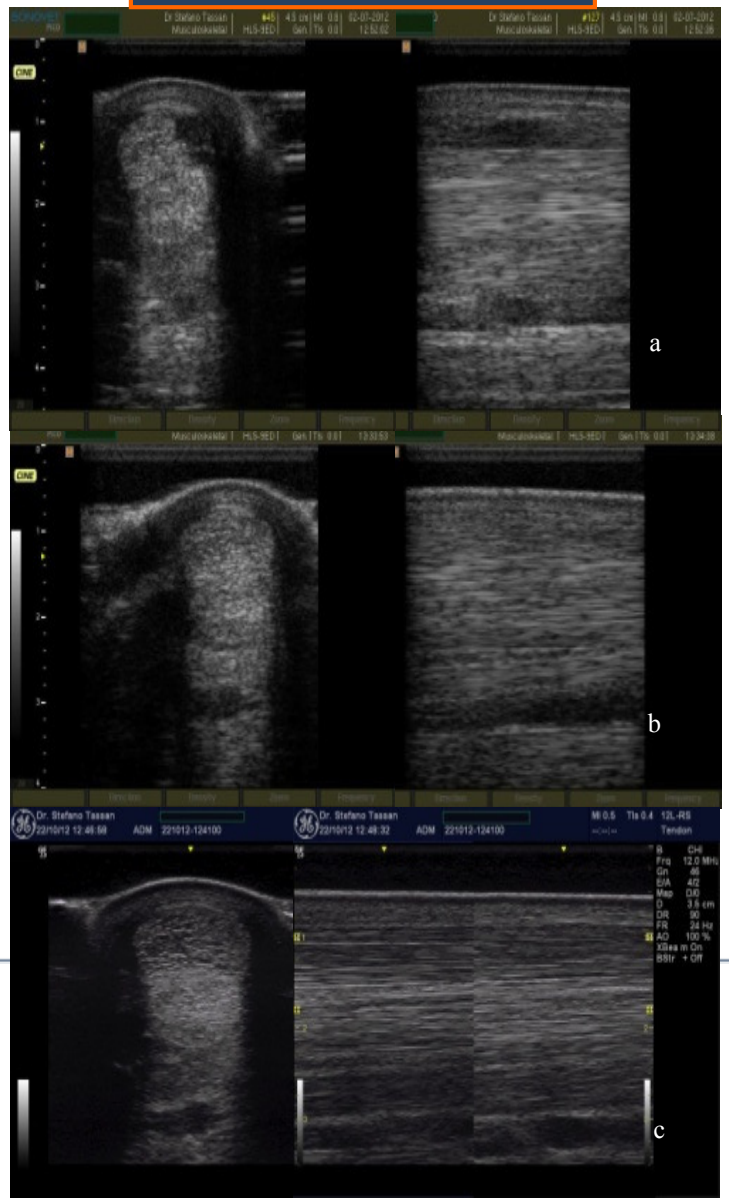
Lange-Consiglio et al., Stem Cell Development, 2013

RESULTS

AMCs- CM treatment



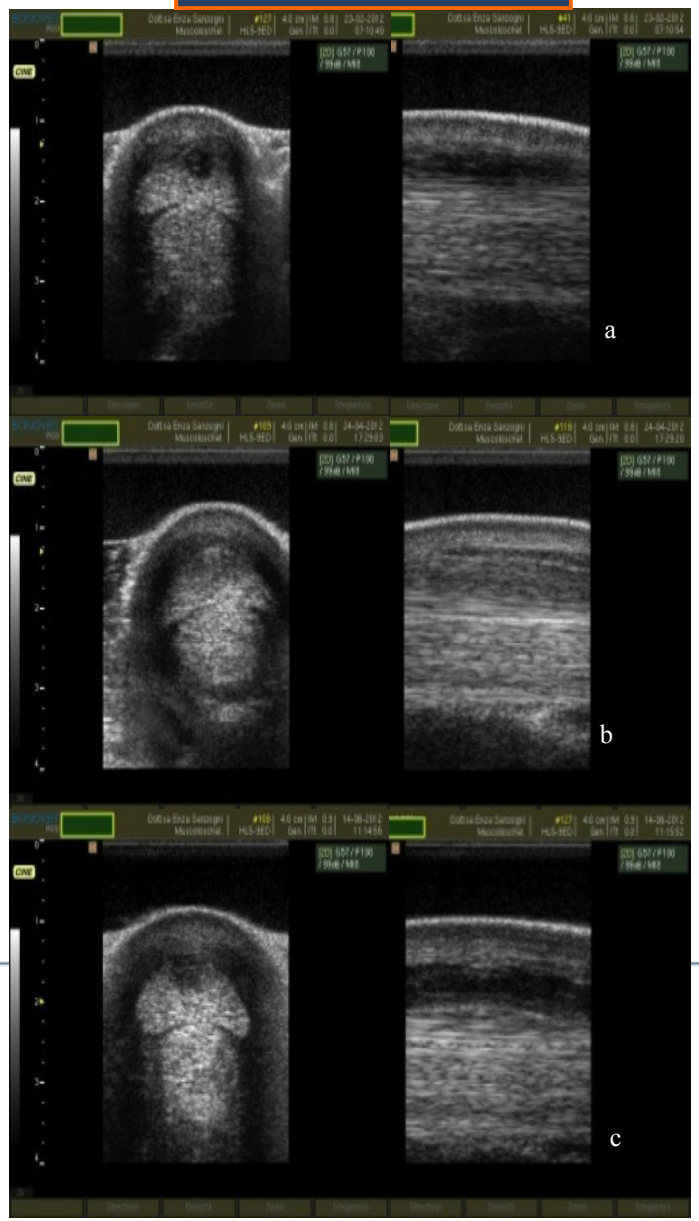
diagnosis

60 days

120 days

9% re-injury

No-CM treatment



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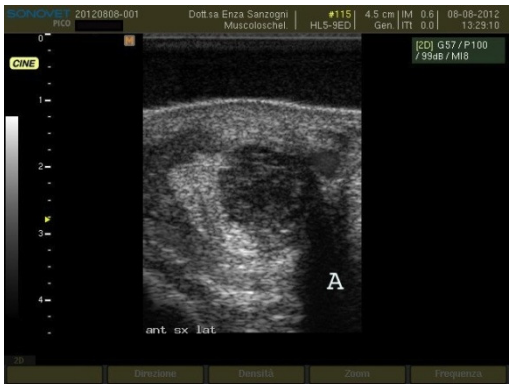
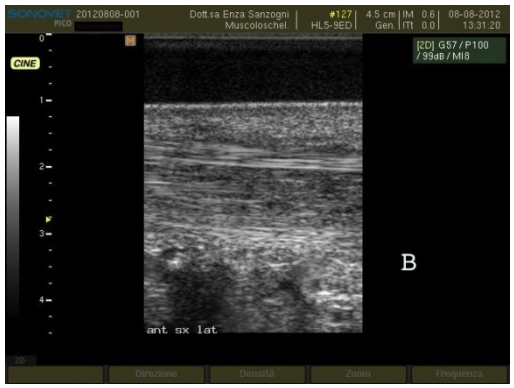
Lange-Consiglio et al., Stem Cell Development, 2013

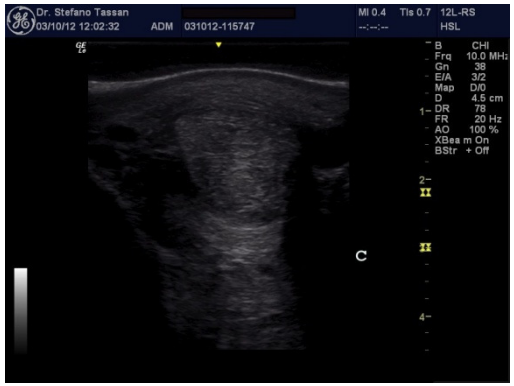
RESULTS

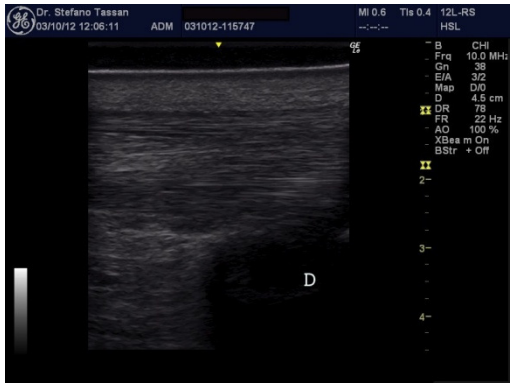


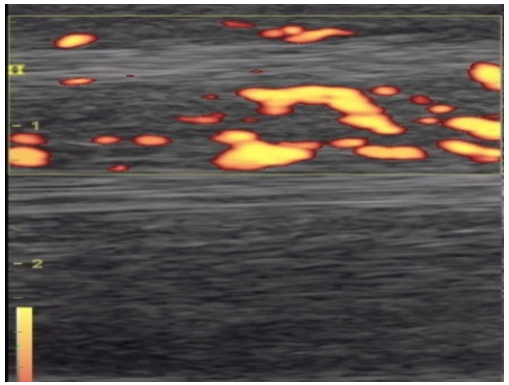


hypoecogenic area involves ⅔ of the ligament section (A)

loss of fibre architecture can be seen (B)

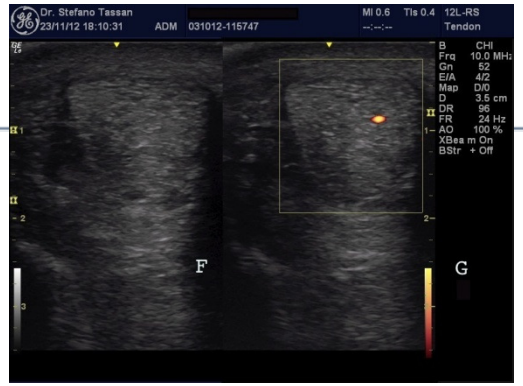






Intraligament **neovascularisation** can be detected, suggesting increased turnover (E)

increase in ecogenicity (C) and fibre alignment (D) can be observed **1 month later**



60 days post-injection, the ligament has reached a normal ecogenicity (F) and blood flow is back to normal (G), confirming an **adequate healing process**

Lange-Consiglio et al., Stem Cell Development, 2013

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In vitro

- AMCs are capable of **inhibiting PBMC proliferation** in a dose-dependent manner, either in cell-cell contact or in transwell system

- This finding suggests that **soluble factors** are implicated

- This hypothesis is supported by PBMC proliferation inhibition also with the **AMCs-CM**

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In vivo

- **Neovascularization**, as a functional stage of tendon healing, was constantly detected after our treatment both in tendons and ligaments

- While improvement in echogenicity and fiber architecture was observed, vessel size and quantity decreased at approximately the fourth month.

- This is clearly correlated with a **positive tissue healing process**, and must be considered as an **important timing predictor** in the rehabilitation

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CONCLUSIONS

AMCs-CM

Can be produced **easily** and in **large quantities**

Can be **stored efficiently** after liophilization

AMCs-CM can be considered a safe, novel biologic cell-free therapeutic agent in regenerative medicine

Can **be administered safely** via intravenous injection, avoiding clot formation and lung capillary entrapment

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Paracrine action of AMCs!

Modalities of cell-cell interaction???

Most of these secreted cannot span

the membranes freely and…

a vehicle should be involved to facilitate the crossing

MSC derived micro-vesicles

have been supposed as shuttles for the functional components for MSC paracrine action

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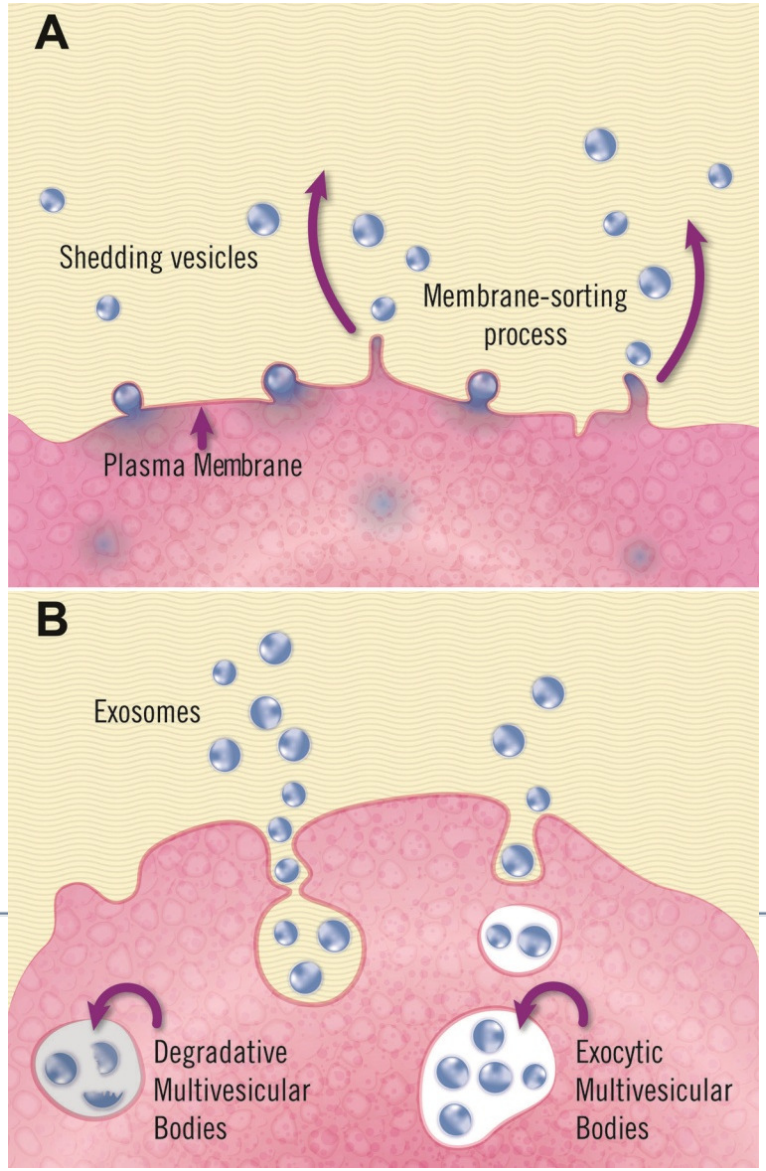
Production and release of microvesicles



A. **Shedding vescicles**, are produced by budding of cell membrane

B. **Exosome**, released by fusion of the exocytic multivesicular bodies with the cell membrane

Camussi G. et al, Am J Cancer Res , 2011

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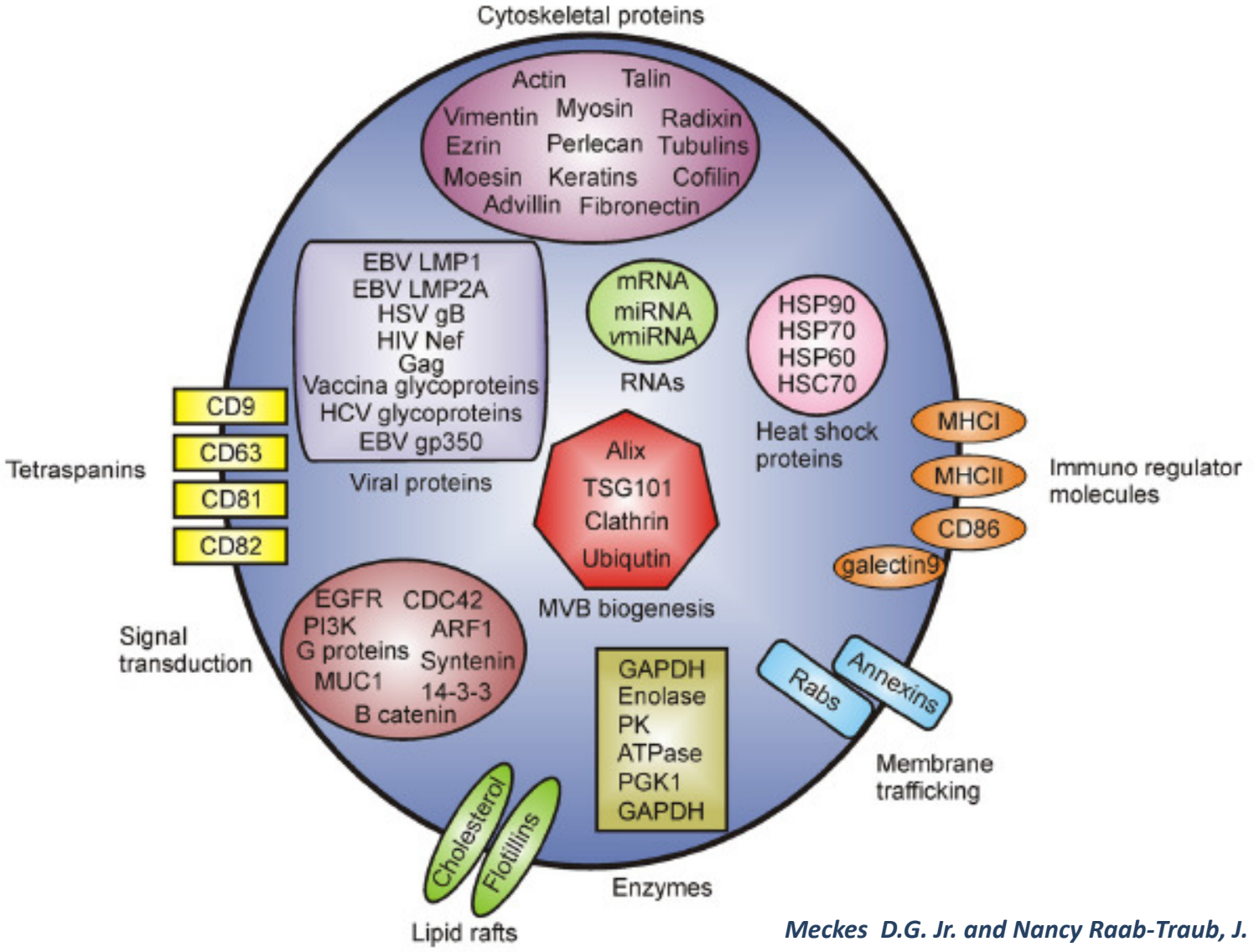
Molecules found in microvesicles



Meckes D.G. Jr. and Nancy Raab-Traub, J. Virol, 2011

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Characteristics of exosomes, shedding vesicles and apoptotic bodies

	Exosomes	Shedding vesicles	Apoptotic bodies
Size (nm)	30–120	100–1000	≥1000
Biogenesis	By exocytosis of multivesicular bodies Process dependent on cytoskeleton activation and Ca^{2+} independent	By budding of plasma membranes. Process dependent on Ca^{2+}, calpain and cytoskeleton reorganization	By blebbing of plasma membranes of dying cells
Markers	CD63, CD81, CD9, Tsg101, Alix, Hsc70 Low exposure of PS Markers specific to the cell of origin, e.g. PECAM in platelet vesicles and EGFRvIII in vesicles from gliomas	Lipid raft-associated molecules (TF, flotillin) High exposure of PS	Exposure of PS
Content	Proteins, lipids, mRNA and microRNA, rarely DNA	Proteins, lipids, mRNA and microRNA, rarely DNA	Fragmented DNA

APPROVED

NO

Biancone et al., Nephrol Dial Transplant, 2012

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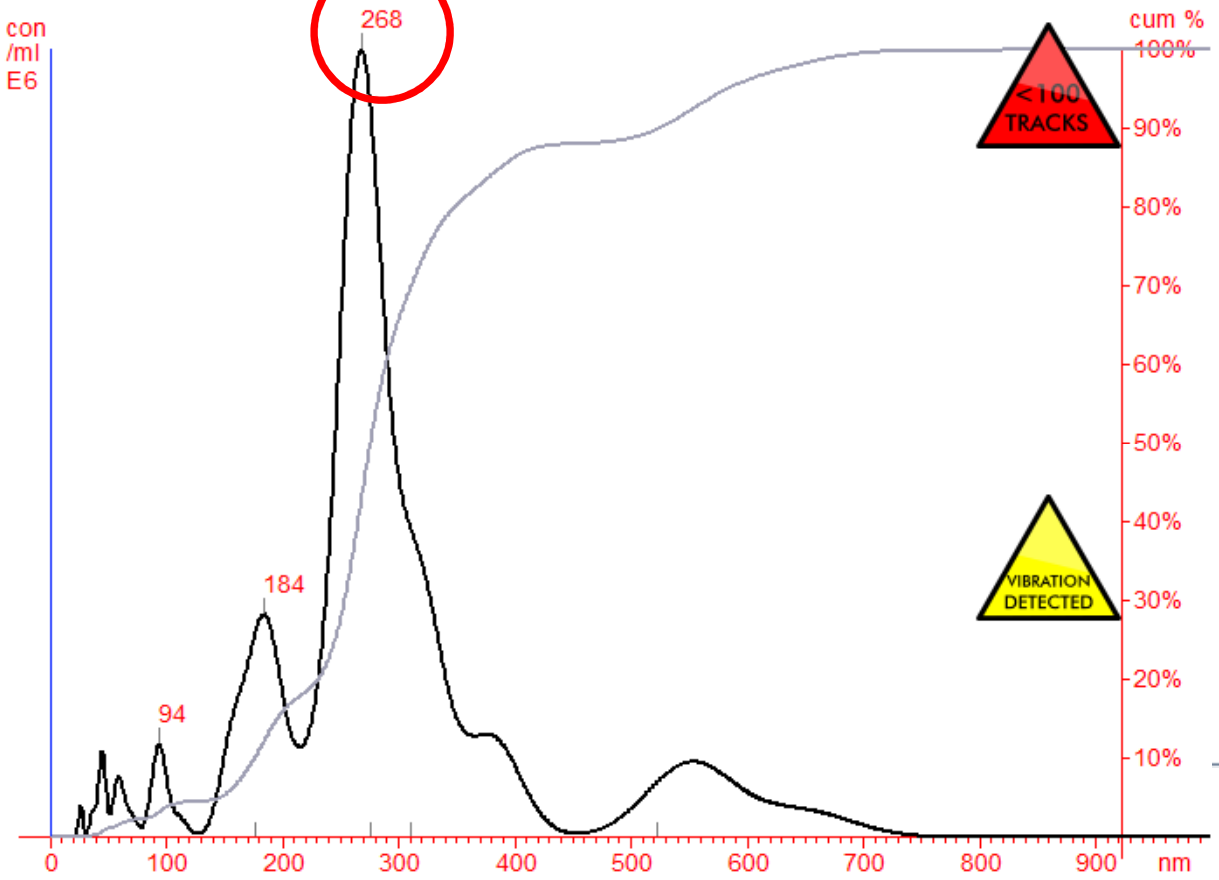
First demonstration of micro-vesicles derived from horse amniotic stem cells conditioned medium

ANALYSIS by Nanoparticle Tracking Analysis (NTA)



Concentration: $200x10^9$ MV/ml

MV production: 242 MV/cell

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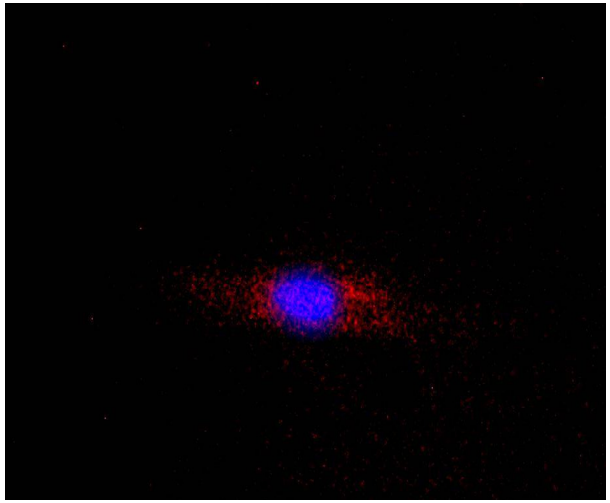
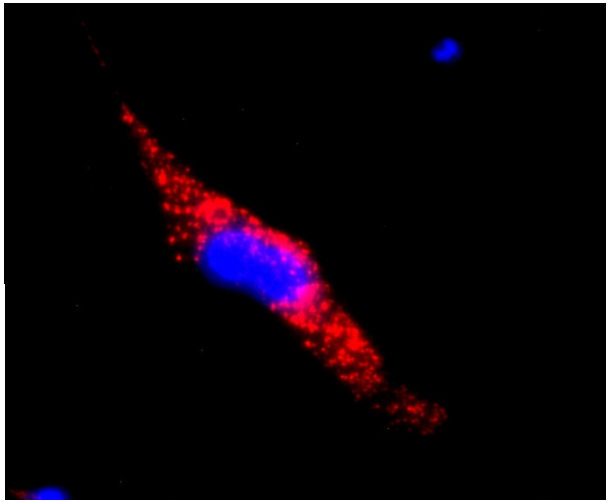
Unpublished data

Incorporation of microvesicles in tendon cells in vitro



24h



72h

Work in progress…….

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Unpublished data

CONCLUSIONS

The results of study *in vitro* lay the foundation for *in vivo* studies in equine tendinopathies

IN STEM CELL BIOLOGY CELLS CAN BE CONSIDERED AS A BIOLOGICAL LABORATORY FOR THE PRODUCTION OF THERAPEUTIC SUBSTANCES

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***Dr Stefano Tassan**, private practitioner,* for *in vivo* study

*Many graduate and undergraduate **students***

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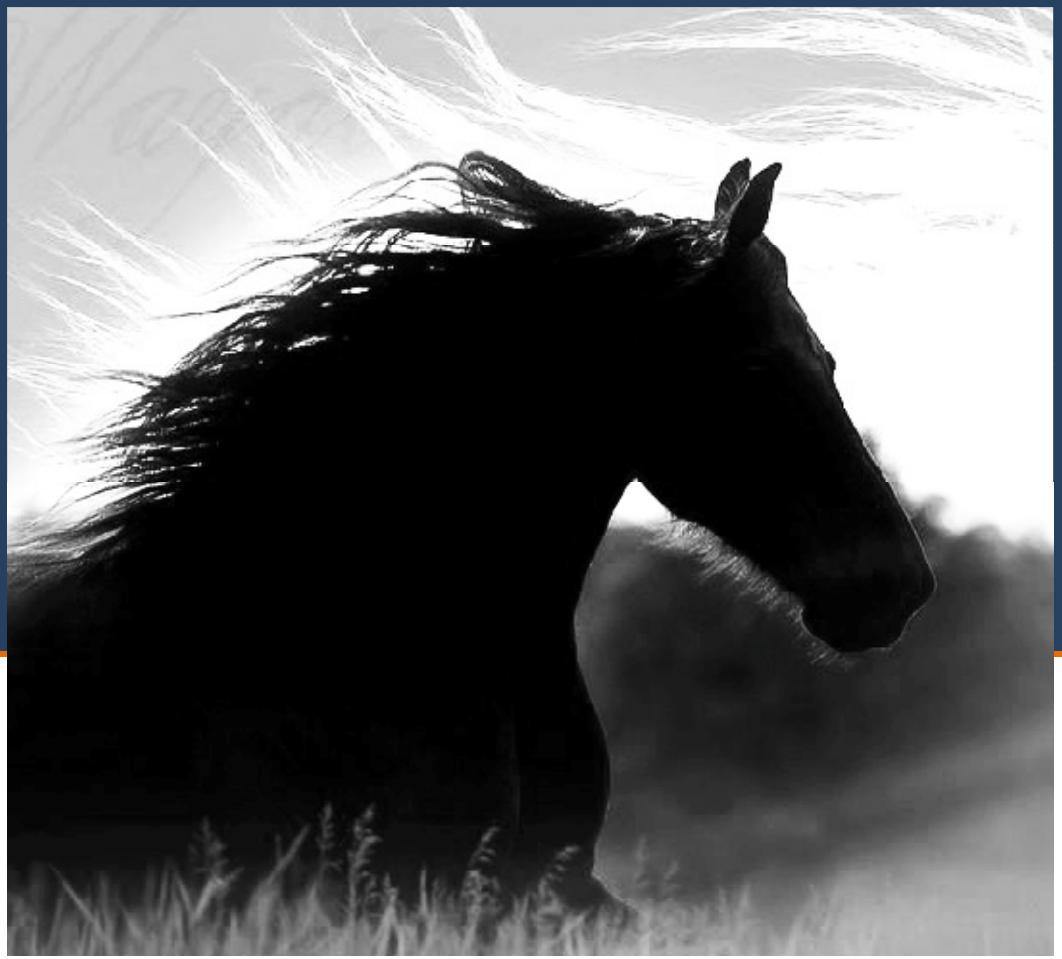
Thank you for your attention



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