PÔLE ÉQUIN

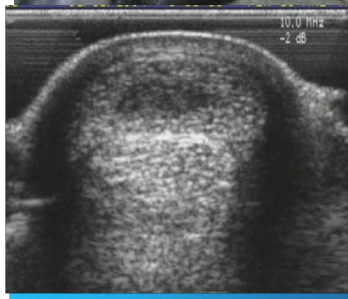
Royal Veterinary College
University of London

Biological (Regenerative) Medicine for Tendon and Ligament Injuries

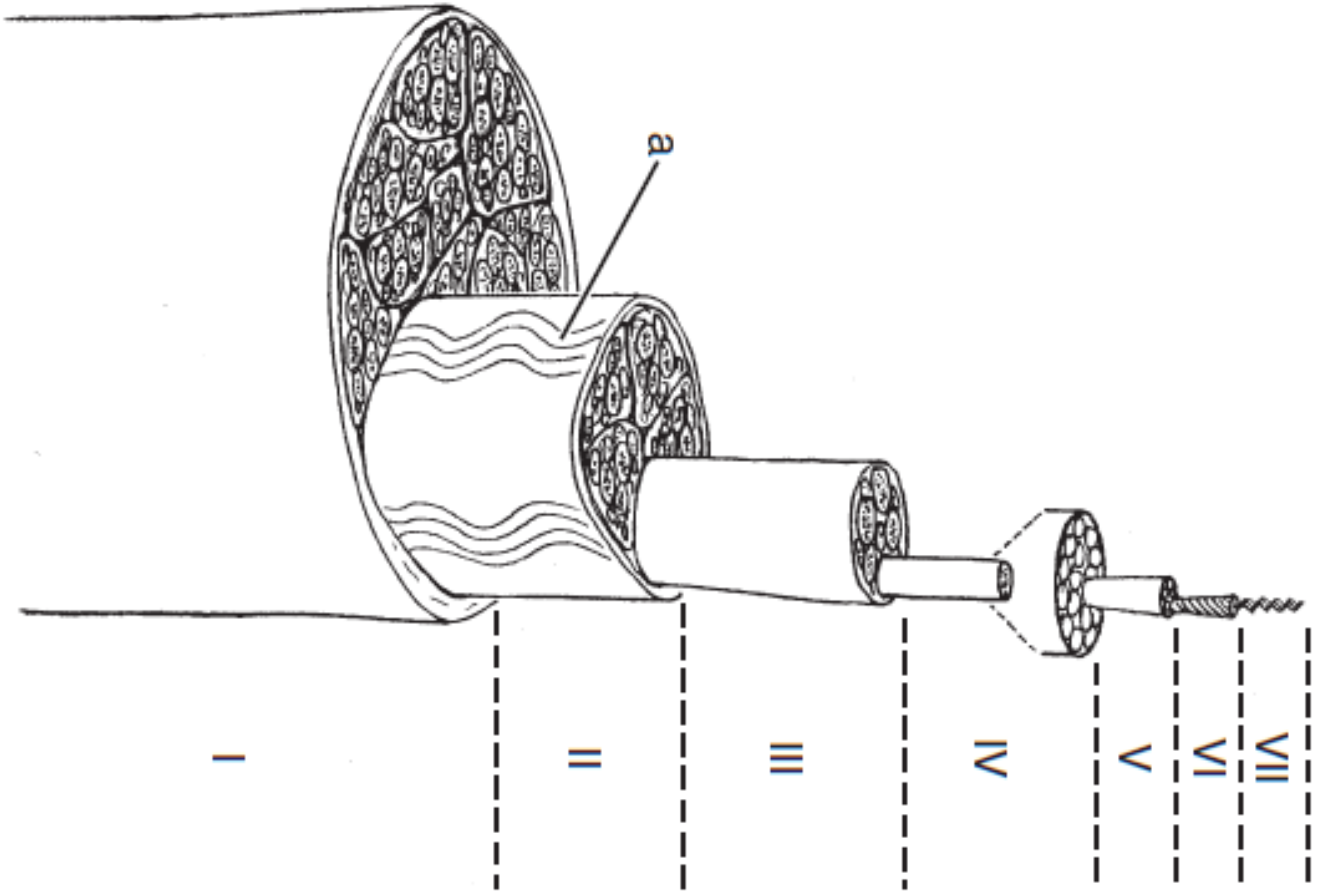
Michael Schramme and Roger Smith

Tendinopathy and Desmopathy

- Very common in horses
  - 46% of limb injuries at racecourses involve flexor tendons/suspensory ligament (Williams et al.)
  - 43% of NH racehorses in training have ultrasonographic evidence of tendon disease (Pickergill and Marr)
  - 23% of NH racehorses in AHT/RVC National Hunt study (Ely, et al.)



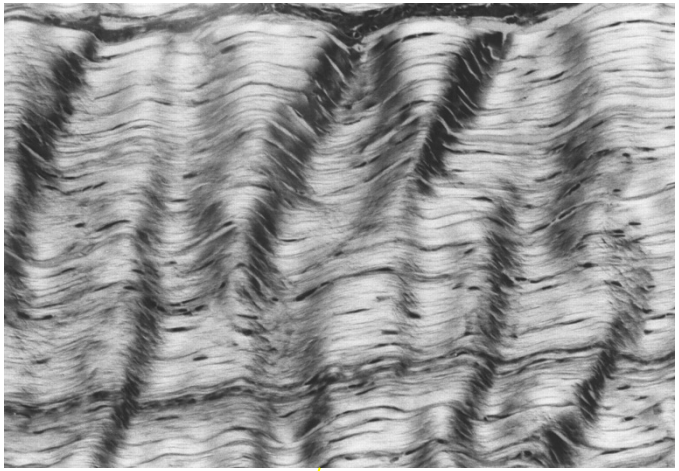
Hierarchy of Increasingly Smaller Subunits

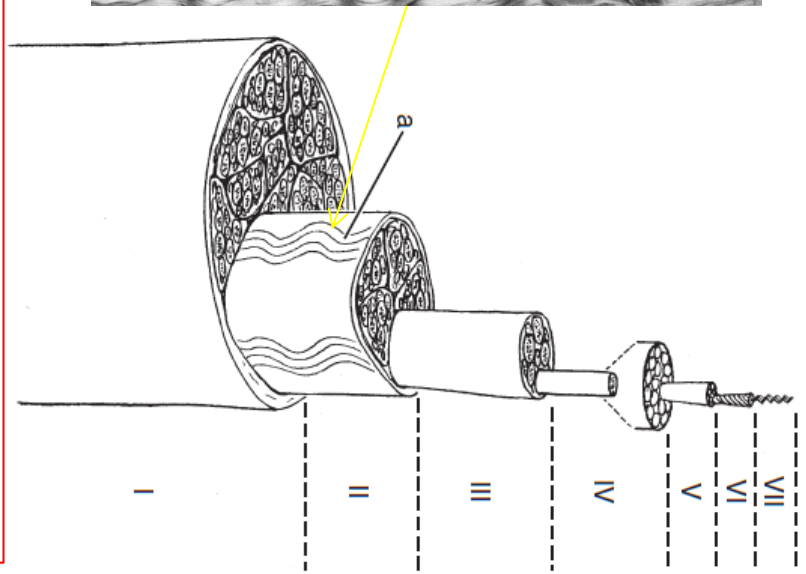


I tendon, II-IV fascicles, V collagen fiber, VI fibril, VII triple helix of collagen alpha chains

Tendon Elongation

- At rest, fascicles have a wave form known as crimp, that stretches with elongation.
- Majority of tendon elasticity/elongation comes from sliding of fascicles over each other rather than stretching of fascicles
- Important role for endotenon in sliding
- Paratenon layer outside the tendon stretches considerably with elongation





I tendon, II-IV fascicles, V collagen fiber, VI fibril, VII triple helix of collagen alpha chains

Mechanism of Clinical Injury

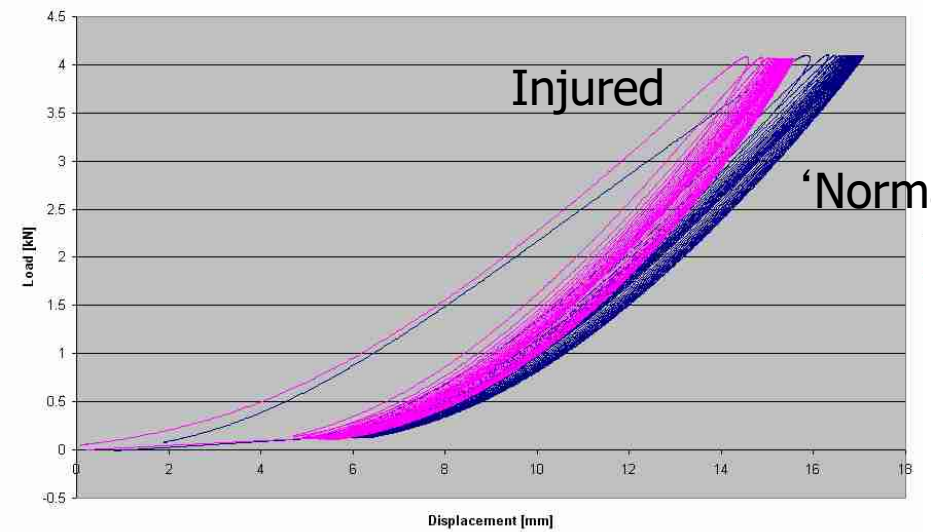
- Once the peak load on the tendon overcomes its structural strength, there is physical disruption of the matrix:
  - Fibrillar slippage (breakage cross-links)
  - Fibrillar rupture
  - Complete separation fibers and fascicles

→ HAEMORRHAGE → INFLAMMATION

- → FIBROPLASIA → SCAR

Rationale for the use of regenerative medicine in tendon disease

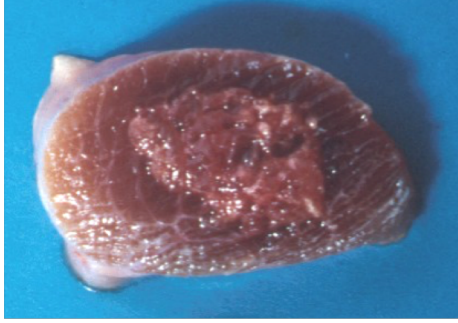
- Consequences of injury
  - Stiffer, less elastic tendon
  - Reduced performance
  - High frequency of re-injury
    - 56% for NH race horses (Dyson, 2004)
- Treatment must be aimed at optimising function:
  - SCAR-FREE WOUND HEALING
  - REGENERATION instead of FIBROUS REPAIR



Ex-vivo tendon mechanics

PROMOTING REGENERATION vs. REPAIR

Regenerative Medicine



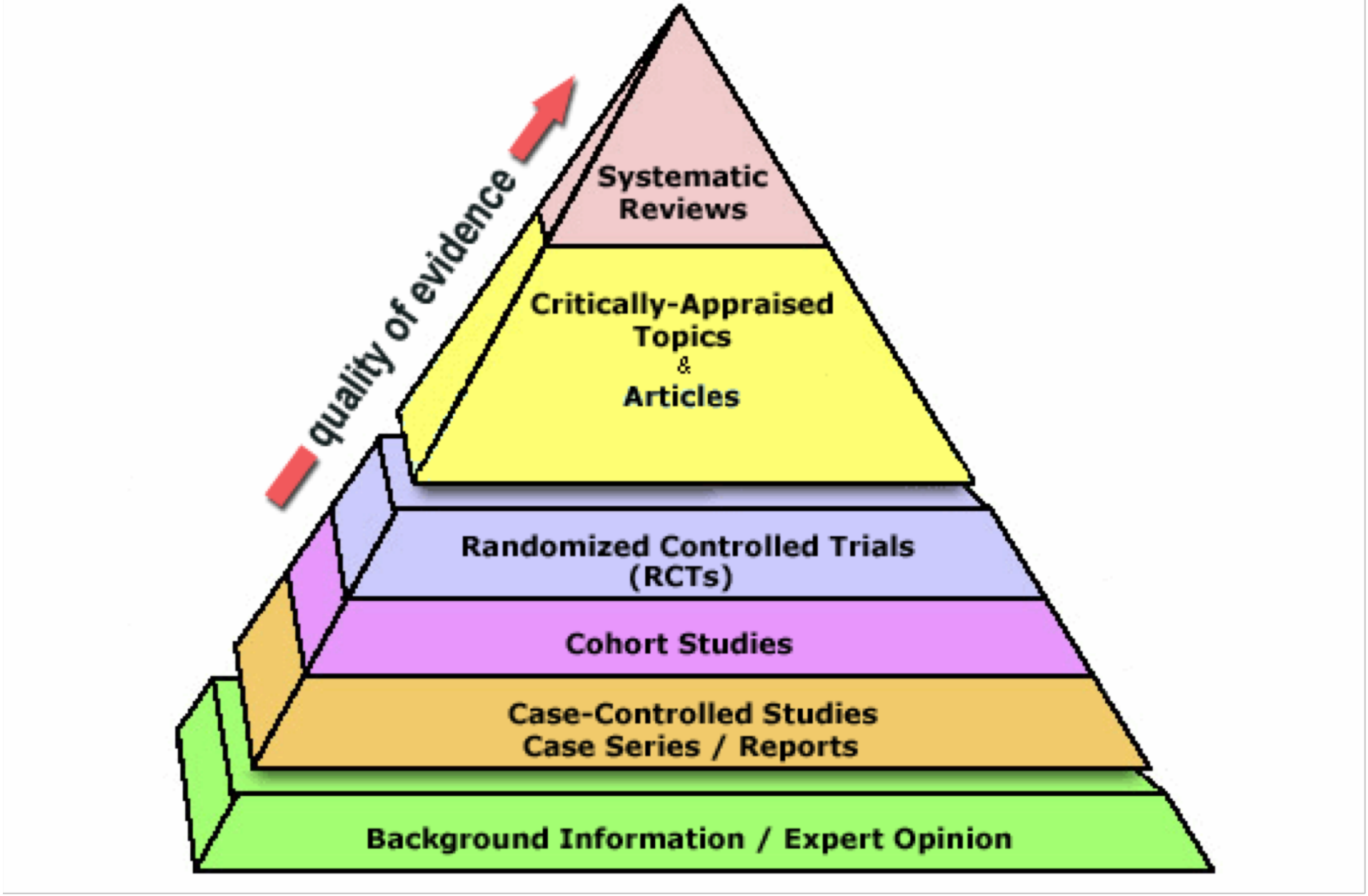
- Four components
  - Growth Factors
  - Cells
  - Scaffolds
  - Mechanical environment

Acute (inflammatory) phase

'Biologics'

Subacute (proliferative) phase

Chronic (remodelling) phase

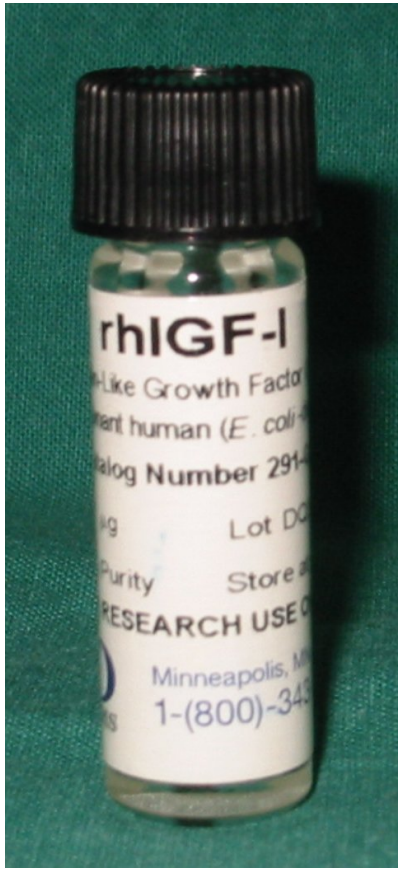


PYRAMID OF EVIDENCE-BASED MEDICINE

Individual Growth Factors: IGF-I

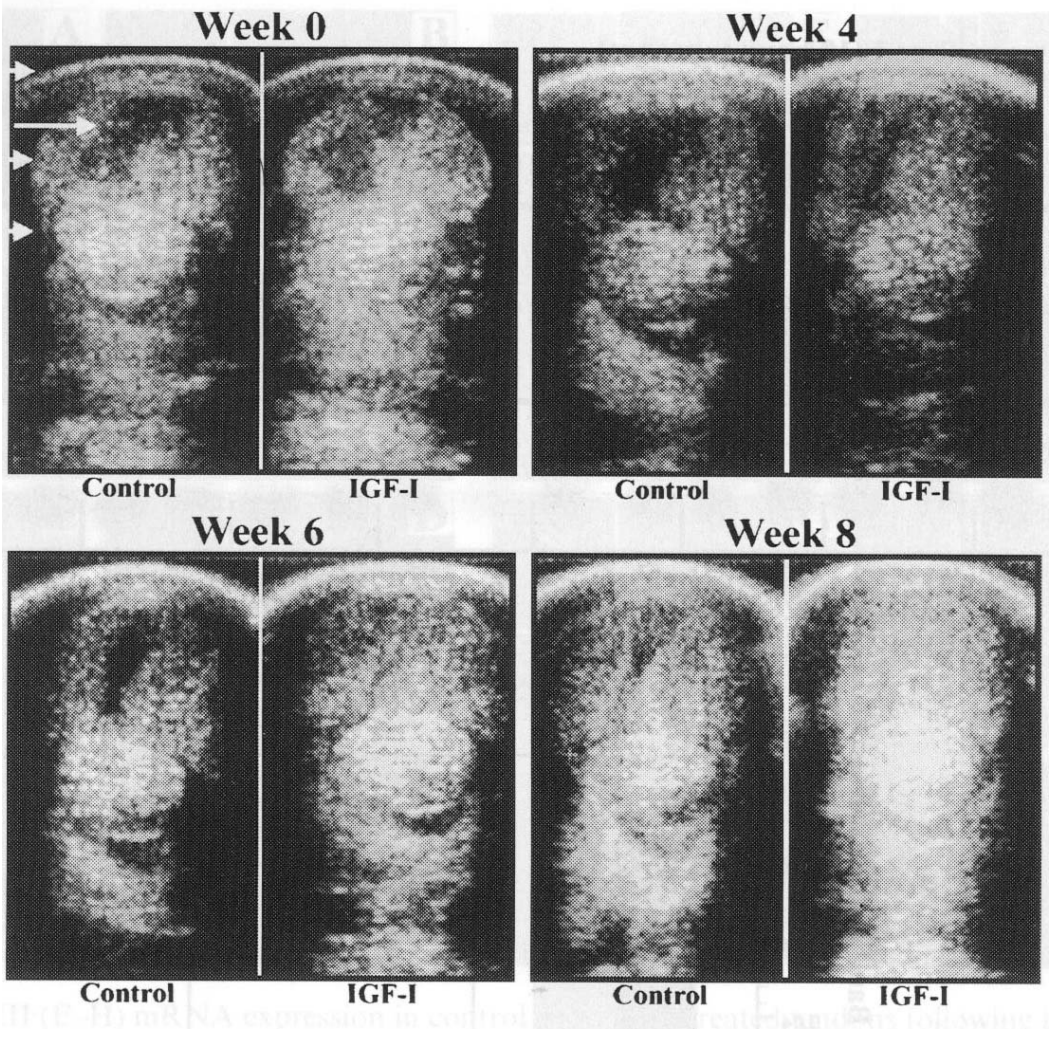
IGF1
(Dahlgren, Witte, Nixon, et al.)

- Collagenase model
- 2µg rhIGF-1 e.o.d. for 10 injections
- IGF1 treated
  - Reduced swelling
  - Lesion smaller at 3 and 4 weeks after treatment (but not afterwards)
  - Cell proliferation and collagen content increased
  - 'Trend' towards increased stiffness (p=0.1)



IGF1 treatment

BUT - ? Just more scar tissue



Intralesional injection of insulin-like growth factor-I for treatment of superficial digital flexor tendonitis in Thoroughbred racehorses: 40 cases (2000–2004)

Thomas H. Witte, BVetMed, PhD, DACVS; Amy E. Yeager, DVM, DACVR; Alan J. Nixon, BVSc, MS, DACVS

JAVMA 2011

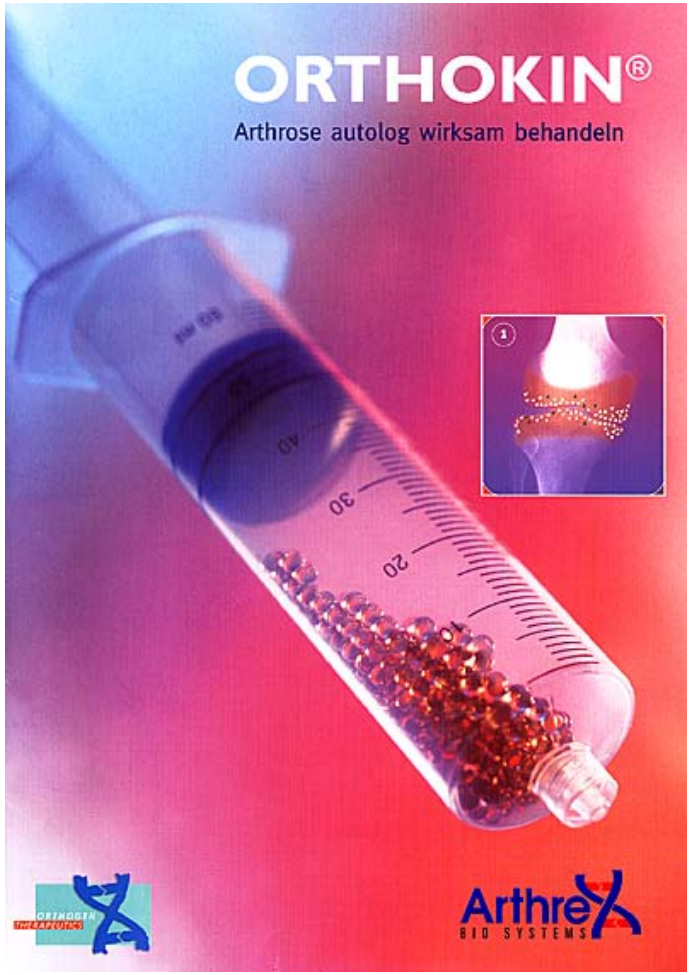
- 40 TB racehorses with SDF tendonitis
- treated within 3 m by intralesional injection of IGF-I (25 or 50 µg every other day for 4 or 5 treatments)
- 26 horses underwent superior check ligament desmotomy.
- 62% raced at least once after treatment,
  30% raced between 1 and 4 times,
  32% raced ≥ 5 times.
- Thirteen of 28 (46%) horses had a recurrence of tendonitis or developed tendonitis elsewhere.
- IGF-I did not improve the prognosis for racing

IRAP Therapy

Orthokine^{R} IRAP

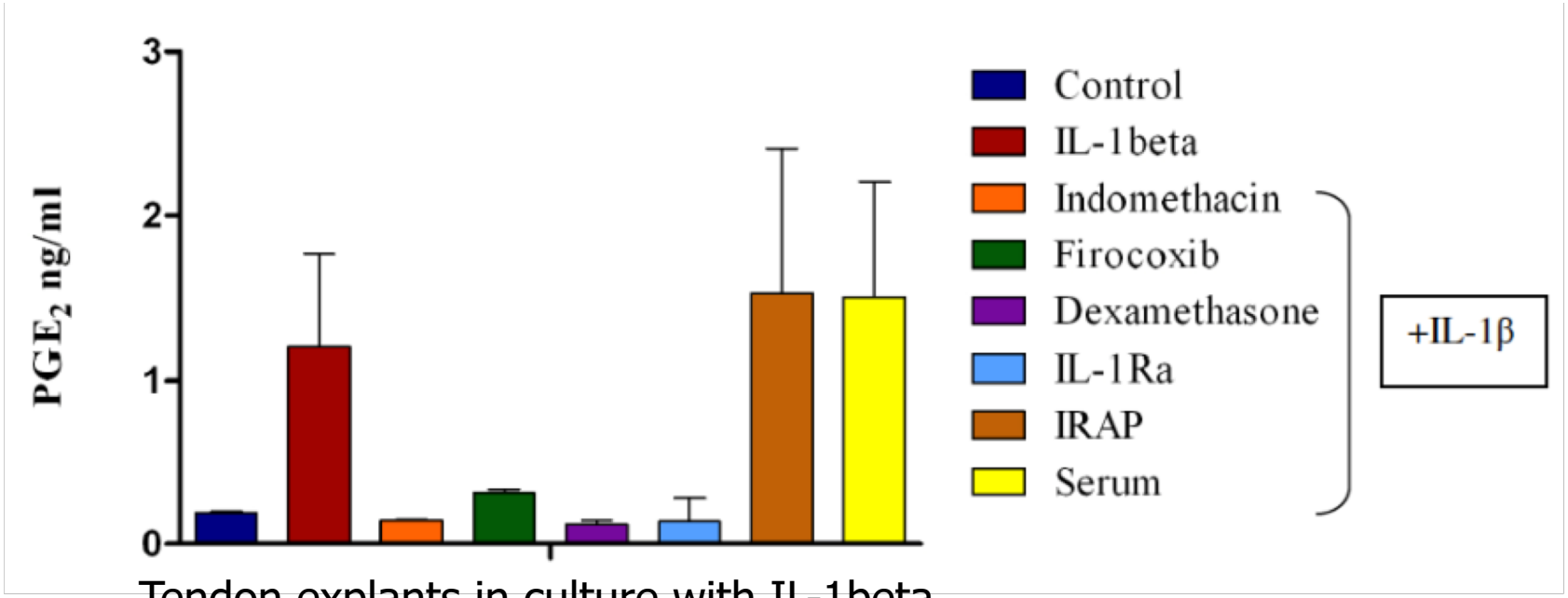
Meijer et al. 2003

- Human leukocytes exposed to medical-grade glass beads coated with chromium sulfate stimulates:
  - IL-4, IL-10
  - IL-1Ra (x 140!)
  - FGF-1,hepatocyte growth factor, and TGF-β1
  - NO increase IL-1β or TNF-α.



Orthokine™/IRAP/Autologous conditioned serum (ACS)

- RESULTS –
  - Not convincing
  - ?Growth factors
- Not well evaluated for tendons and ligaments
- Does it really contain IRAP?



Tendon explants in culture with IL-1beta

AUTOLOGOUS CONDITIONED SERUM STIMULATES AN ANABOLIC RESPONSE IN EQUINE TENDON EXPLANTS Linda A. Dahlgren, Stewart C. Harvey. Virginia-Maryland Regional College of Veterinary Medicine, Blacksburg, VA, United States

Dahlgren, ACVS 2007

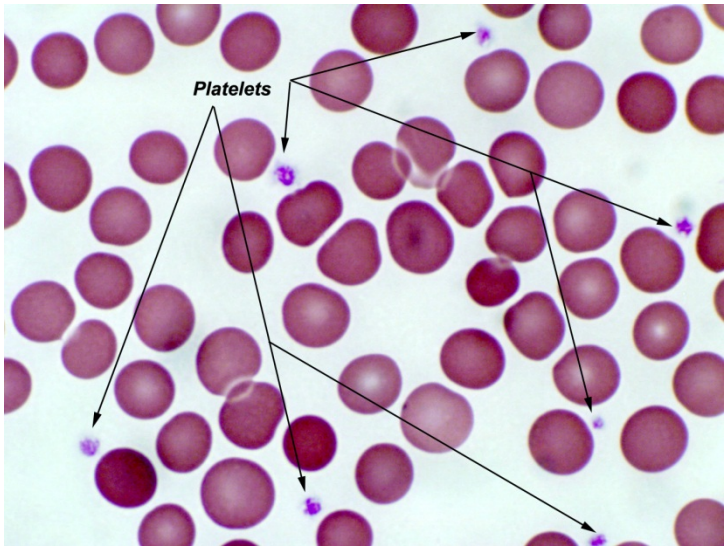
- Tendon explants from 6 adult horses in medium containing 10% FBS, and 10%, 50%, and 100% normal equine serum and Orthokine (IRAP).
- No significant differences for the levels of IGF-I, FGF-2 and TGF-β1 between normal serum and Orthokine.
- Treatment of tendon explants with both normal serum or Orthokine generally increased gene expression compared to 10% FBS.
- There were no significant differences between normal serum and Orthokine for expression of collagen type I, type, III, COMP, or MMP13 between normal serum and Orthokine.
- Orthokine may be beneficial in the treatment of tendon injury.

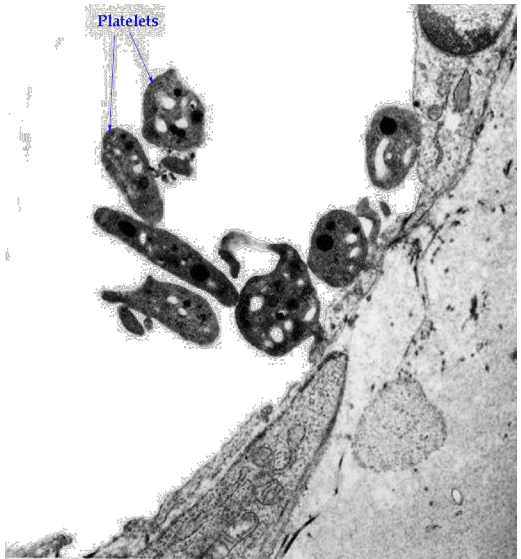
No clinical information available!

PRP Therapy

PLATELET-RICH PLASMA

- Any preparation which concentrates platelets above the levels in peripheral blood (varies!)
- Platelets are not cells but cytoplasmatic fragments of precursor cells of the bone marrow (large megakaryocytes).
- Initiate clotting cascade
- α-granules rich in bioactive proteins!





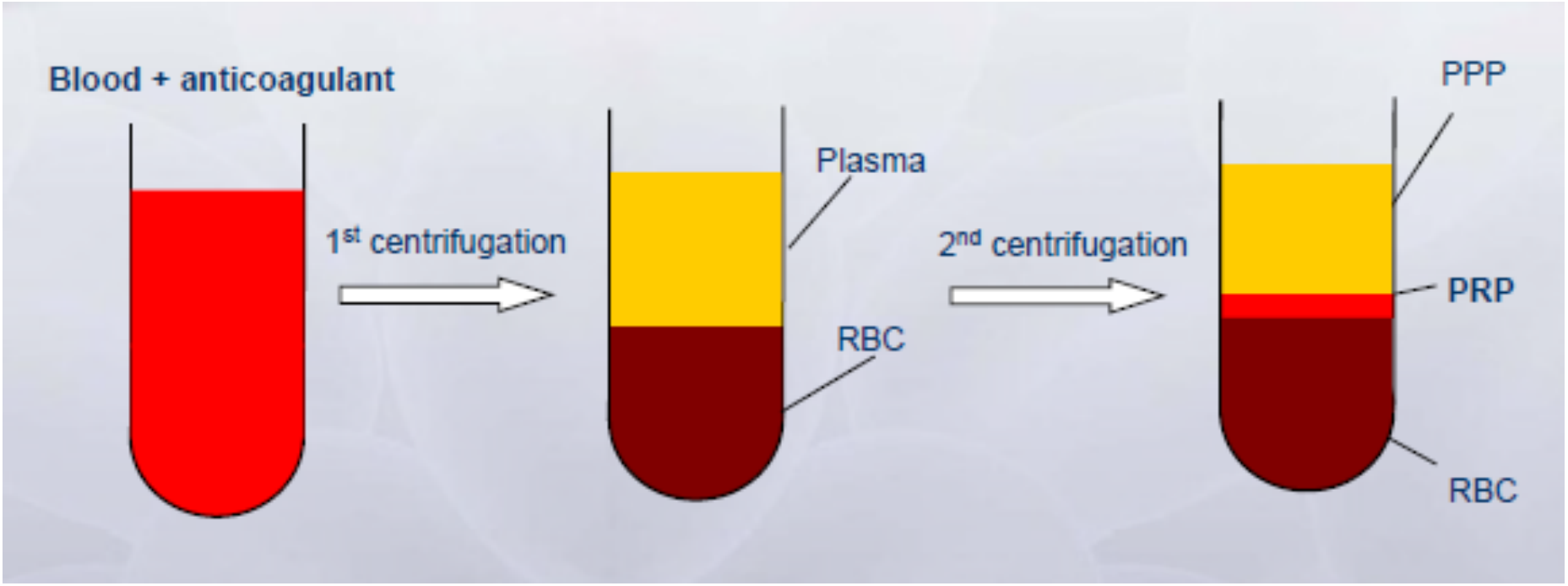
Platelet-contained Growth Factors (GF)

- TGF-ß, PDGF, EGF, VEGF, IGF, HGF,… (>30)
- Control differentiation of stem cells and stimulate cell proliferation
- **PDGF-BB** (Platelet Derived Growth Factor)
  - Mitosis, angiogenesis, wound contraction & remodeling
- **TGF-$ß_1$** (Transforming Growth Factor)
  - Cell proliferation & differentiation, angiogenesis

PLATELET RICH PLASMA

Preparation

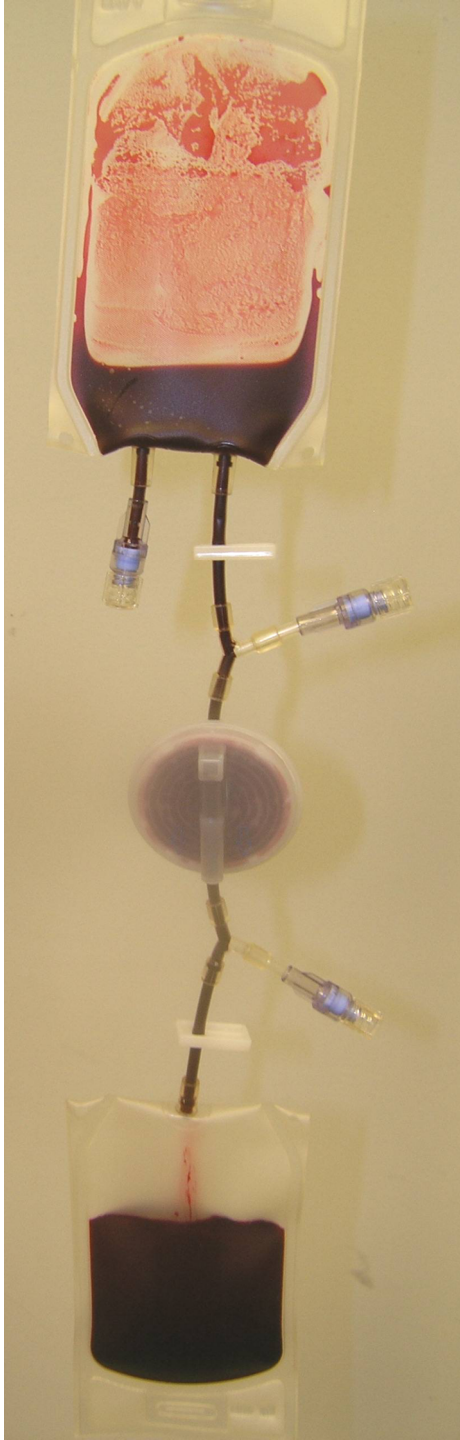
- Double centrifugation/separation



PLATELET-CONCENTRATE

Filtration Based System

- e-PET™ (Pall Corporation)
- Closed, field system
- Optimised for equine blood
- Sufficient volume to treat most tendon or ligament injuries

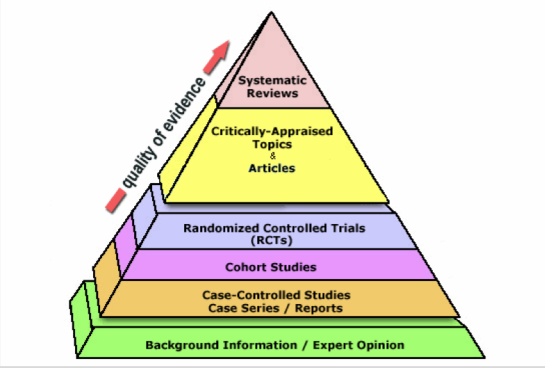


WBCs Good or Bad?

- Concentration correlates with increase in expression of catabolic genes

	Platelet Concentration			WBC* Concentration		
	r	r^2	p	r	r^2	p
COL1A1:COL3A1	0.79	0.62	<0.01	-0.55	0.30	<0.01
COMP	0.73	0.53	<0.01	-0.40	0.16	<0.01
MMP-3	-0.37	0.14	<0.01	0.65	0.42	<0.01
MMP-13	-0.76	0.58	<0.01	0.45	0.20	<0.01

McCarrel & Fortier, JOR, 2010



Efficacy in humans: CLINICAL

Platelet-Rich Plasma Treatment for Ligament and Tendon Injuries

Justin Paoloni, MBBS, PhD, Robert J. De Vos, MD,† Bruce Hamilton, MBChB,*‡ George A. C. Murrell, MD, DPhil,§ and John Orchard, MBBS, MD¶*

Clin J Sport Med • Volume 21, Number 1, January 2011

- Based on the limited publications to date and theoretical considerations, the potential risks involved with PRP are fortunately very low.
- However, benefits remain unproven to date, particularly when comparing PRP with other injections for ligament and tendon injuries.

J Orthop Res. 2013 Jan;31(1):111-8. doi: 10.1002/jor.22199. Epub 2012 Aug 6.

The role of platelets in the treatment of Achilles tendon injuries.

Sadoghi P[1], Rosso C, Valderrabano V, Leithner A, Vavken P.

Abstract

To systematically review the current in-vivo evidence for the use of platelet-concentrates (PRP) in the treatment of Achilles tendinopathy and Achilles tendon ruptures in animal models and human applications. A systematic search of PubMed, CINAHL, EMBASE, CCTR, and CDSR was performed for animal and human studies on the effect of platelet-concentrates in the treatment of Achilles tendinopathy and ruptures using the terms "Achilles tendon and platelet." The systematic search revealed a total of 149 papers. After excluding duplicates and cases of overlapping data, studies not focusing on in vivo evidence in terms of treatment or outcome, studies without any intervention, studies with unacceptable high attrition, one Chinese and one Swedish study, the remaining 14 manuscripts were included. The key finding of our study is evidence in support of a statistically significant effect of platelet concentrates in the treatment of Achilles tendon ruptures in vivo in animal models and human application, consistent with a medium to large sized effect. This effect is most likely attributable to fastened and enhanced scar tissue maturation. There was no evidence for a beneficial effect of platelets in Achilles tendinopathy.

Contents lists available at ScienceDirect

Foot and Ankle Surgery

20(2014) 2-9

journal homepage: www.elsevier.com/locate/fas

Review

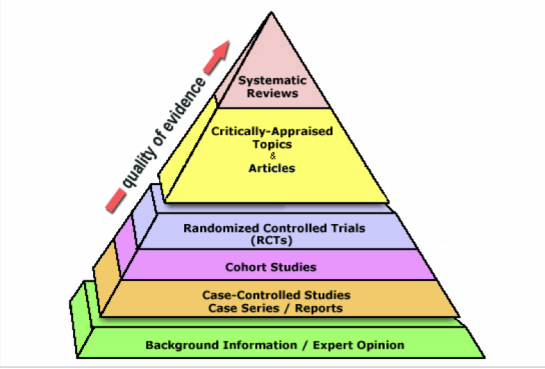
Platelet-rich plasma for foot and ankle pathologies: A systematic review

F. Vannini [a], B. Di Matteo [b,*], G. Filardo [b], E. Kon [b], M. Marcacci [b], S. Giannini [a]

[a] I Orthopaedic Clinic and Movement Analysis Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano n. 1/10, 40136 Bologna, Italy
[b] II Orthopaedic Clinic and Biomechanics Laboratory, Rizzoli Orthopaedic Institute, Via di Barbiano n. 1/10, 40136 Bologna, Italy

Results: A total of 17 studies fulfilled the inclusion criteria. Nine papers dealt with Achilles tendon management, 2 articles with plantar fasciitis, 3 papers with talar osteochondral lesions, 2 with PRP application in total ankle replacement, and 1 article with PRP in foot and ankle fusions. The overall evaluation of the results reported does not clearly demonstrate the potential of PRP treatment in any of the specific fields of application.

Conclusions: Considering the literature currently available, no clear indications for using PRP in the foot and ankle district emerged.



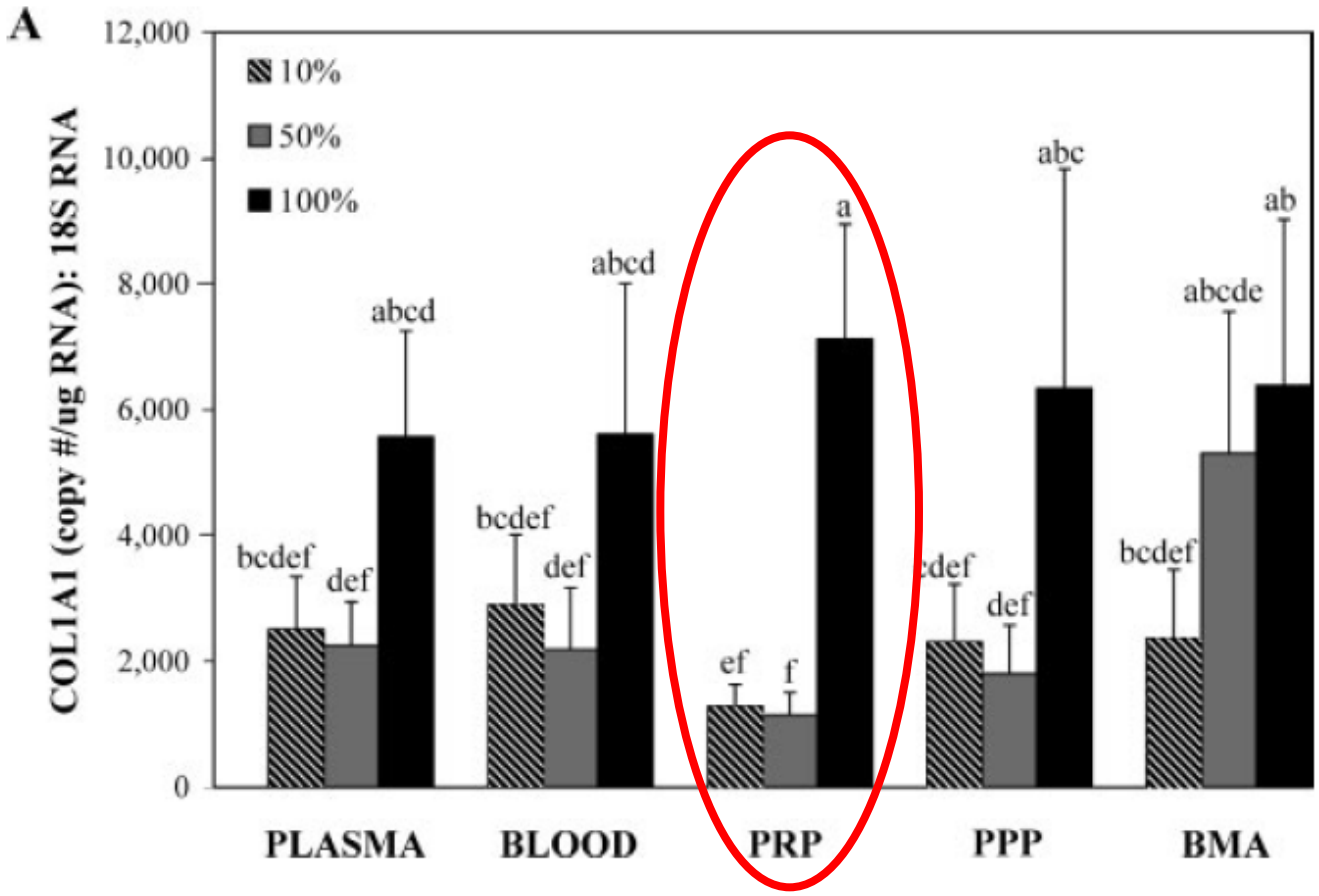
Efficacy in horses: IN VITRO

Platelet Rich Plasma (PRP) Enhances Anabolic Gene Expression Patterns in Flexor Digitorum Superficialis Tendons

Lauren V. Schnabel,[1] Hussni O. Mohammed,[2] Brian J. Miller,[1] William G. McDermott,[1] May S. Jacobson,[3] Kelly S. Santangelo,[1] Lisa A. Fortier[1]

Journal of Orthopedic Research (2007) 25, 230-240.

- This study examined gene expression patterns, DNA, and collagen content of equine tendon explants cultured with
  - whole blood,
  - plasma,
  - patelet-rich plasma,
  - platelet-poor plasma and
  - bone marrow aspirate.



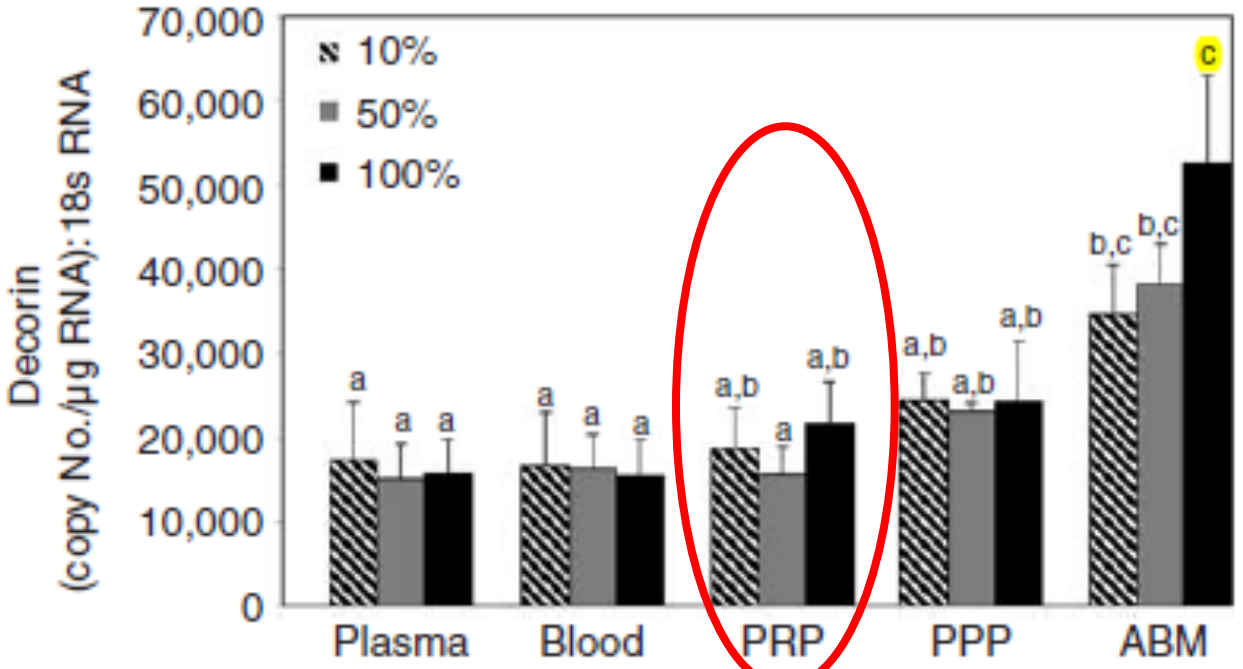
- All blood products stimulated gene expression, but overall PRP 100% stimulated the greatest number of genes (Collagen types I and III, and COMP).

Effects of platelet rich plasma and acellular bone marrow on gene expression patterns and DNA content of equine suspensory ligament explant cultures

EQUINE VETERINARY JOURNAL
Equine vet. J. (2008) **40** (3) 260-265

L. V. SCHNABEL, H. O. MOHAMMED†, M. S. JACOBSON‡ and L. A. FORTIER*

Departments of Clinical Sciences, and †Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, New York 14853; and ‡Children's Hospital, Harvard Medical School, Boston, Massachusetts 02115, USA.



- PRP did not enhance anabolic gene expression patterns in equine suspensory ligament explants.
- Acellular bone marrow aspirate at 100% stimulated decorin and COMP mRNA synthesis

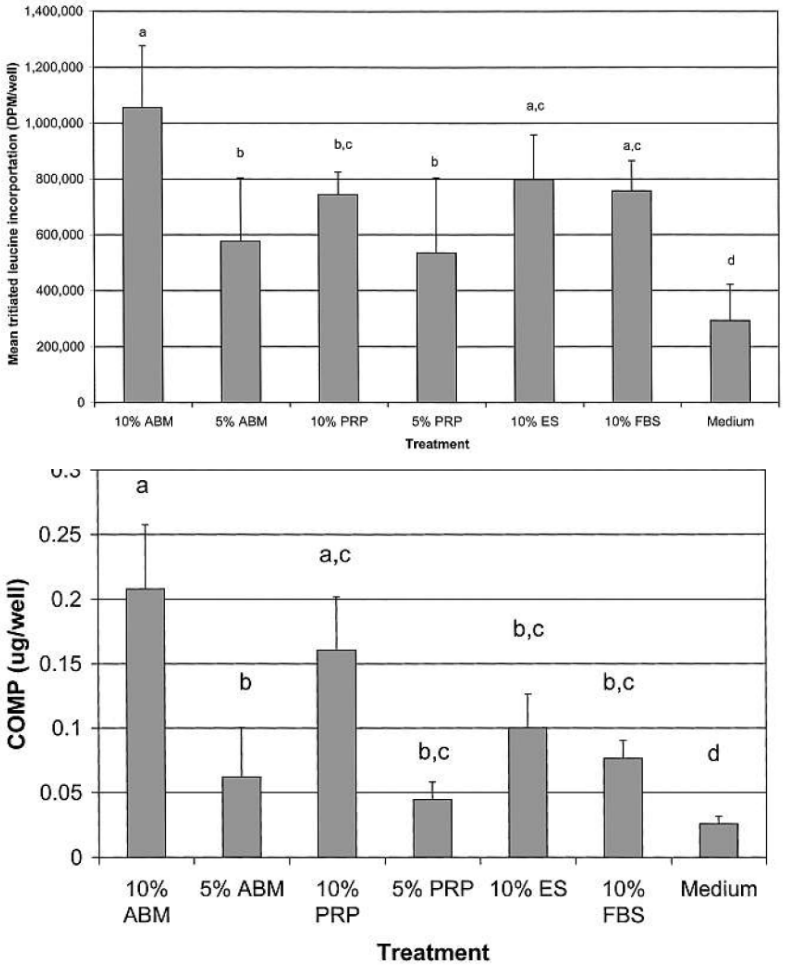
Anabolic effects of acellular bone marrow, platelet rich plasma, and serum on equine suspensory ligament fibroblasts *in vitro*

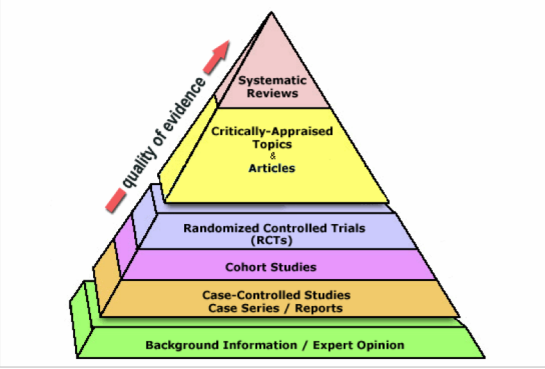
J. J. Smith[1], M. W. Ross[1], R. K. W. Smith[2]

Vet Comp Orthop Traumatol 1/2006

Equine ABM, PRP, and serum:

- All contain anabolic factors that promote matrix synthesis by suspensory ligament fibroblasts *in vitro*
- ABM had greatest effect





Efficacy in horses: EXPERIMENTAL

Effects of Platelet-Rich Plasma on the Quality of Repair of Mechanically Induced Core Lesions in Equine Superficial Digital Flexor Tendons: A Placebo-Controlled Experimental Study

Gerco Bosch,[1] Hans T. M. van Schie,[1,2] Mark W. de Groot,[3] Jennifer A. Cadby,[1,2] Chris H. A. van de Lest,[1,4] Ab Barneveld,[1] P. René van Weeren[1]

JOURNAL OF ORTHOPAEDIC RESEARCH FEBRUARY 2010

- Increased collagen, GAG, DNA content (cellularity), neovascularisation, strength and elastic modulus
- Better organisation

- Mechanism of action?
  - TGFbeta1 pro-scarring?
  - Exaggerated fibrosis?
  - Less appropriate for energy-storing tendons (eg SDFT)?

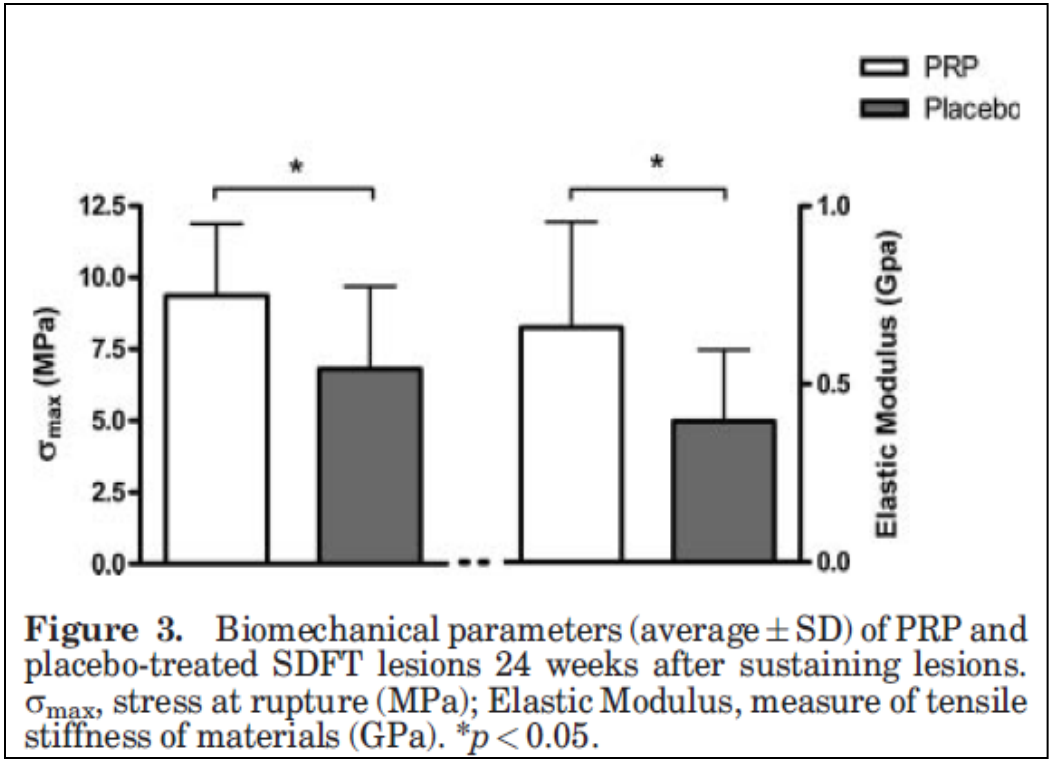


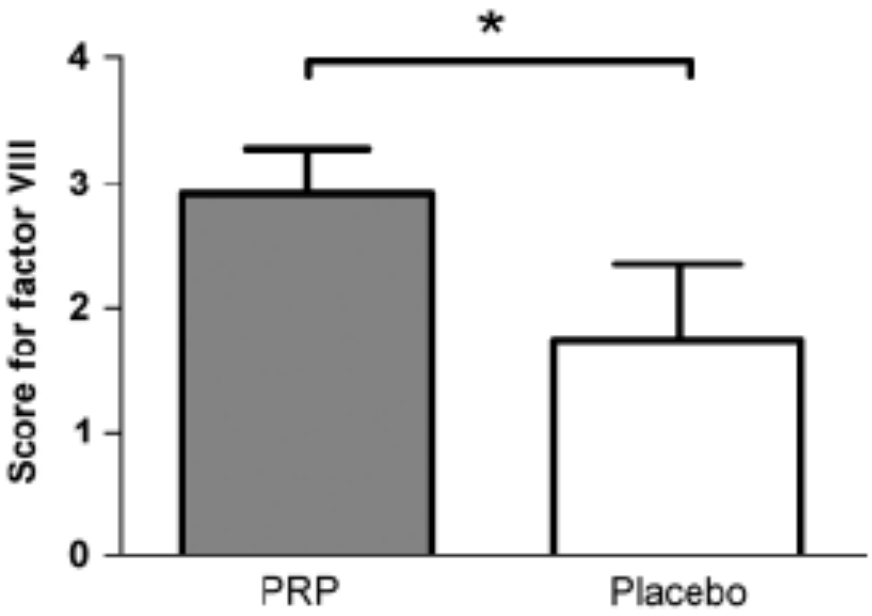
Figure 3. Biomechanical parameters (average ± SD) of PRP and placebo-treated SDFT lesions 24 weeks after sustaining lesions. σ_{max}, stress at rupture (MPa); Elastic Modulus, measure of tensile stiffness of materials (GPa). $^{*}p < 0.05$.

The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions

Scand J Med Sci Sports 2010
doi: 10.1111/j.1600-0838.2009.01070.x

G. Bosch[1], M. Moleman[1], A. Barneveld[1], P. R. van Weeren[1], H. T. M. van Schie[1,2]

- Ultrasound tissue significantly better organisation in PRP treated horses
- PRP induced significantly more neovascularization
- VEGF-induced neovascularisation may explain some of the beneficial effects of PRP



Vet J. 2011 Jan;187(1):92-8. doi: 10.1016/j.tvjl.2009.10.014. Epub 2009 Nov 20.

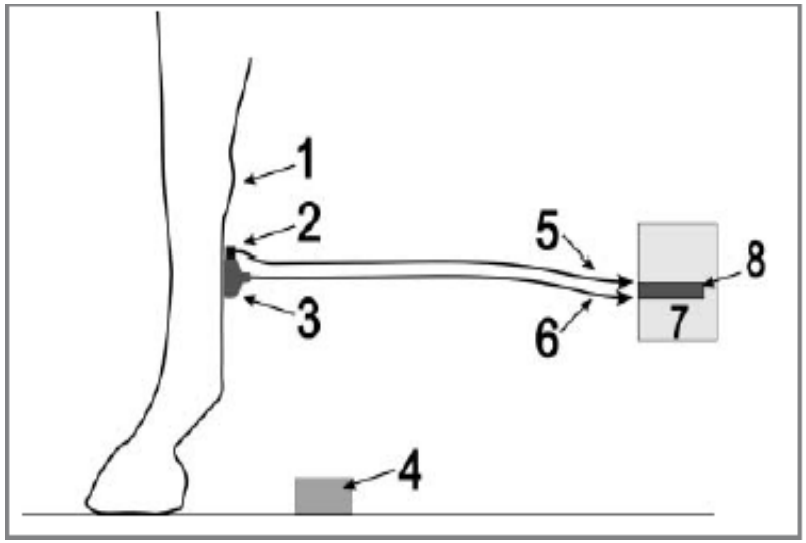
Computerised analysis of standardised ultrasonographic images to monitor the repair of surgically created core lesions in equine superficial digital flexor tendons following treatment with intratendinous platelet rich plasma or placebo.

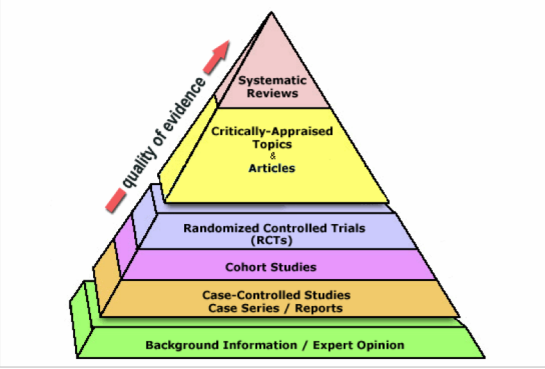
Bosch G, René van Weeren P, Barneveld A, van Schie HT.

Department of Equine Sciences, Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands. g.bosch@uu.nl

- Ultrasonographic tissue characterisation (UTC) for intra-vital monitoring of the healing trajectory of standardised tendon lesions

- At end stage, over 80% of pixels showed correct alignment in the PRP group, compared with just over 60% in the placebo group ($P<0.05$).





Efficacy in horses: CLINICAL

J Am Vet Med Assoc. 2008 May 15;232(10):1515-20. doi: 10.2460/javma.232.10.1515.

Intralesional injection of platelet-rich plasma followed by controlled exercise for treatment of midbody suspensory ligament desmitis in Standardbred racehorses.

Waselau M, Sutter WW, Genovese RL, Bertone AL.

Department of Veterinary Clinical Sciences, The Ohio State University, Columbus, OH 43210, USA.

- 9 horses with at least 15% of midbody SL affected (4 front, 5 hind)
- Treatment with single intralesional injection PRP
- All horses returned to racing after 26-68 weeks
- 89% raced in year 1, 100% in year 2, 56% in year 3.
- Earnings significantly reduced in year 1 only.
- "PRP can be safely used in horses and may represent a novel, valuable alternative and/or adjunctive treatment option in horses with midbody SLD"

Evaluation of a filter-prepared platelet concentrate for the treatment of suspensory branch injuries in horses.

G. Castelijns[1]; A. Crawford[2]; J. Schaffer[3]; G. A. Ortolano[3]; T. Beauregard[4]; R. K. W. Smith[2]

VCOT 2011

- 11 cases suspensory ligament branch desmitis
- Lameness grade average (2/10) reduced to 0 in 10/11 horses at 3 months
- All lesions resolved ultrasonographically in 3 months
- 5/11 horses returned to original level of performance

Injection of platelet- and leukocyte-rich plasma at the junction of the proximal sesamoid bone and the suspensory ligament branch for treatment of yearling Thoroughbreds with proximal sesamoid bone inflammation and associated suspensory ligament branch desmitis

JAVMA, Vol 243, No. 1, July 1, 2013

Katherine S. Garrett, DVM, DACVS; Lawrence R. Bramlage, DVM, MS, DACVS;
Deborah L. Spike-Pierce, DVM; Noah D. Cohen, VMD, PhD, DACVIM

- 39 yearling Thoroughbreds with sesamoiditis and suspensory branch desmitis
- 20 injected with PRP; 19 with saline
- PRP horses were significantly more likely to start at least 1 race during the 2-year-old racing year
- No significant differences for the 3- and 4-year-old racing years.
- No significant differences regarding earnings for any racing year.
- PRP treatment protocol evaluated in this study did not seem to improve future racing performance

No evidence of clinical benefit available yet in tendons nor joints in horses!

Why?

Important questions:

What's in PRP?

Veterinärmedizinische Fakultät

UNIVERSITÄT LEIPZIG

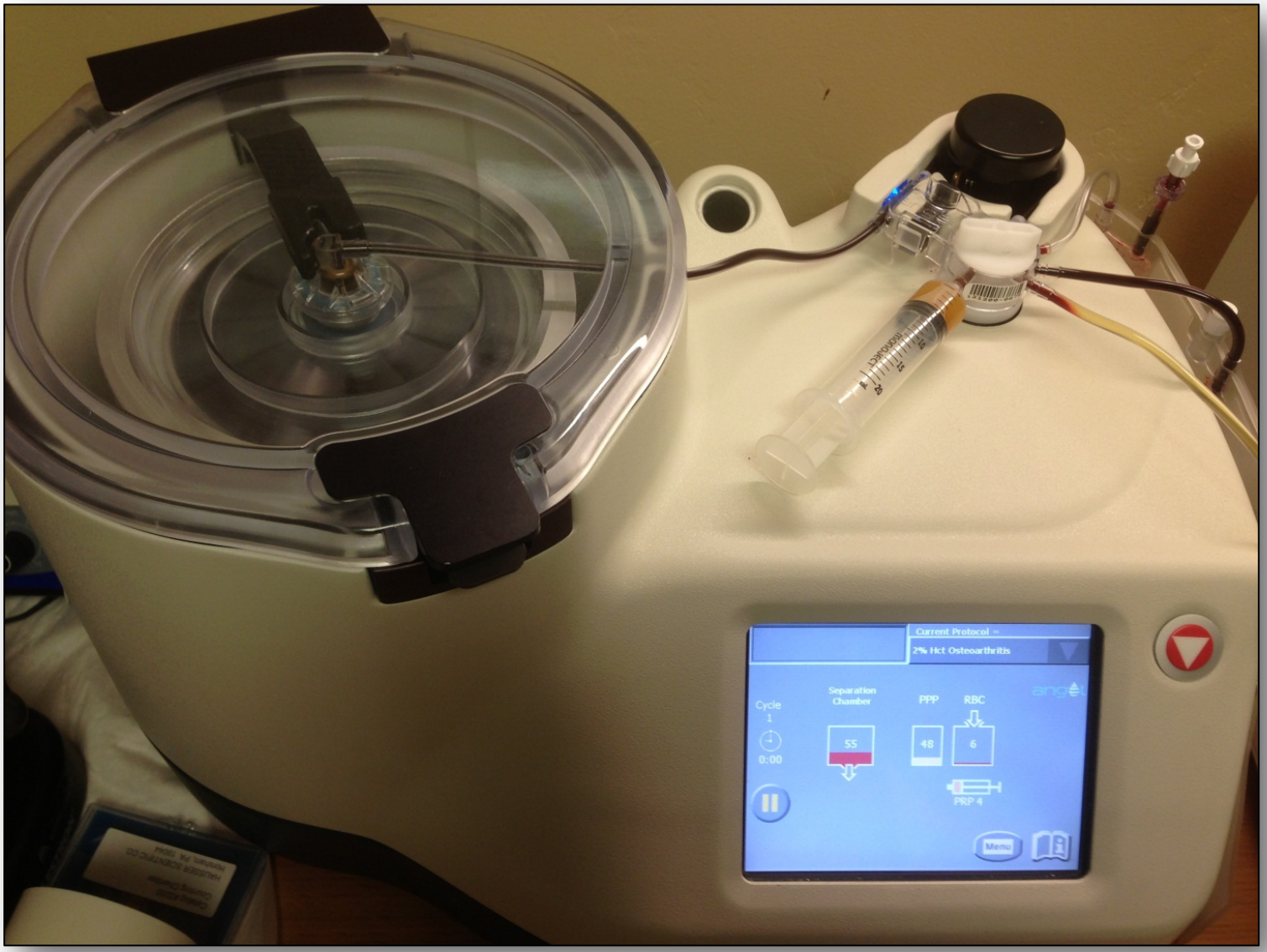
Universiteit Utrecht

EQUINE AUTOLOGOUS PLATELET CONCENTRATES: A COMPARATIVE STUDY BETWEEN 5 DIFFERENT SYSTEMS

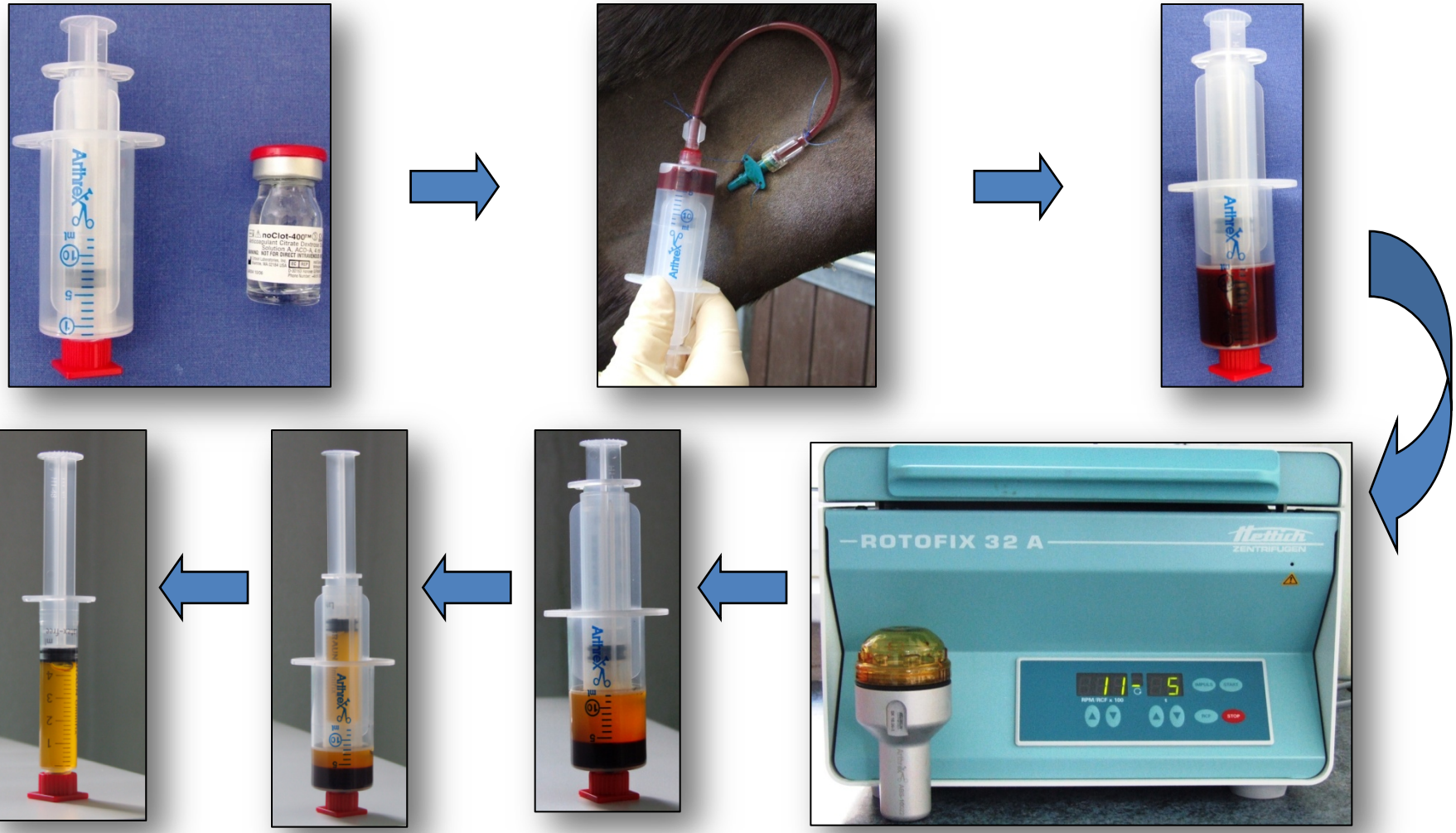
J.-C. Ionita[1], L. Hessel[2], G. Bosch[2], R. van Weeren[2]

[1] Large Animal Clinic for Surgery, University of Leipzig, Leipzig, Germany
[2] Faculty of Veterinary Medicine, University of Utrecht, Utrecht, The Netherlands

Angel® by Cytomedix



ACP® by Arthrex

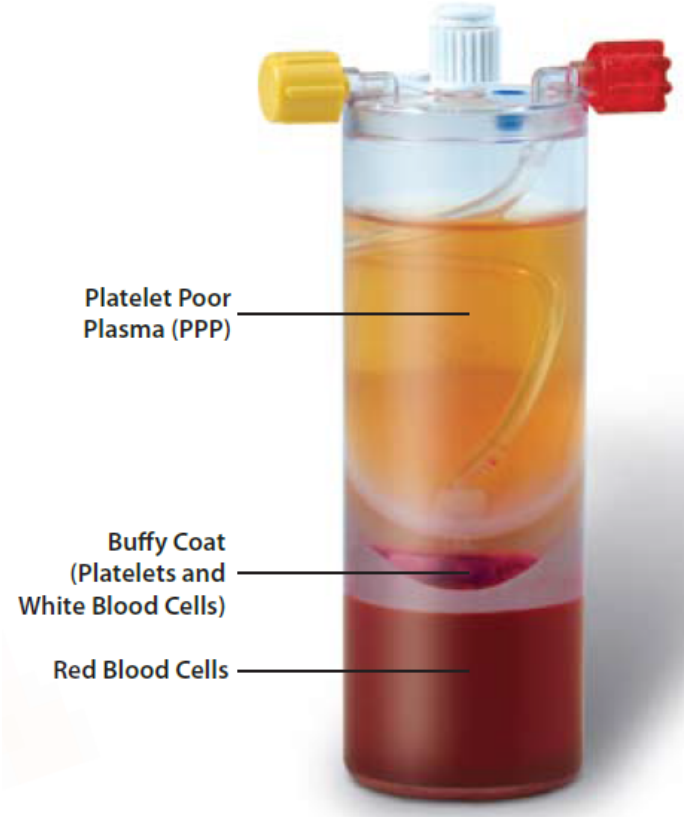


GPS®III by Biomet

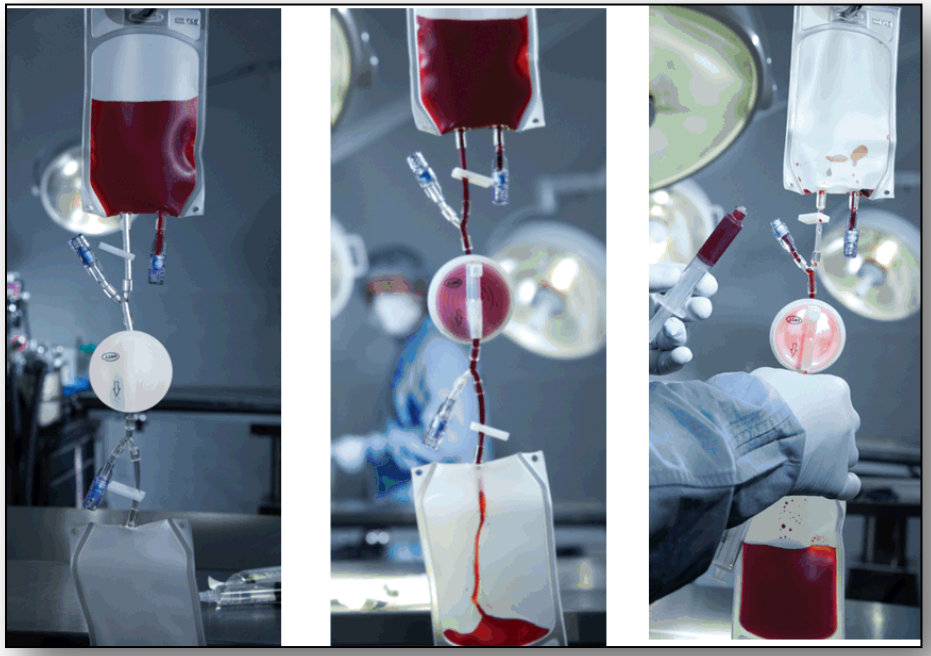
Load blood

Spin

Extract platelet rich plasma

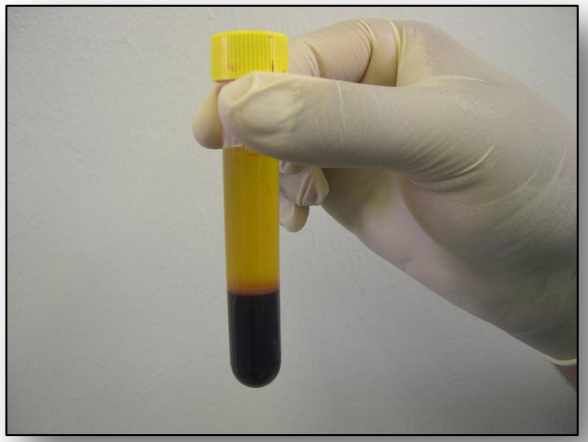


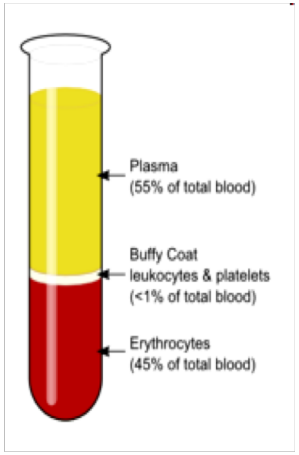
E-PET® by Pall

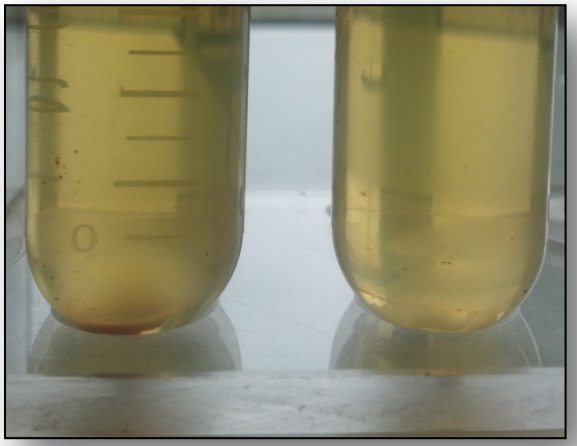


Stall-Side preparation

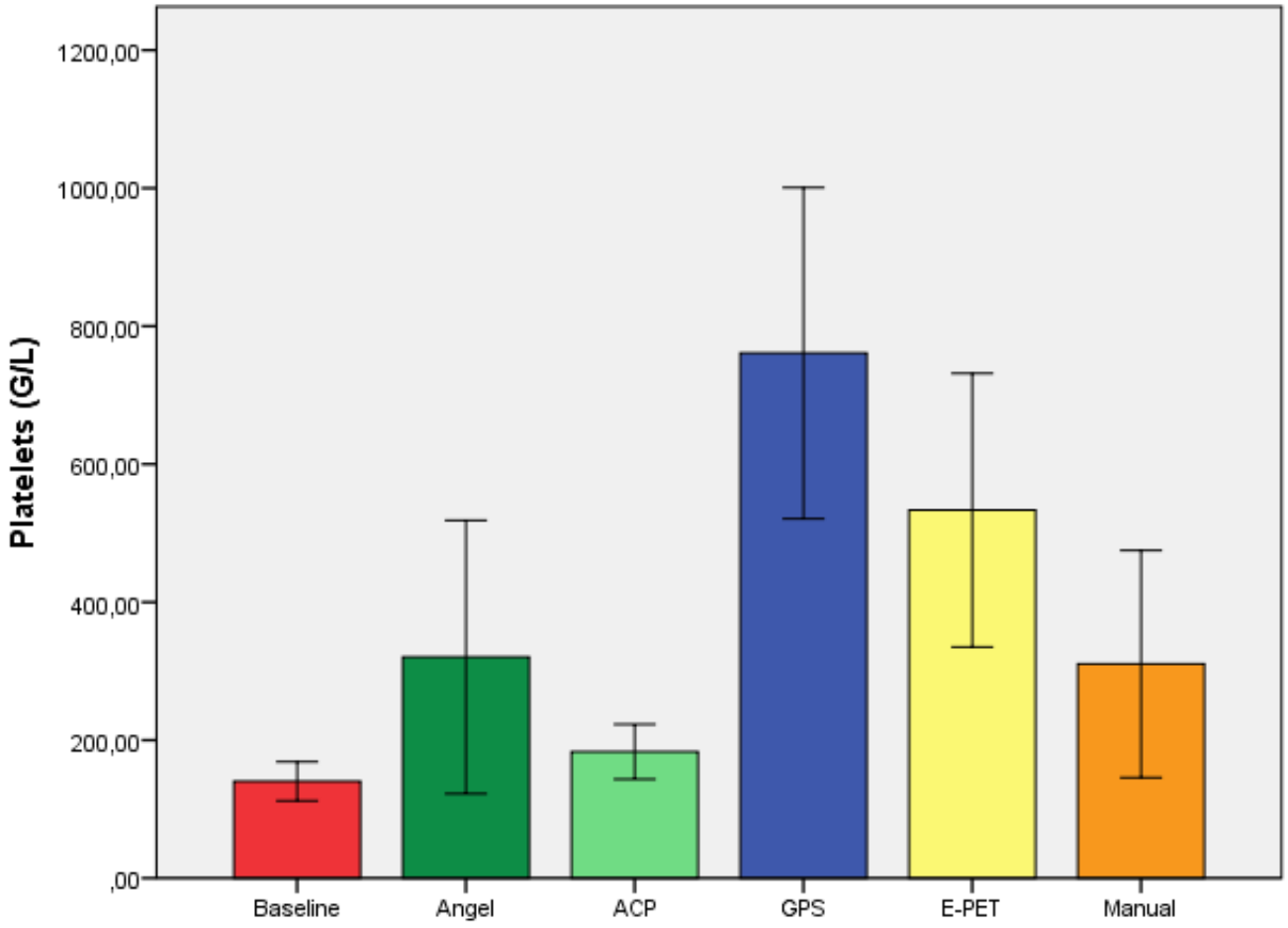
Manual, double tube technique



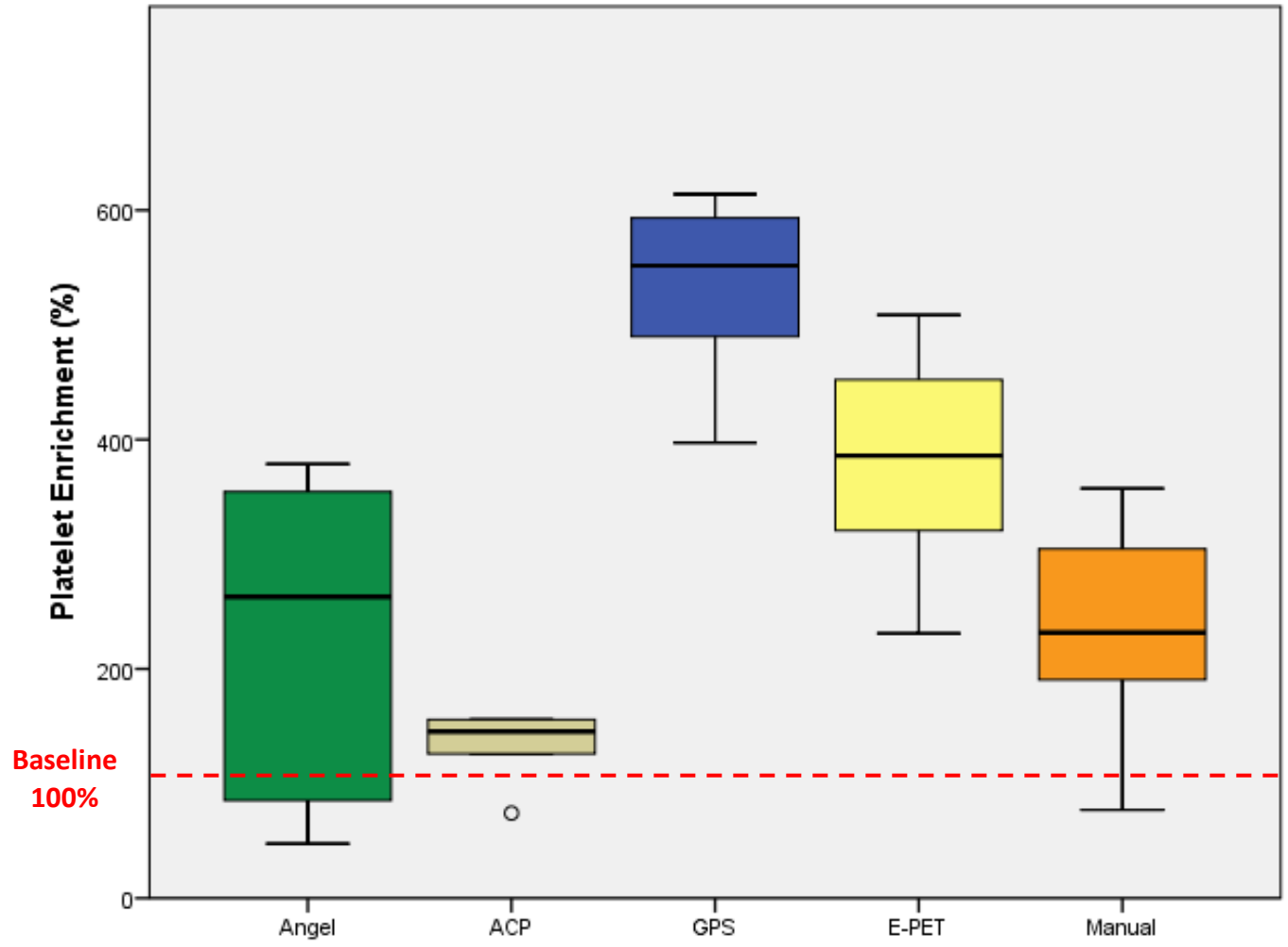




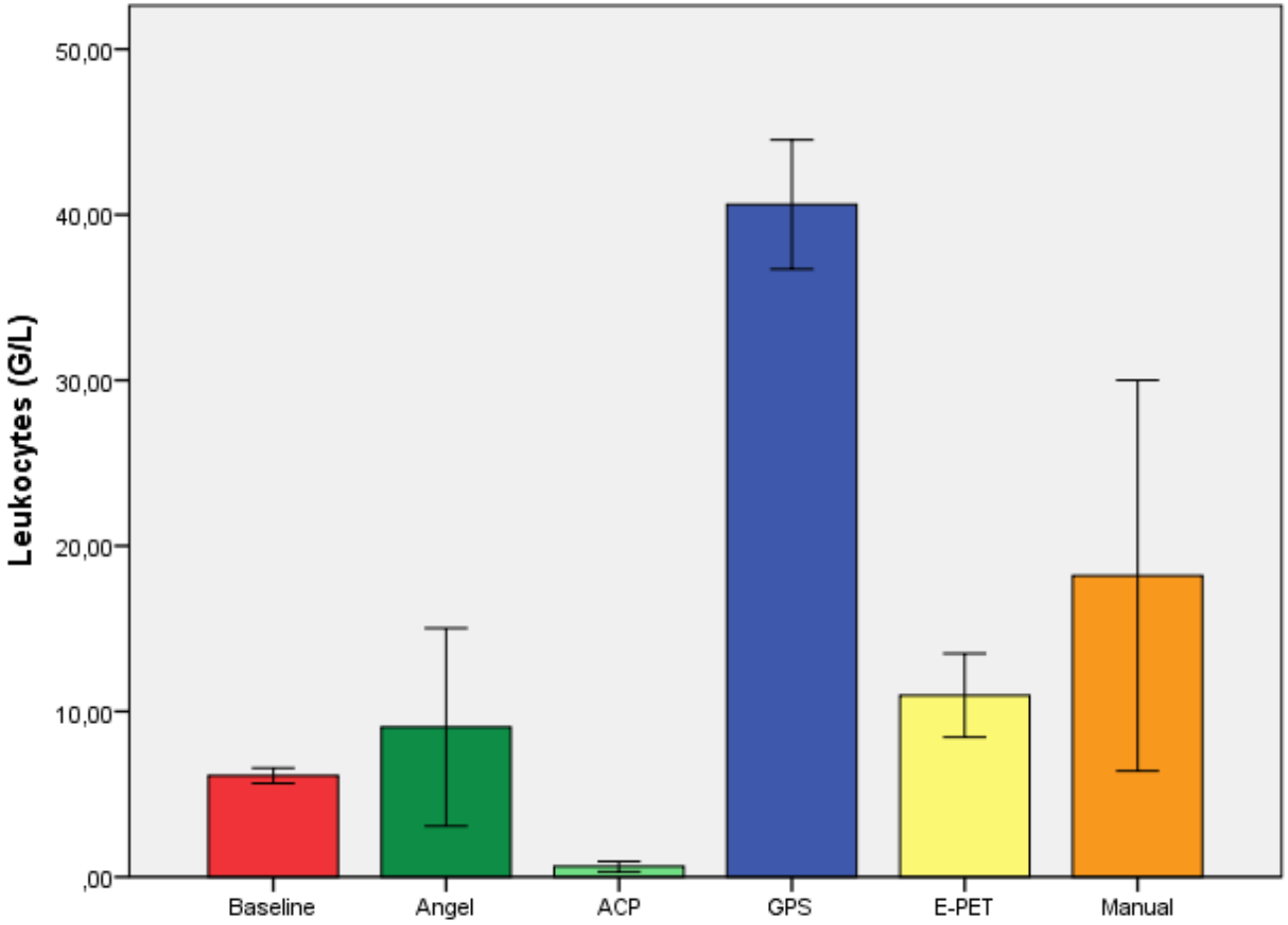
Mean Platelet Concentration



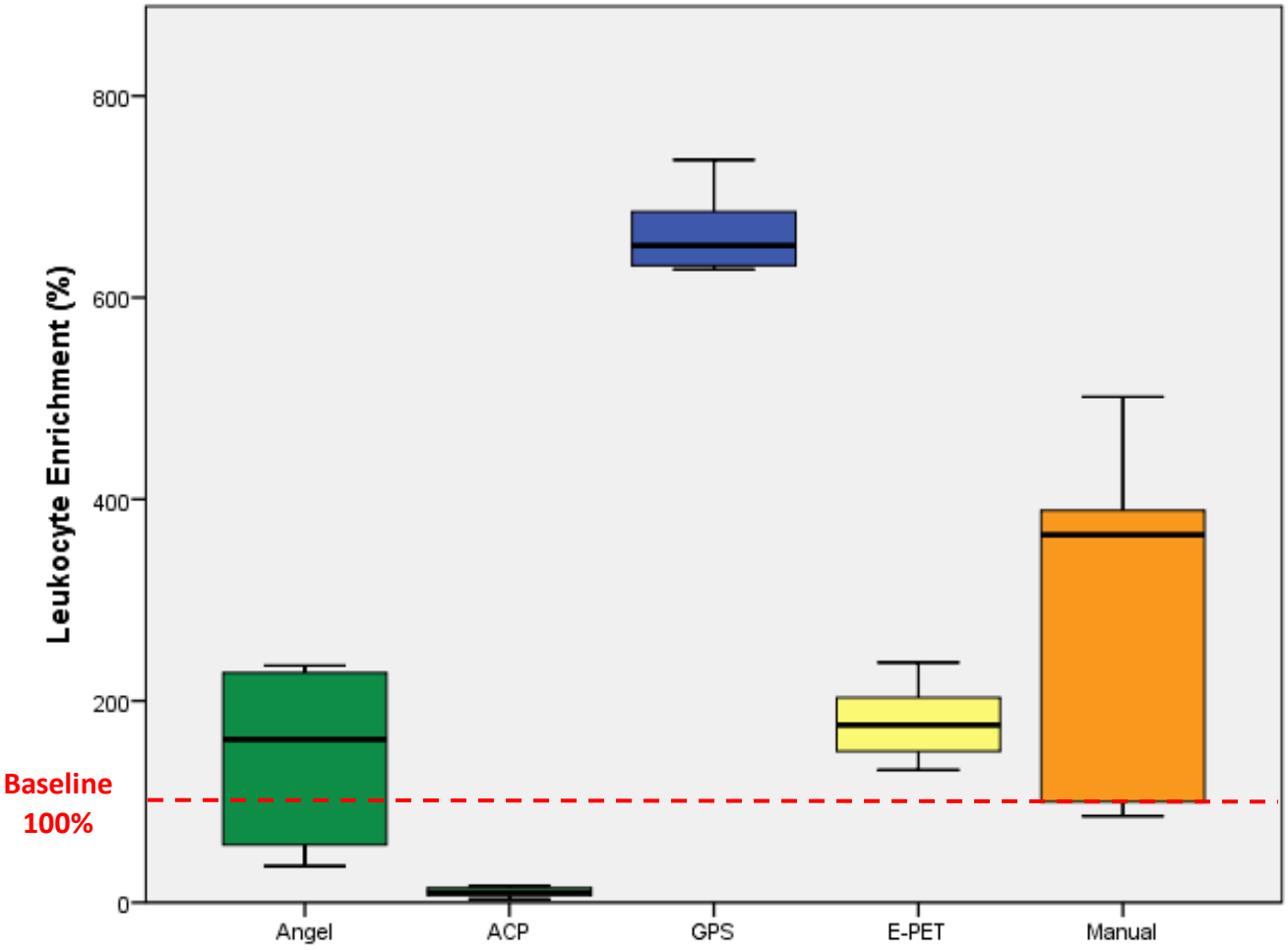
Median Platelet Enrichment



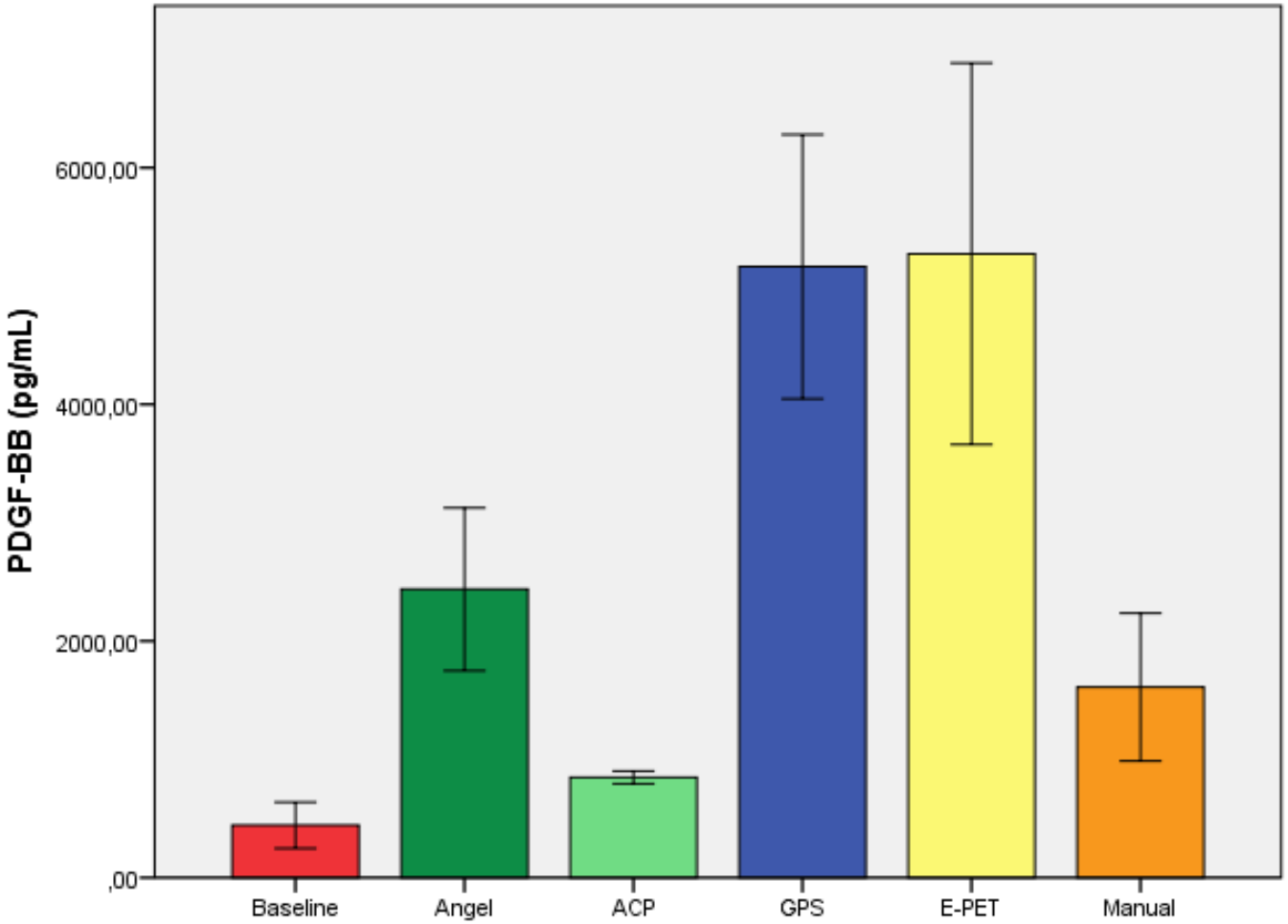
Mean Leukocyte Concentration



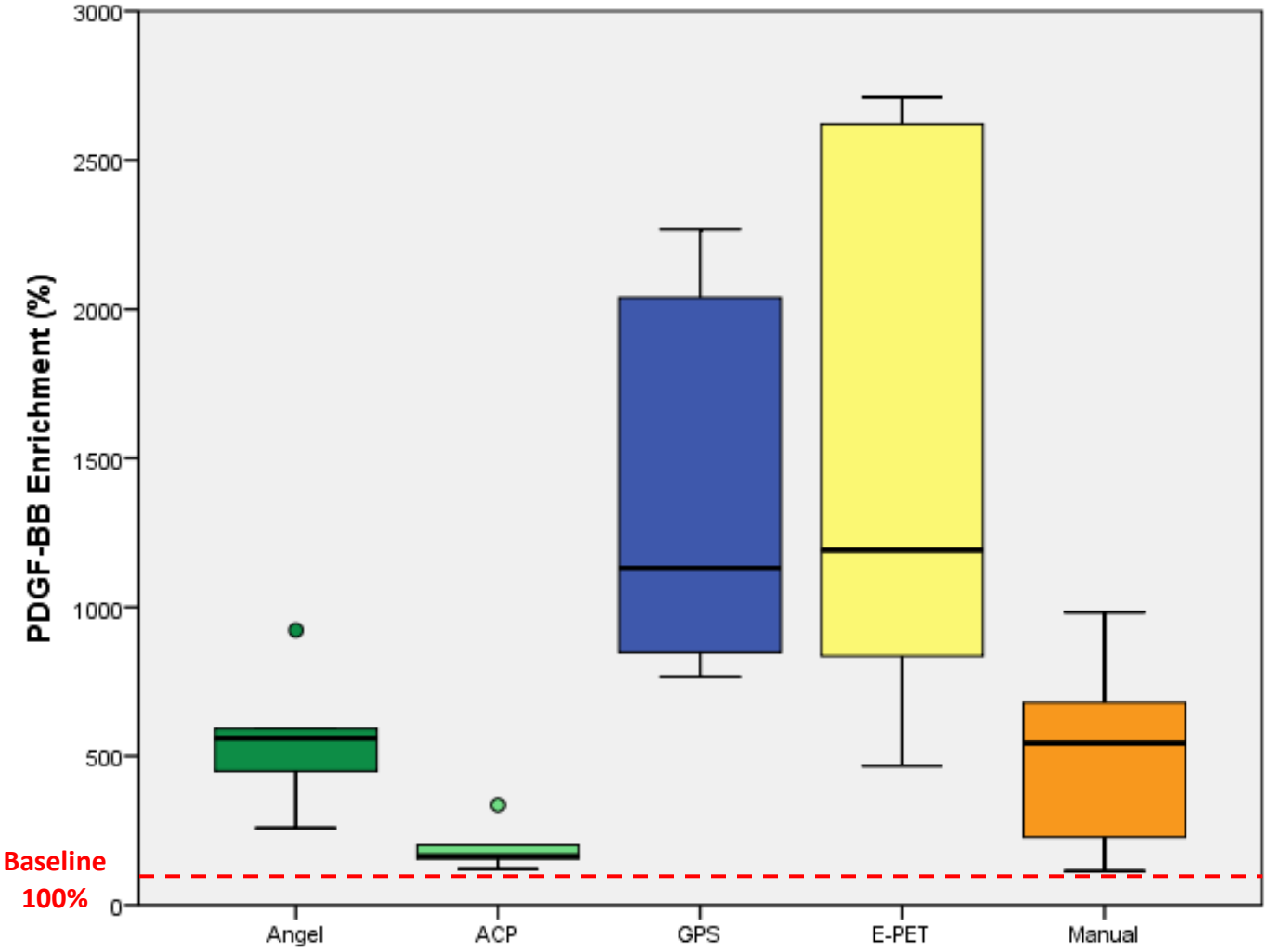
Median Leukocyte Enrichment



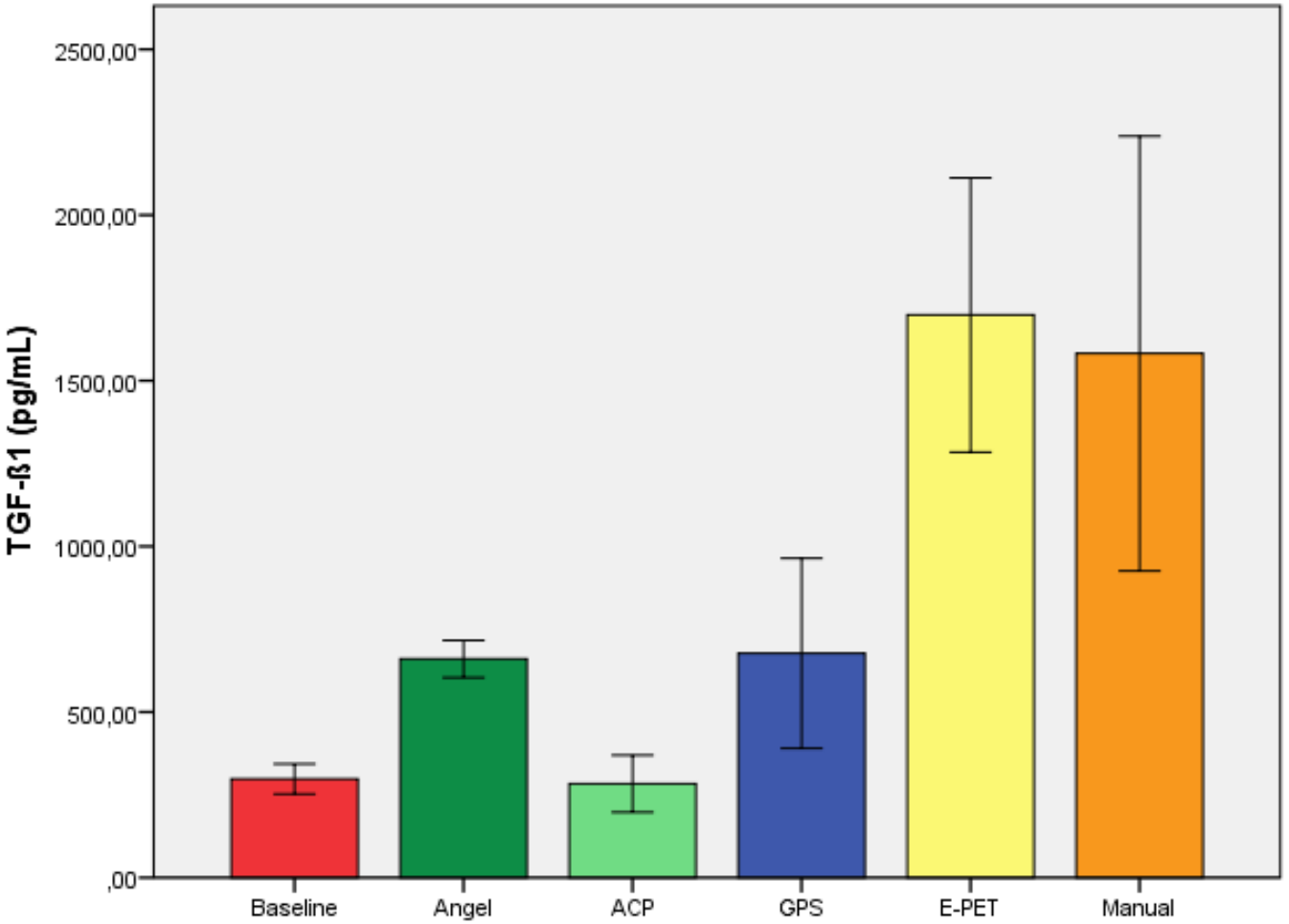
Mean PDGF-BB Concentration



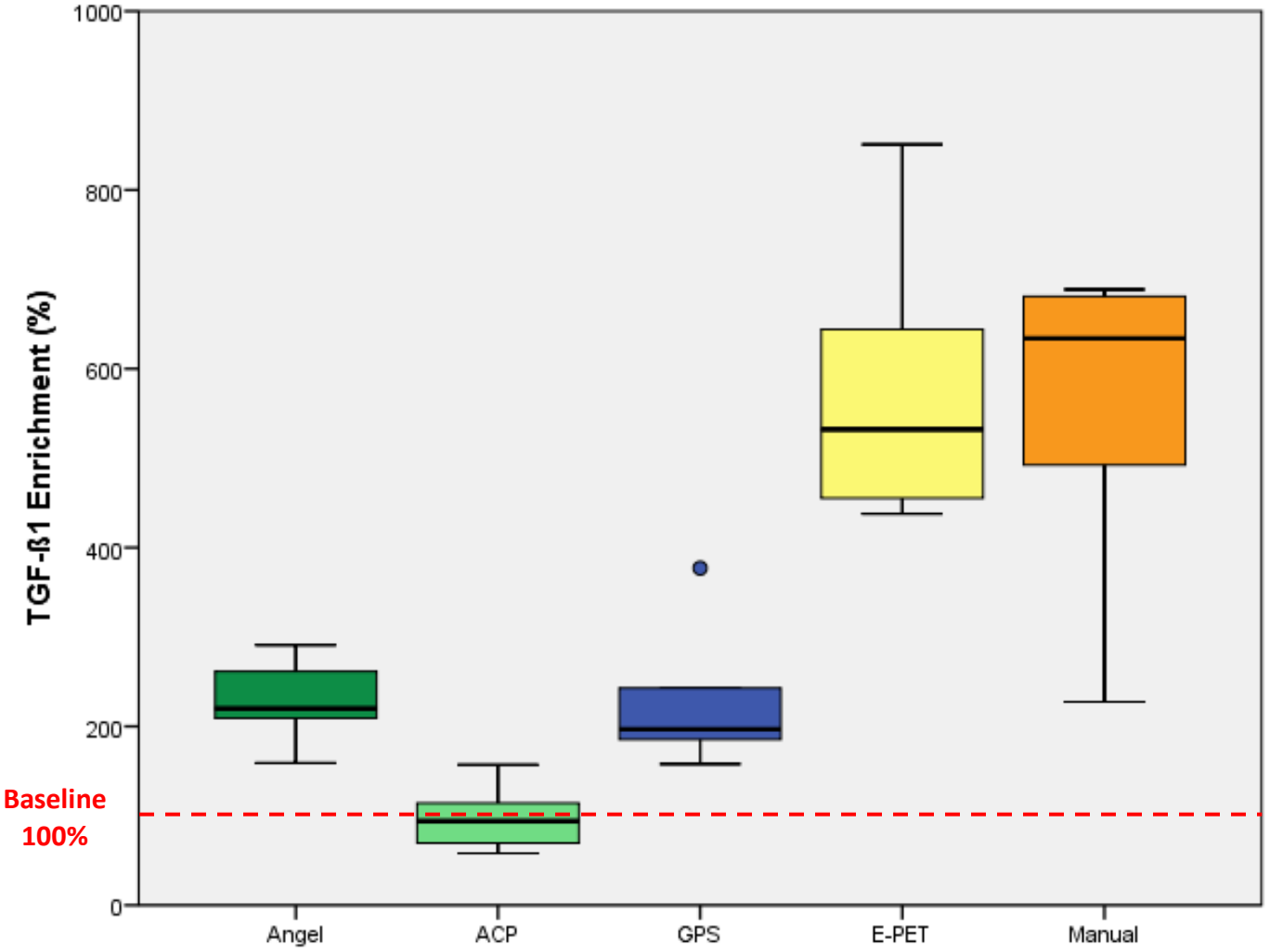
Median PDGF-BB Enrichment



Mean TGF-$ß_1$ Concentration



Median TGF-ß$_1$ Enrichment



Conclusions

- There was strong variability between systems for hematologic and biochemical characteristics
- There was also a high individual variability between horses in platelets and GF concentrations in all systems. PRP is NOT a standardised pharmacological product!
- These discrepancies may have a strong impact on clinical efficacy
- Clinicians should not rely on data relative to human patients when selecting a method for horses
- Further studies are needed to determine the influence of these APC on the quality of tissue regeneration

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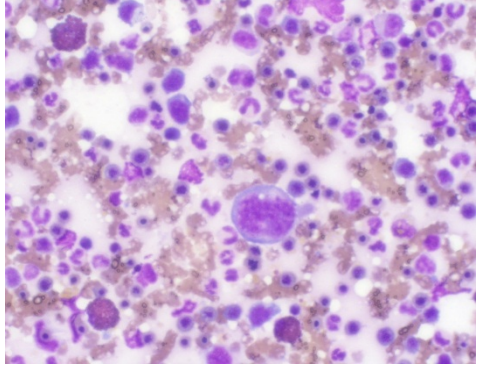
Stem Cell Therapy

Available cell treatments
A bewildering array available!



1. Non-stem cell products
   - Acell: endogenous attraction?
2. 'Minimal' stem cell products
   - Direct bone marrow
   - Bone marrow nucleated cell fraction (Bone marrow aspirate concentrate (BMAC))
   - Fat digest – no culture (Vet-Stem)
   - Umbilical cord blood
3. Enriched stem cell products
   - Cultured bone marrow MSCs (VetCell)
   - Cultured fat MSCs (VetStem)
   - Cultured umbilical cord cells (VetBioBank)
   - Cultured peripheral blood stem cells (GST)
4. Future?
   - Embryonic (Hackett 2011)
   - iPS (Nagy 2011)

The differences between sources is probably smaller than the difference between preparation methods

Method 1 – Ad-MSCs

- Fat recovered from tail head
- Non-adipocytes recovered by collagenase digestion and floatation
- No culture step
- Return of ~50.10^6 cells after 48 hours
  - ?2% MSCs

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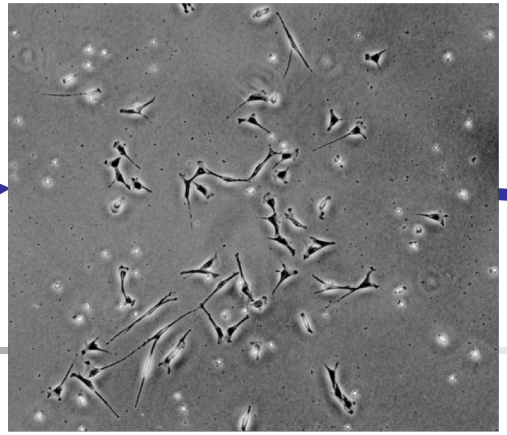
VetCell



•Recovery and expansion of stem cell-rich population – 3 weeks

•Recovery of heparinised bone marrow

Method 2: Bone marrow technique

•Resuspension in citrated supernatant of bone marrow

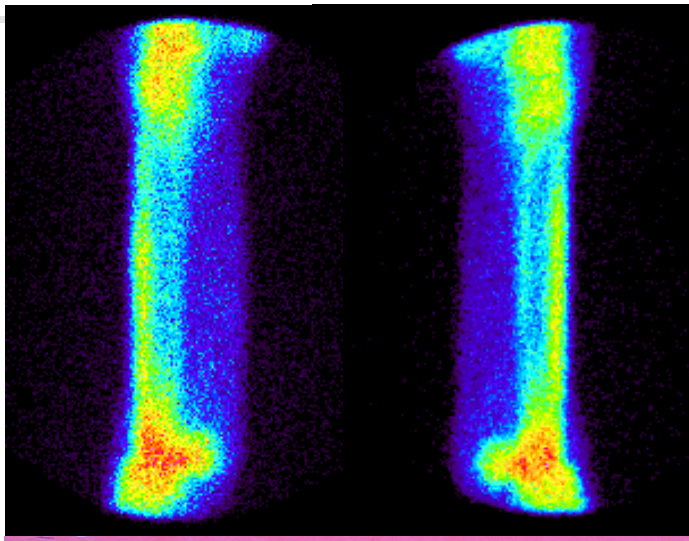
•Implantation under ultrasound guidance (standing)

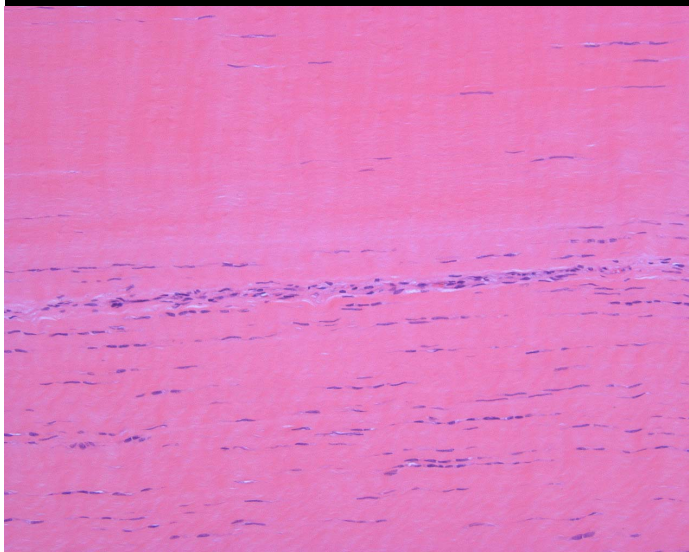
•Post-implantation 48 week rehab programme

Important Questions!

How safe is cell therapy?

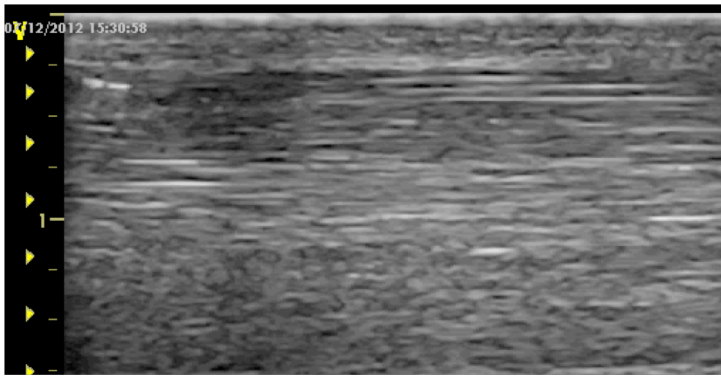
- Excellent safety record
  - Thousands of horses treated world-wide (>2000 by VetCell to 2012)
- Occasional needle tracts
- Adverse reactions rare
  - Bone scan negative at 3 months
  - Occasional 'flares' with intrasynovial administration (9%; Ferris et al. 2014)
  - Mineralisation *outside* the tendon (1)
- Histological evaluation (n=17)
  - Good linear organisation
  - No evidence of abnormal tissue or neoplastic transformation

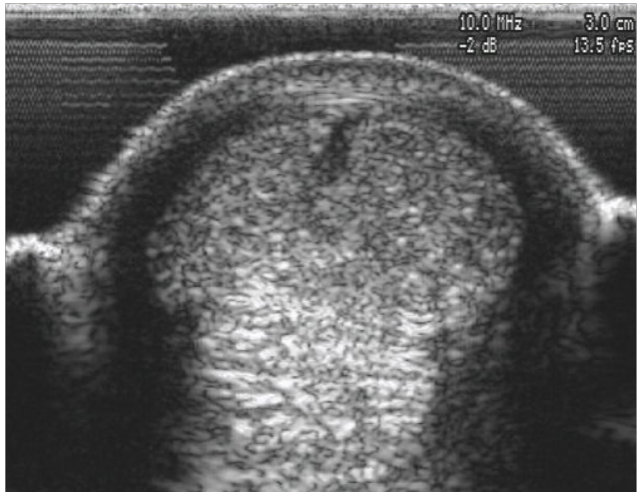




How important is injection technique?

- How should they be implanted?
  - Currently injected under ultrasound guidance
  - See needle tracts commonly post implantation so desire to reduce needle size
  - MSCs considerably smaller than diameter of all needles used clinically BUT could still suffer damage due to shear forces
    - Loss of viability
    - Less severe cellular damage which could influence efficacy
      - Changes in metabolism
      - Delayed cell death (apoptosis)





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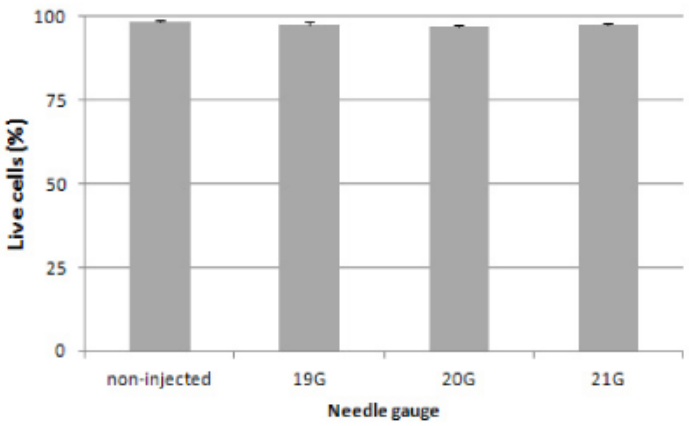
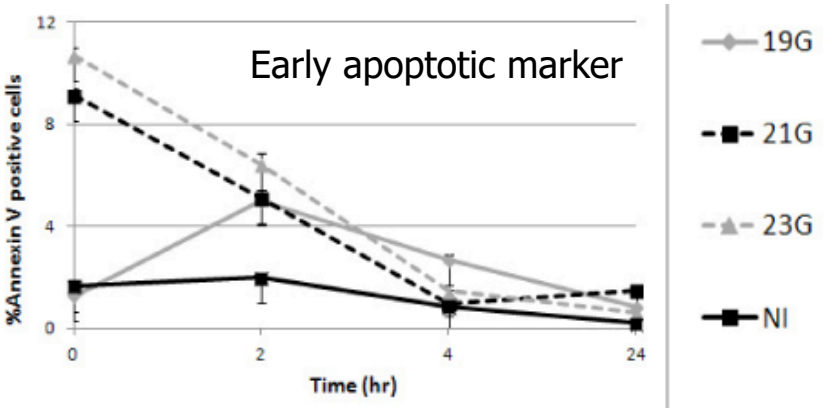
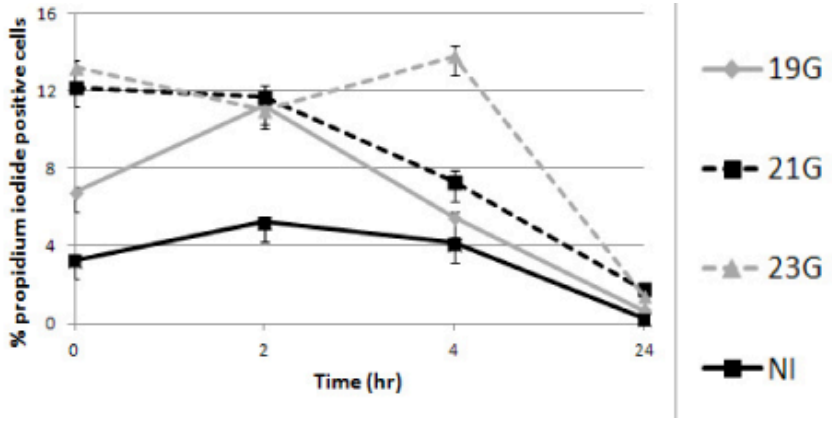
Injection Technique?



- Tested 19G, 21G, and 23G needles
- No loss of viability BUT:
- Decrease in metabolic activity immediately post-injection although cells recovered by 2hrs
- 21G and 23G needles significantly increased apoptotic cells compared to 19G and non-injected controls
- We currently advise 20G needles



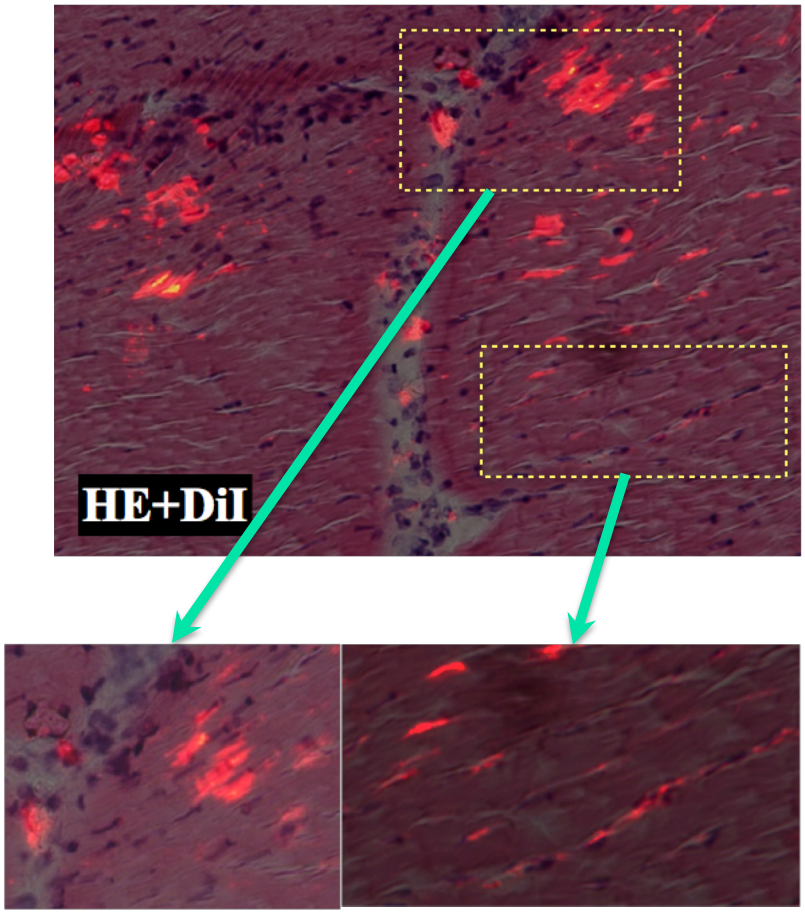


Late apoptotic marker

Garvican et al. submitted

How long do cells stay alive in tendon lesions?

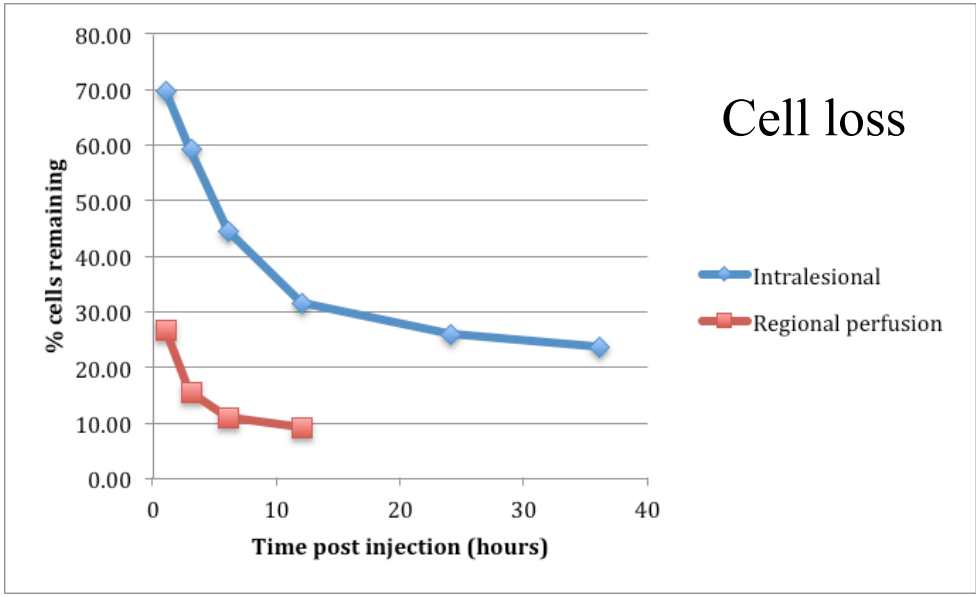
- Labelled MSCs
- 7 horses with tendon injury
  - Labelled MSCs
  - Survival in tendon up to 5 months if paratenon intact
- Guest et al., 2008
  - Surgical lesion
  - +ve cells mainly within lesions
    - Rapid decline in numbers
  - Embryonic stem cells spread more and survived better

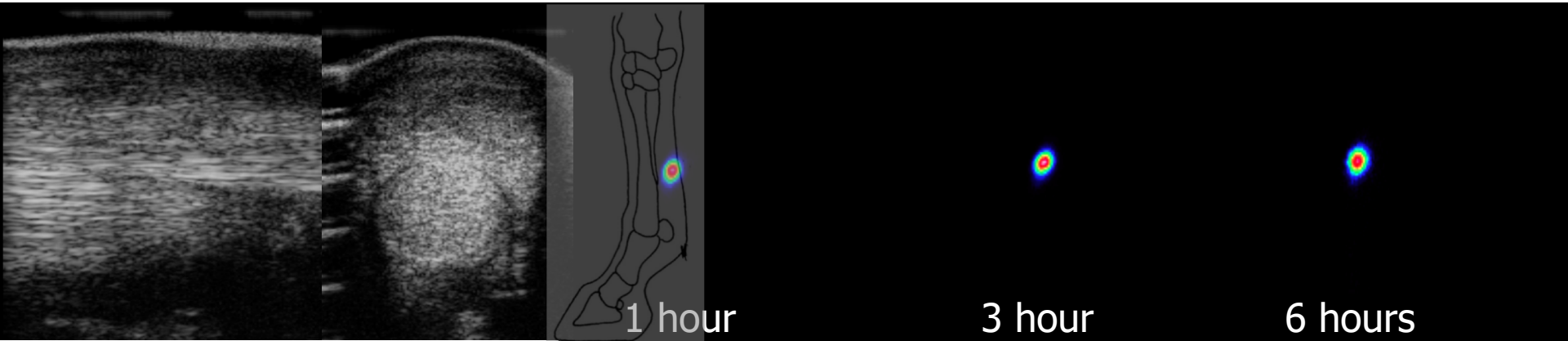


Dr. Kasashima, Equine Research Institute, Japan Racing Association

How many cells stay in the lesion after intralesional injection?

- Tc99m-HMPAO labelling
- Minimal spread
- Only 24% of cells still present in the tendon after 24 hrs
- Only 5% after 10 days



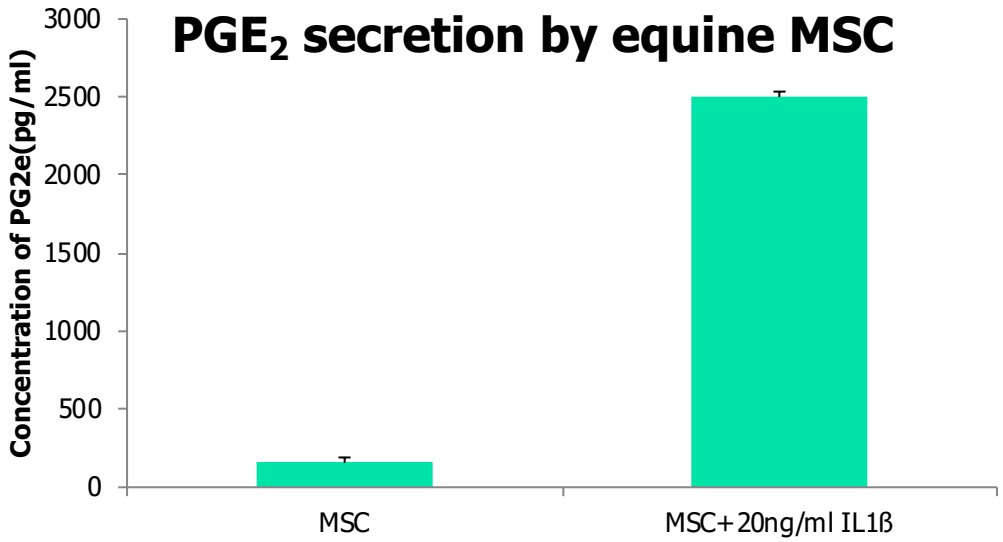


Autologous versus allogenic?

Parameter	Autologous	Allogenic
Ease of technique	Requires two stage procedure	'Off the shelf' treatment
Timing of injection	Governed by culture interval 'Minimally manipulated' can be administered anytime	Can be administered anytime
Cost	Expensive	Cheaper
Risk of immune reaction	Minimal	?Possible
Legislation	Allowed (UK) Requires licensed product (USA)	Illegal (UK) Requires licensed product (USA)

Is allogenic use a possibility?

- MSCs can be immunomodulatory
  - They secrete immunosuppressive factors – eg PGE_2
  - Can suppress T cell proliferation (Carrade Holt et al. 2014)
- BUT positive MHC-II mismatched equine MSCs cause T cell proliferation (Schnabel et al. 2014)



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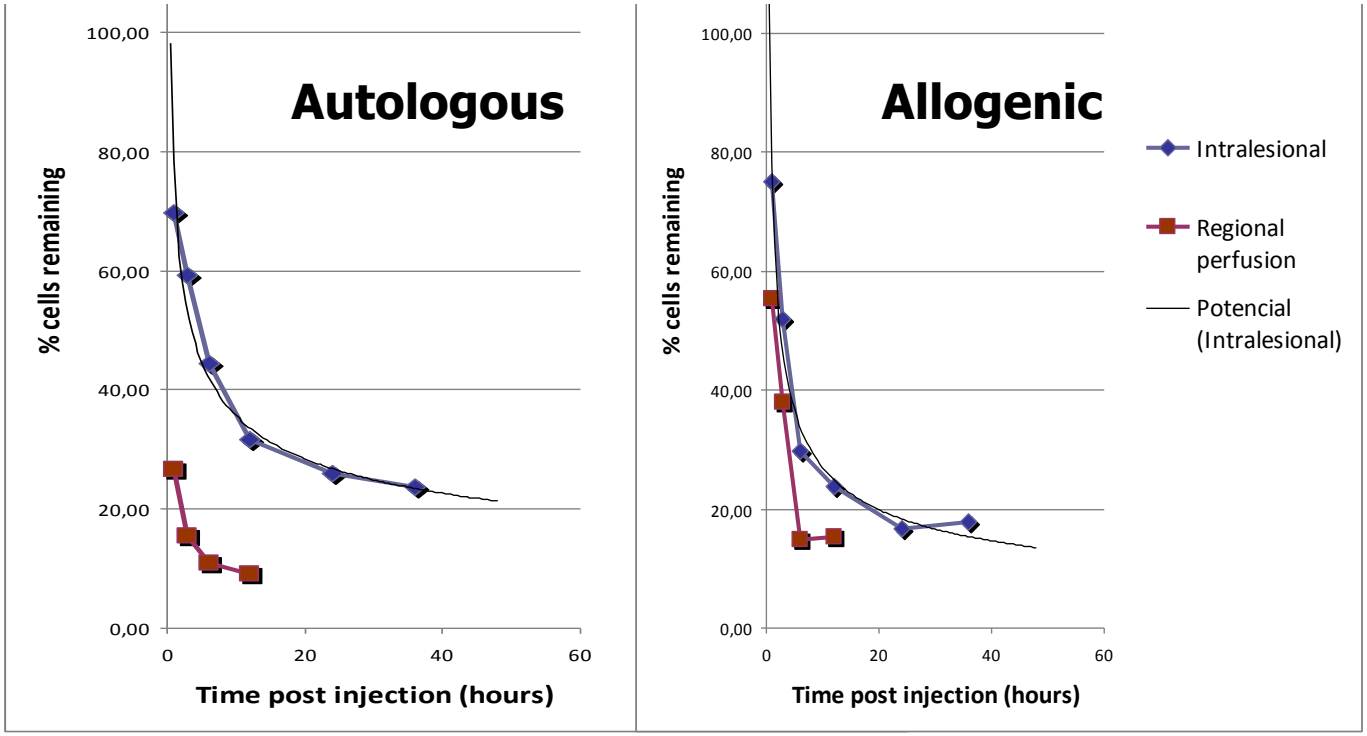
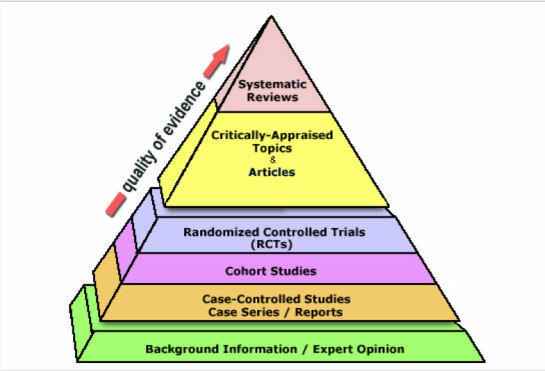
Is there evidence that allogenic cells clear more rapidly than autologous?

- No difference in short-term retention between autologous and allogenic cell implantation
- Allogenic use a real possibility but caution still urged, especially when implanting in 'inflammation-sensitive' organs (eg joints)

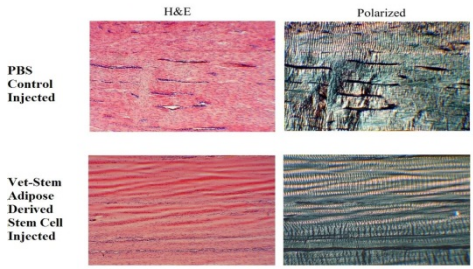




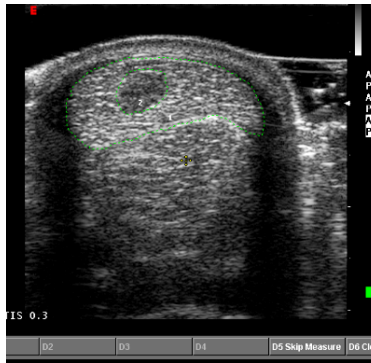
Efficacy in horses: EXPERIMENTAL

Efficacy - Experimental studies – horses

- Collagenase
  - Fat-derived MSCs (Vet-Stem; USA)
    - Pilot study suggested improved organisation
  - Fetal MSCs (Watts et al., 2011)
  - BM-MSCs (Schnabel et al., 2009; Crovace et al., 2010)
    - No significant compositional differences
    - Increased COMP, reduced CollIII
    - Significantly improved histological scores

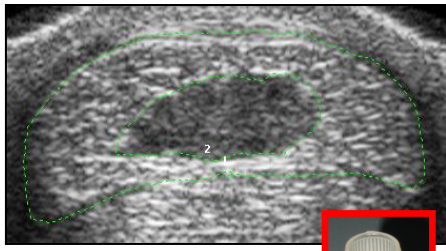


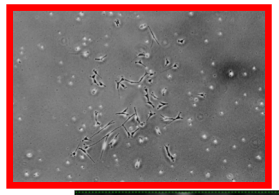
- Mechanical injury
  - BM-MSCs (Caniglia, Schramme, Smith, 2012)
    - No effect on collagen fibril diameters at 12 wks

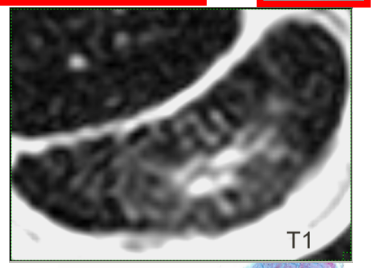


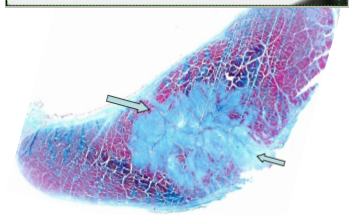
The effect of intralesional injection of bone marrow derived mesenchymal stem cells and bone marrow supernatant on collagen fibril size in a surgical model of equine superficial digital flexor tendonitis

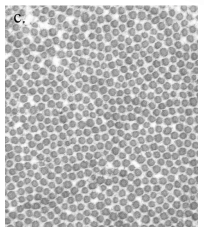
C. J. CANIGLIA, M. C. SCHRAMME* and R. K. SMITH†

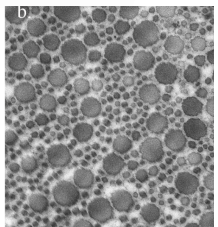












- 6 horses
  - Standardised surgical core lesions created in SDFT of both forelimbs
  - Treatment of 1 forelimb with 10 million stemcells in bone marrow supernatant; control limb with 2 ml bone marrow supernatant
  - Euthanasia 12 weeks after treatment
- Results treated vs. control:
  - No difference in ultrasound scores at 2, 4, 6, 8, 10, 12 weeks
  - No difference collagen fibril composition at 12 weeks
  - No difference MRI signal at 12 weeks
  - No difference histological parameters of composition and organisation

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PLOS ONE

Beneficial Effects of Autologous Bone Marrow-Derived Mesenchymal Stem Cells in Naturally Occurring Tendinopathy

Roger Kenneth Whealands Smith[1], Natalie Jayne Werling[2], Stephanie Georgina Dakin[1], Rafiqul Alam[1], Allen E. Goodship[3], Jayesh Dudhia[1*]

- 12 horses with naturally-occurring injuries within one month of injury
  - Destined for euthanasia on financial grounds
  - Severe
  - Usually recurrent
- Sequentially assigned to MSC and saline injected control groups
- Given 6 months of walking and trotting exercise before euthanasia
- Tendons analysed for mechanics, organisation and composition

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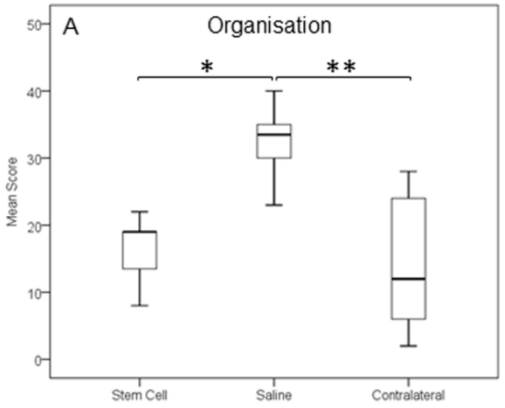
Beneficial Effects of Autologous Bone Marrow-Derived Mesenchymal Stem Cells in Naturally Occurring Tendinopathy

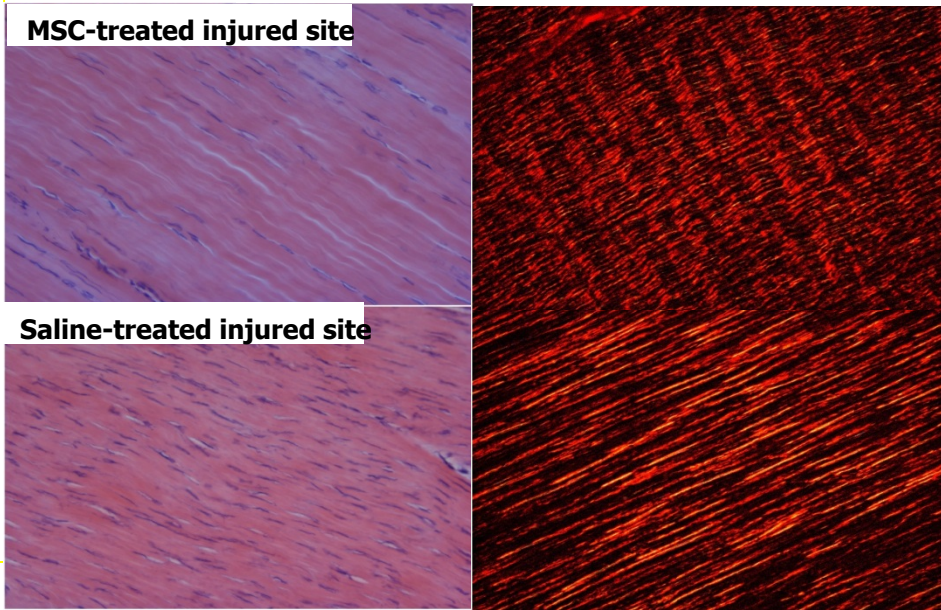
Roger Kenneth Whealands Smith[1], Natalie Jayne Werling[2], Stephanie Georgina Dakin[1], Rafiqul Alam[1], Allen E. Goodship[3], Jayesh Dudhia[1*]

SIGNIFICANT IMPROVEMENTS:

P<0.05

- Improved mechanics
  - Reduced CSA
  - Reduced stiffness
- Improved organisation and crimp
- Reduced cellularity
- No effect on collagen (hydroxyproline) content
- Lower GAG content





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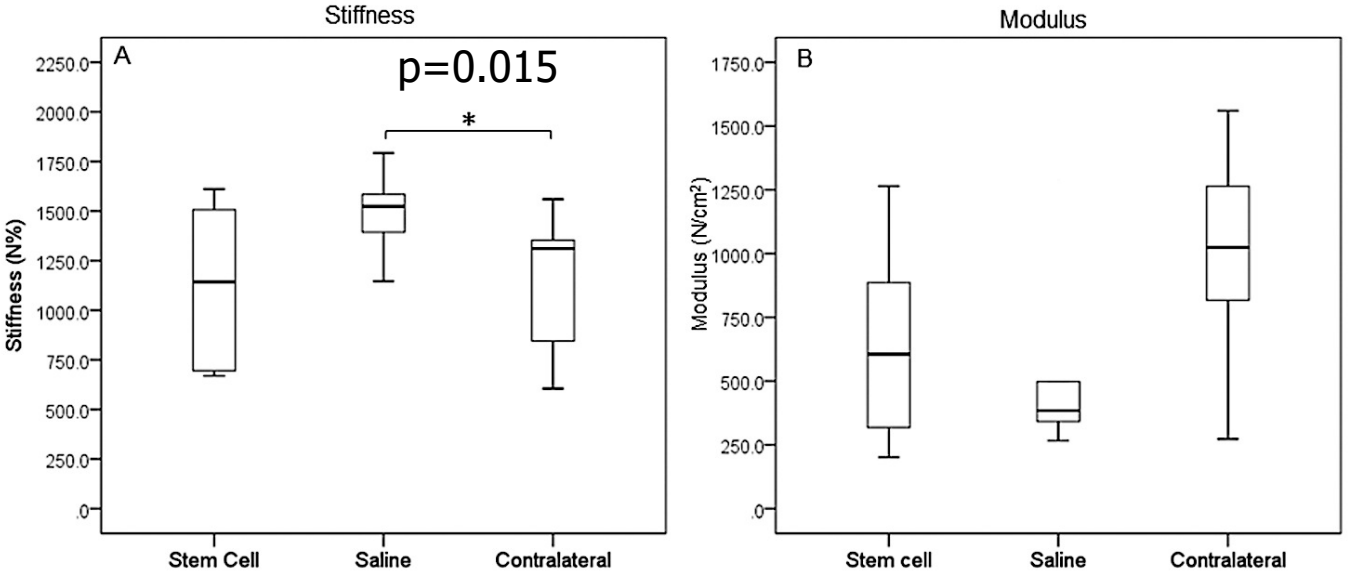
Structural stiffness of the superficial digital flexor tendon

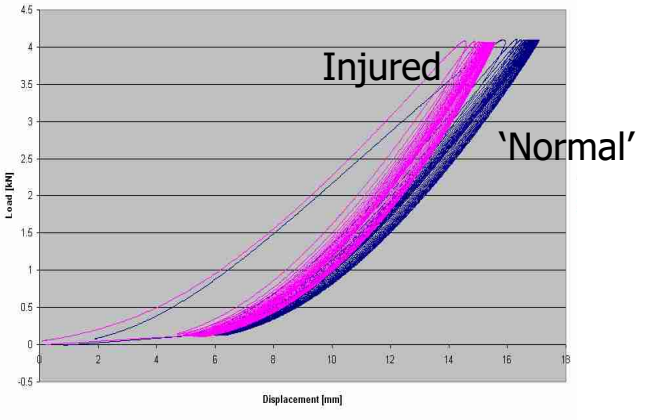
- *In vitro non-destructive* structural strength test of the tendon at 6 months
- Reduced stiffness (increased elasticity) with same modulus

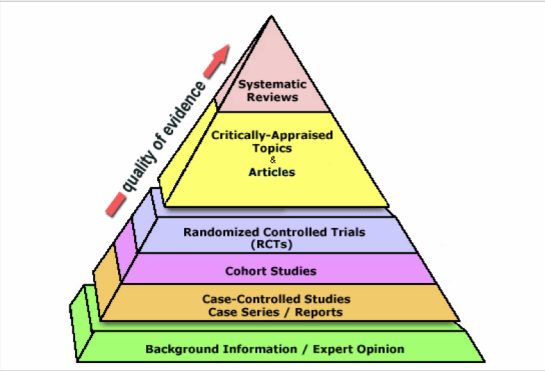
Stem cell vs Saline p=0.061



Conclusions – tissue effects

- Significantly improved histological scores and most biochemical parameters
- Trends (p=0.05-0.1)
  - Reduced proteoglycan content
  - Reduced structural stiffness (normal modulus)
- 'Normalisation' of matrix
- ?Modulation of repair





Efficacy in horses: CLINICAL

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THE VetCell STUDY

Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon

E. E. GODWIN, N. J. YOUNG, J. DUDHIA, I. C. BEAMISH and R. K. W. SMITH*

Department of Veterinary Clinical Sciences, The Royal Veterinary College, Hatfield, UK.

- 113 Racing Thoroughbreds with 3 year follow-up
- comparison with historical data from horses used for same discipline, treated with conventional management
  - Dyson, 2004 – Study 1: conservative management only
  - O' Meara et al., 2010: IGF1, SCLD, firing (cohort matched for % CSA)
- Data independently analysed from VetCell's records
  - Re-injury rate

Equine Veterinary Journal

Implantation of bone marrow-derived mesenchymal stem cells demonstrates improved outcome in horses with overstrain injury of the superficial digital flexor tendon

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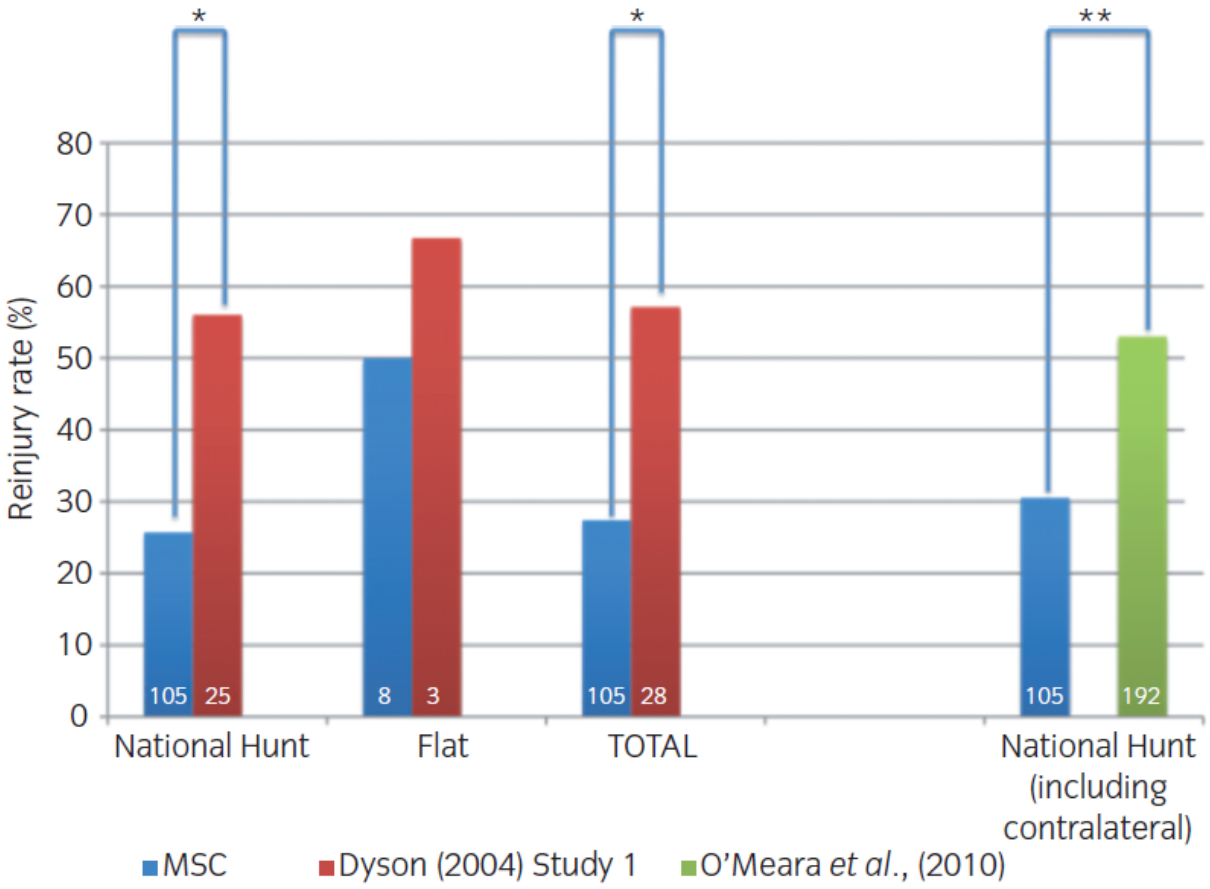
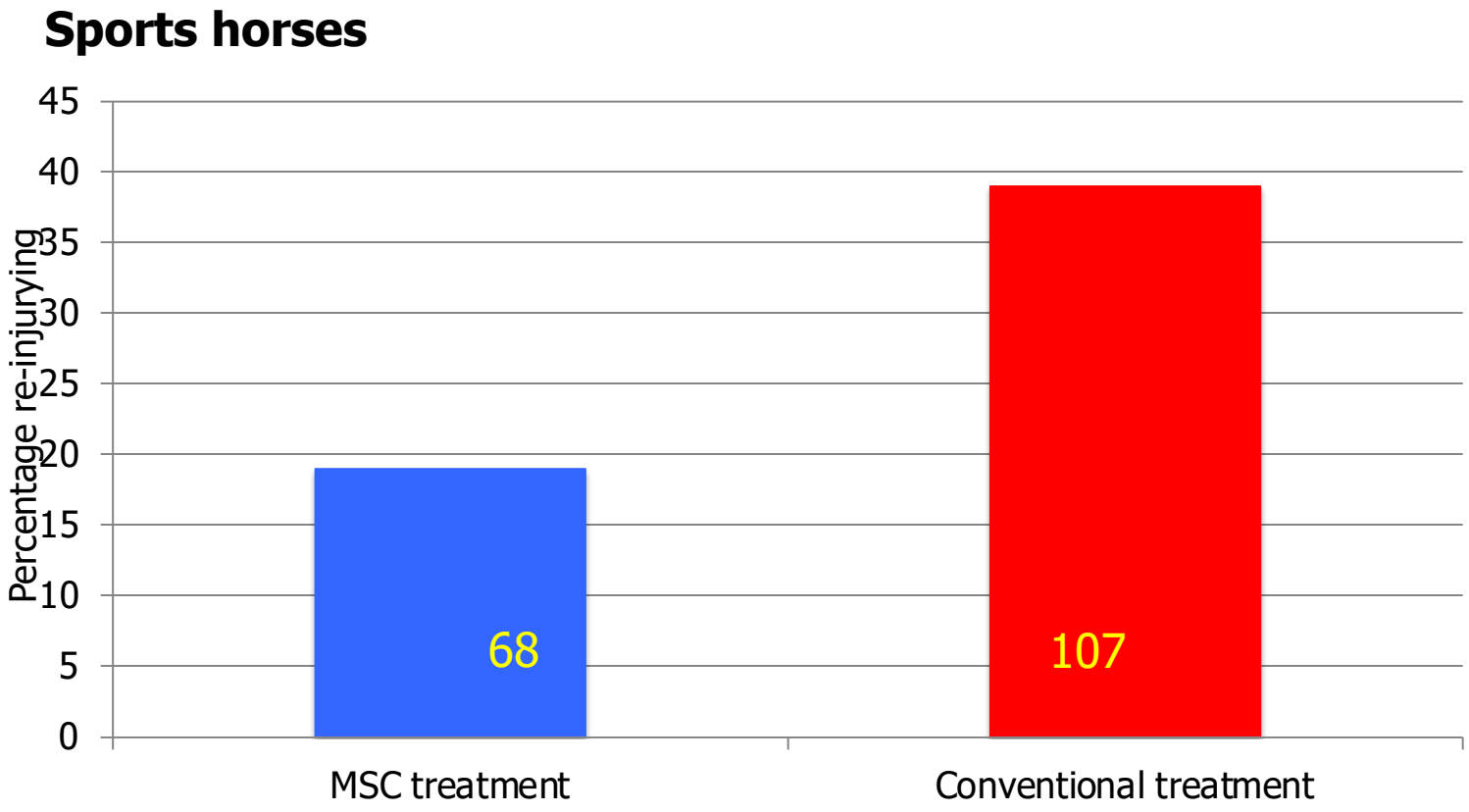
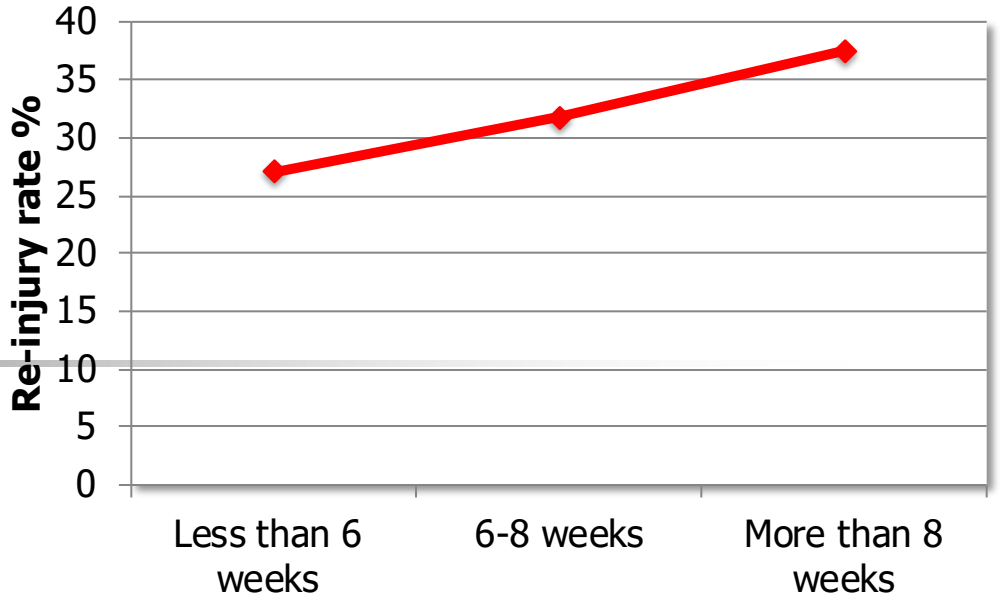


Re-injury rate – Sport Horses

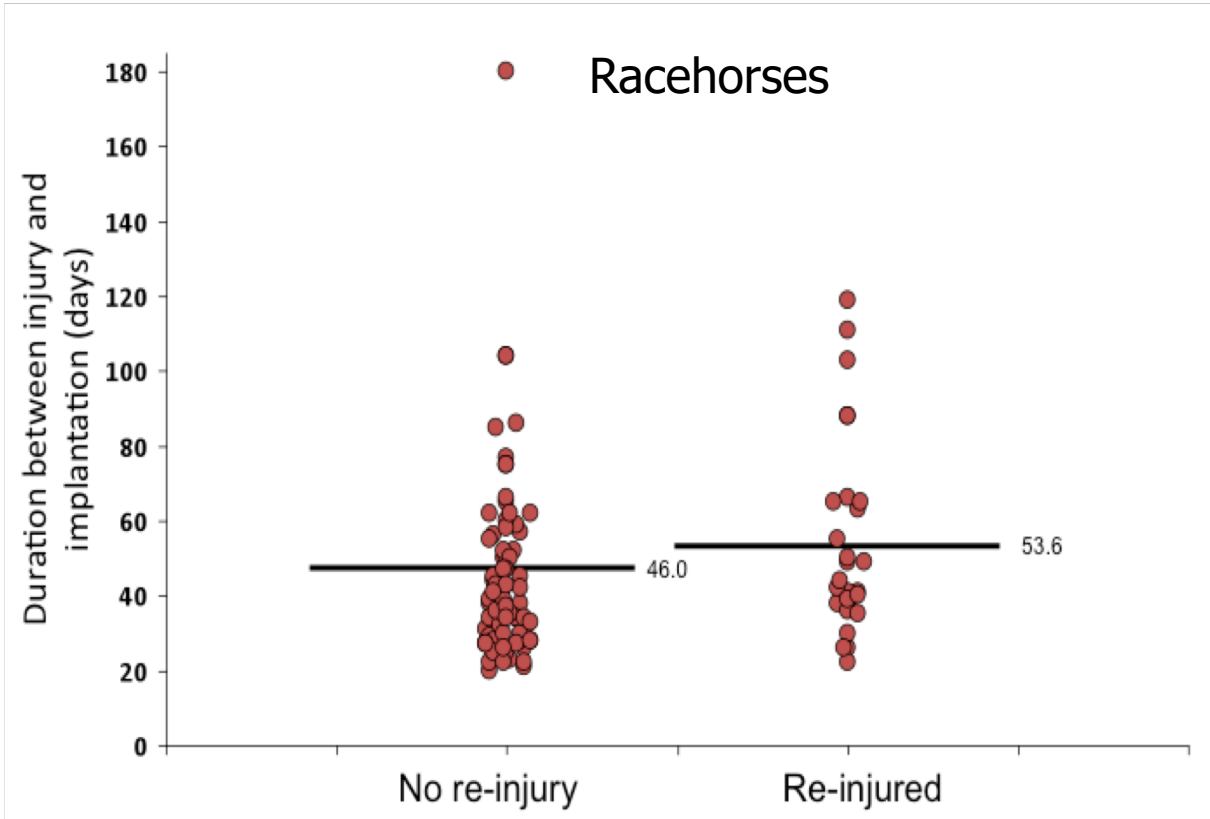


Dyson, 2004
74% Event horses

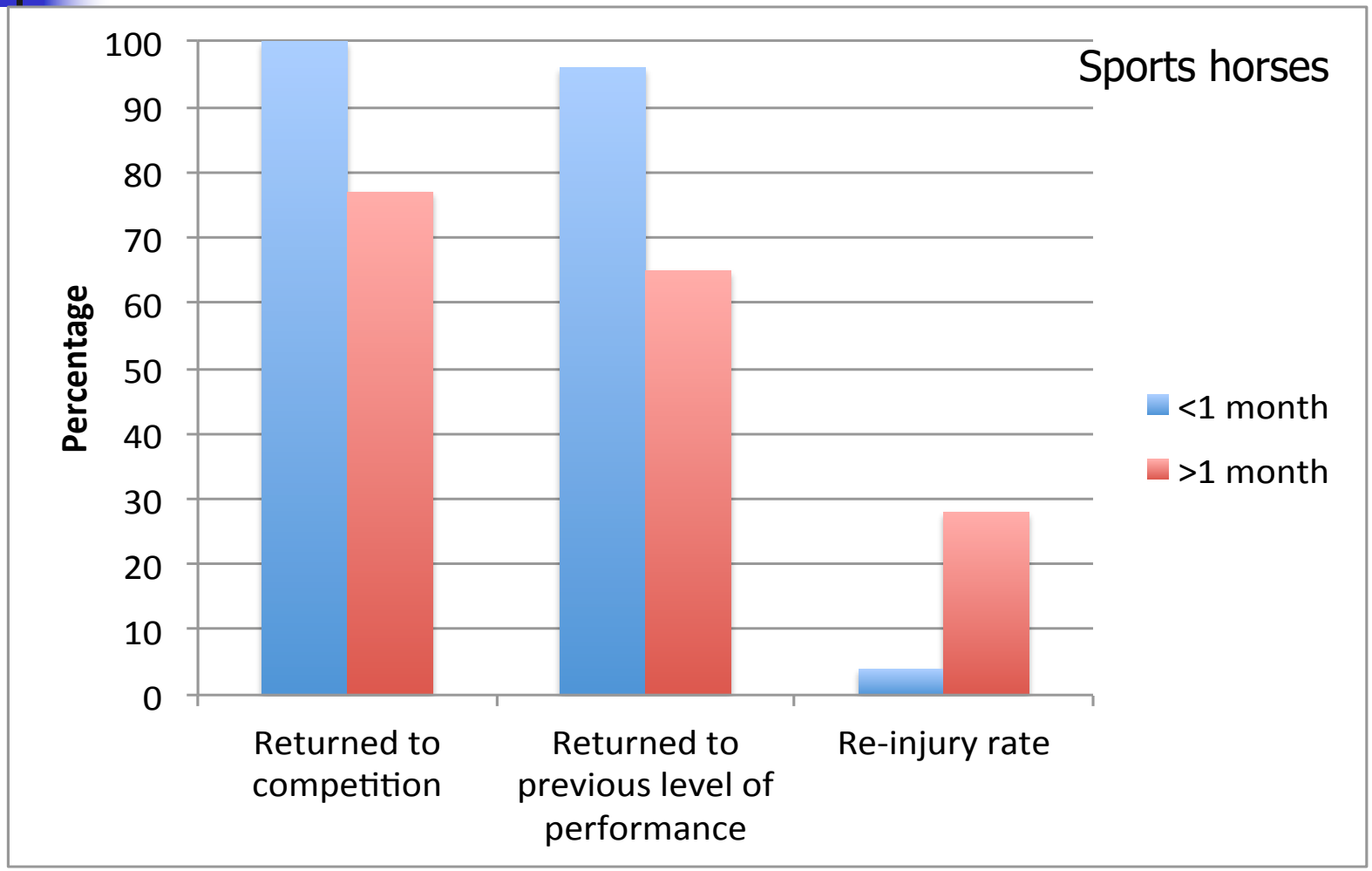
Effect of injection time



- Increasing re-injury rate with increasing age of the lesion
  - Not statistically significant

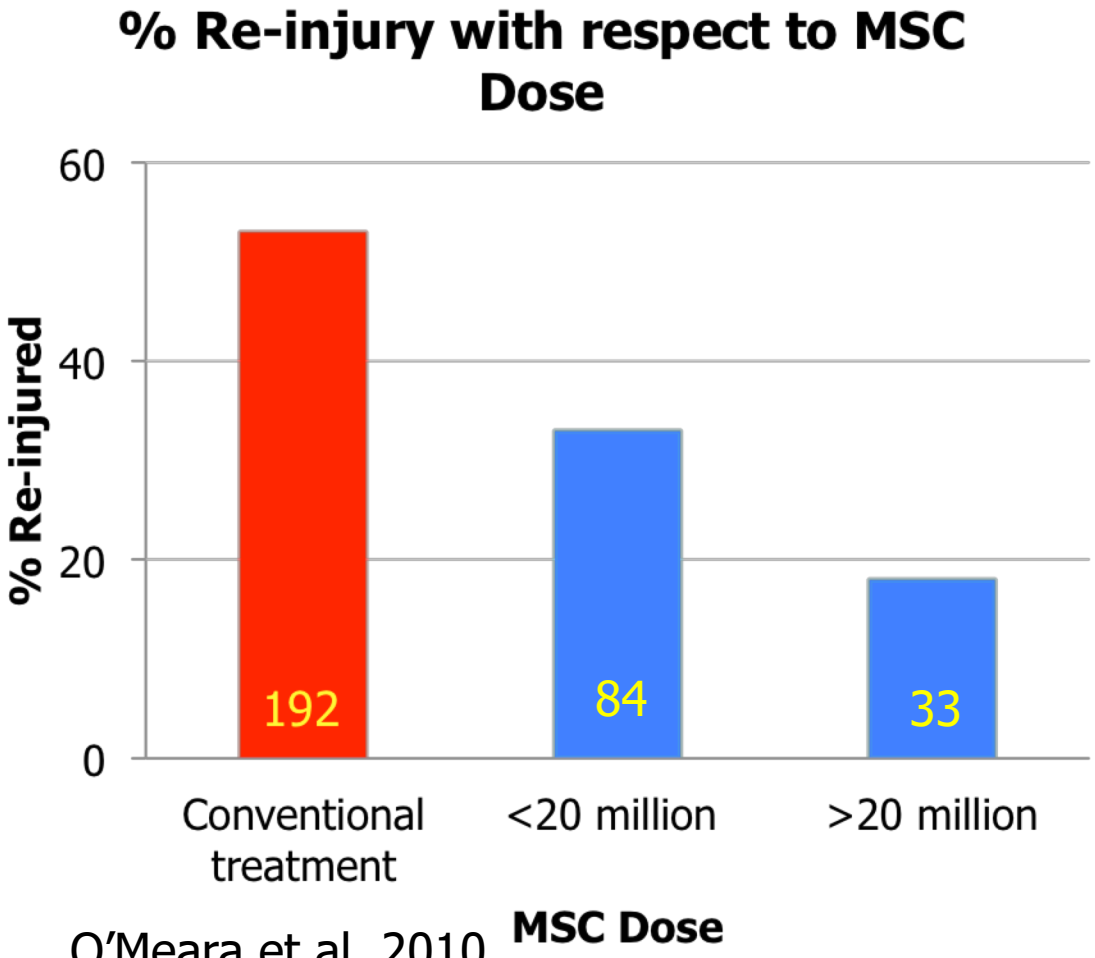


Effect of Injection Time



Effect of dose

- Reduced re-injury rate with higher cell dose
  - Influenced by lesion size
  - ?Better for larger lesions



O'Meara et al. 2010

Effect of dose

- Reduced re-injury rate with higher cell dose
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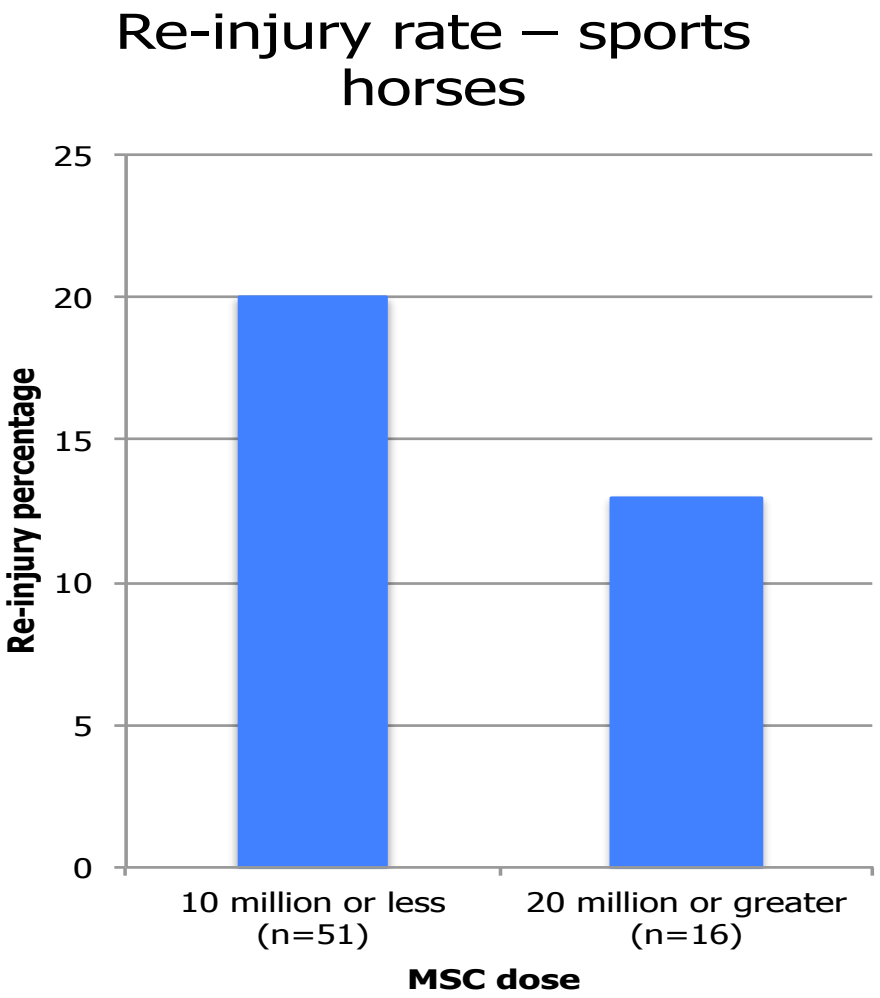
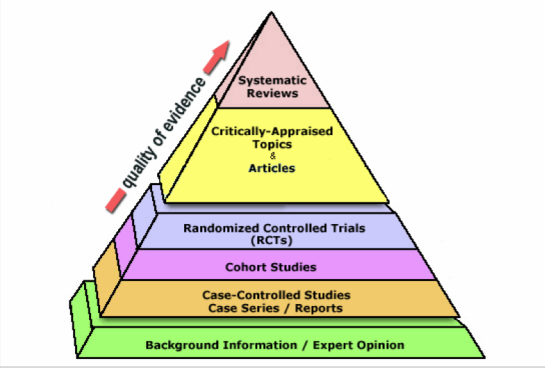
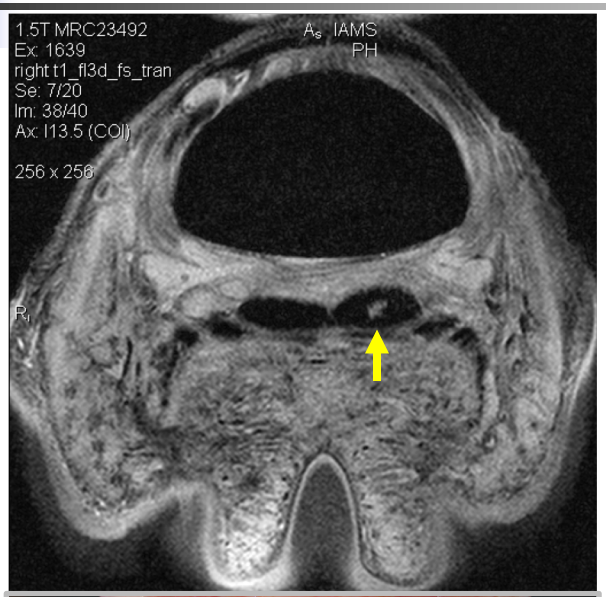
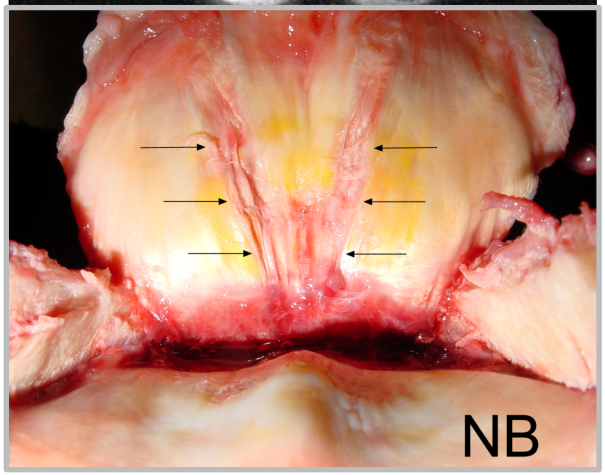




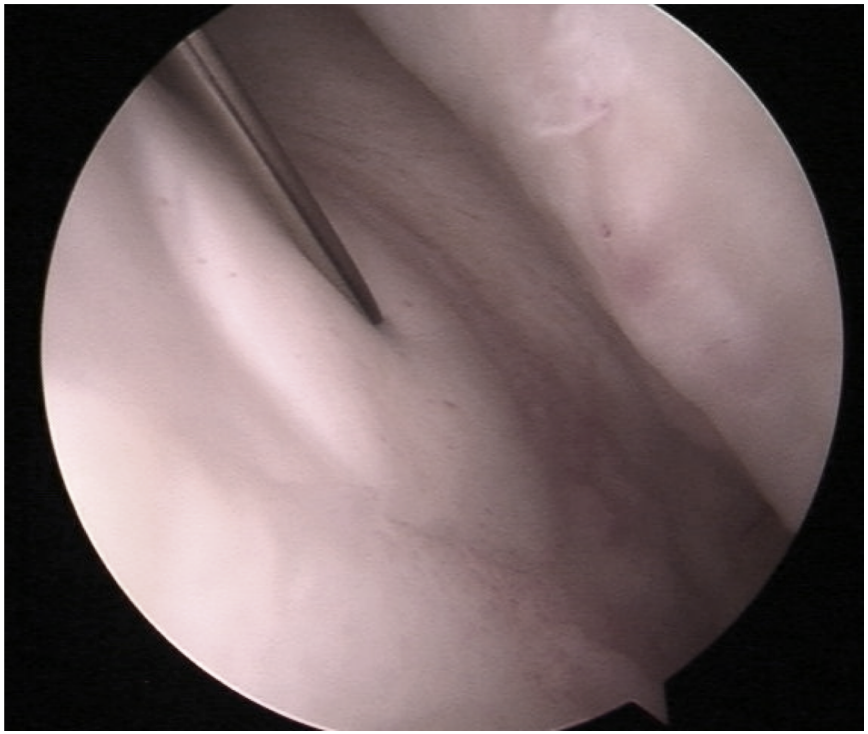
Other clinical studies in horses?

Bursoscopy and Stem Cells for treatment of DDFT tendinopathy

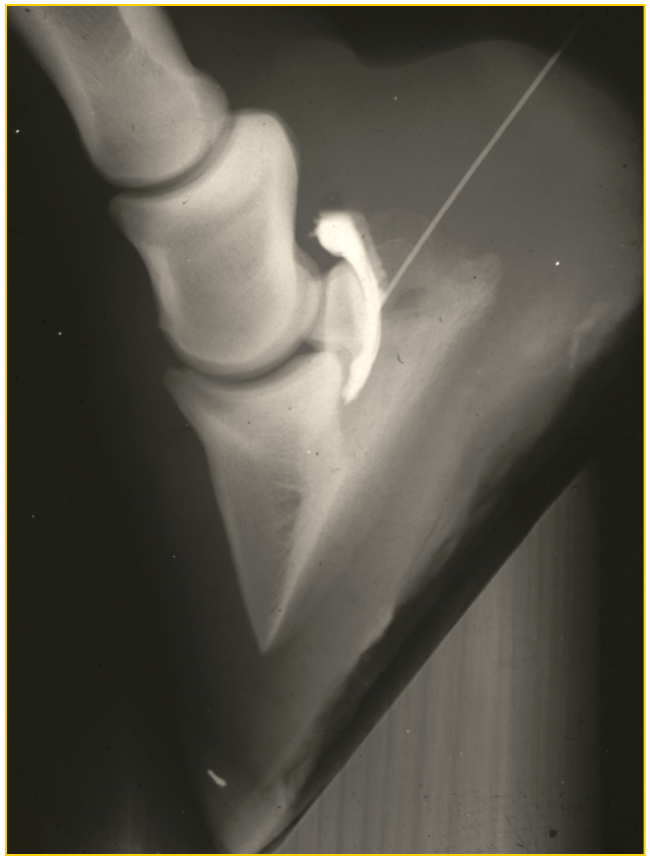




Stem Cell Injection



Intra-operative



Intra-bursal

Results

- 29 horses with DDFT tears
  - 23 BURSOSCOPY + MSCs
    - 17 intralesional, 6 intrabursal
    - 18 bone marrow-derived, 5 fat-derived
  - 6 MSCs without bursoscopy
    - 3 bone marrow-derived, 3 fat-derived
- 6 months' stall rest followed by a graduated return to exercise.
- Follow-up time: 6 months – 5 years

Results

Treatment	Sound for intended use	Sound for lower level use	Persistent lameness	Convalescing	Not Available
Stem cell therapy alone	3	1	2	0	0

Results

Treatment	Sound for intended use	Sound for lower level use	Persistent lameness	Convalescing	Not Available
Stem cell therapy alone	3	1	2	0	0
Bursoscopy + Stem cell therapy	6	8	7	0	2

Results

Treatment	Sound for intended use	Sound for lower level use	Persistent lameness	Convalescing	Not Available
Stem cell therapy	3	1	2	0	0
Bursoscopy + Stem cell therapy	6 28%	8 38%	7 33%	0	2

Conclusions?

REGENERATIVE MEDICINE: REVOLUTION OR HYPE?

What is best?

- Most evidence for stem cells (if still weak)
  - But only for superficial digital flexor tendons!
  - Technology can and must improve.
- 'Jury's out' for the rest!
  - No evidence of efficacy for IRAP, serum, bone marrow, etc.
  - PRP: stronger rationale: may be an effective way of provoking fibrosis (TGF-β)
    - Beware of combining with MSCs – no synergistic effect (Martinello et al. 2013)

Mesenchymal Stem Cells

GUEST EDITORIAL

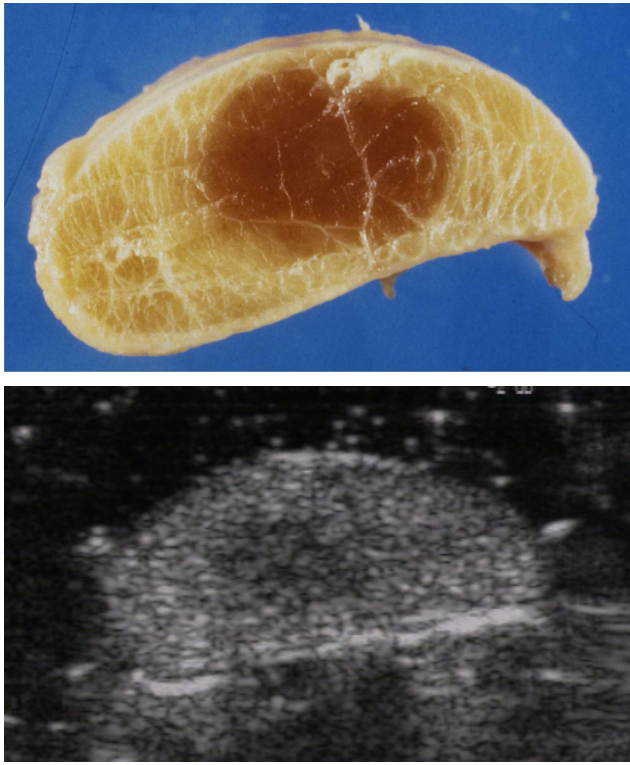
Is 'Stem Cell Therapy' Becoming 21st Century Snake Oil?

Veterinary Surgery 41 (2012) 189–190

THE END

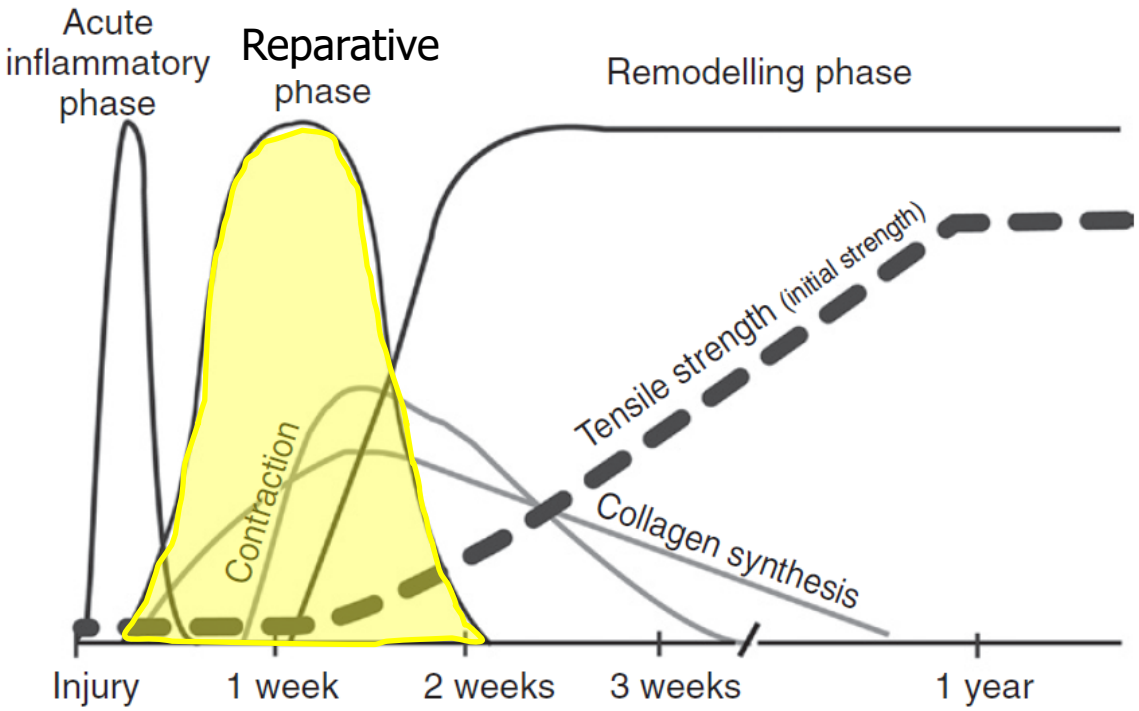
Etiopathogenesis of Tendon Injury

- OVERSTRAIN injury that is preceded by
- a phase of molecular degeneration, which
  - induces neither a clinically evident inflammatory reaction
  - nor any reparative response, but instead
  - progressively weakens the tendon.



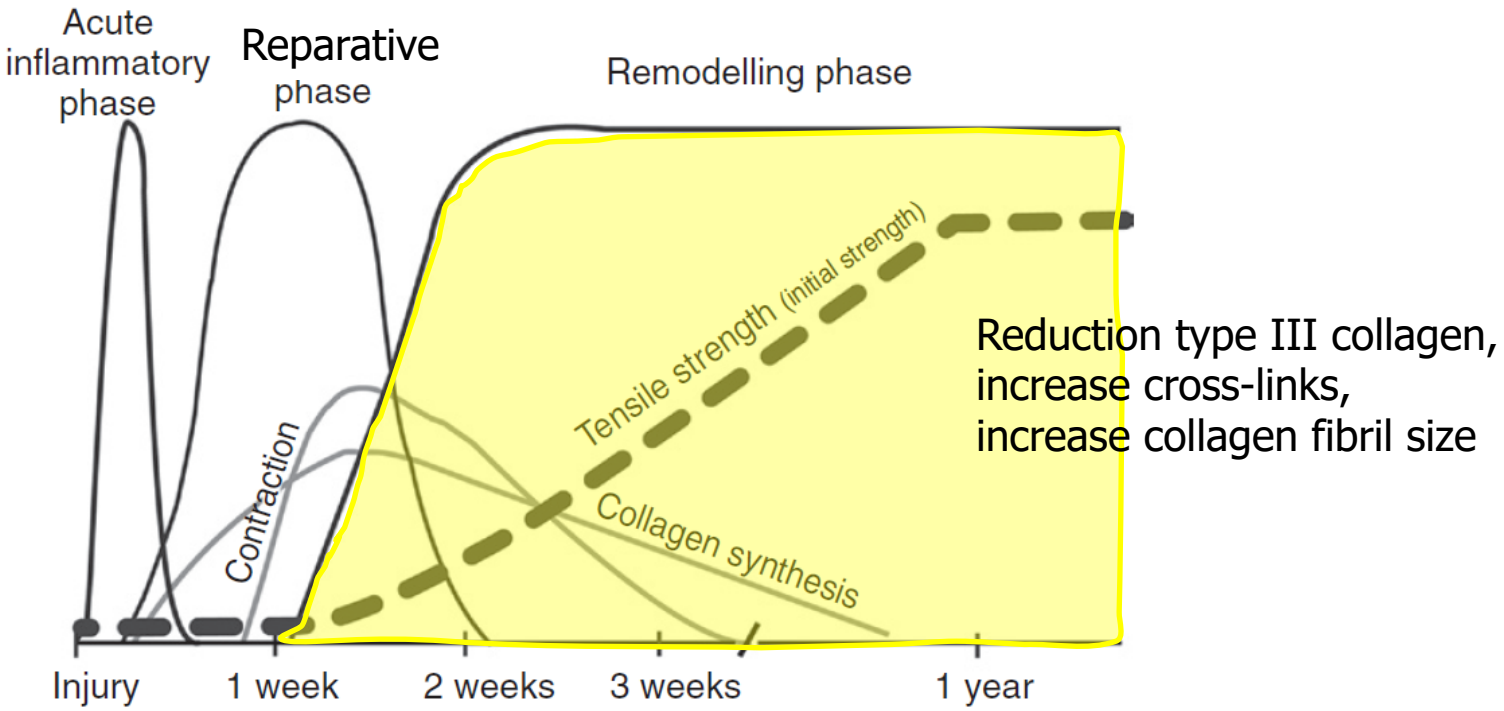
Obervation of asymptomatic Lesions in racehorses. Webbon 1977; Goodship 1994

Mechanism of Clinical Injury



- Hemorhage, fibrin, inflammation, oedema, neutrophils, proteolytic enzymes…..
- Fibroblasts result in synthesis of scar tissue with a higher ratio of disorganized collagen III to collagen I, a higher hydration, and higher levels of GAGs, lack of fascicles.

Mechanism of Clinical Injury



- The scarred tendon is often stiffer than the original. As a result, the healed tendon becomes strong but it is functionally inferior (less elastic) than normal tendon.
- This predisposes it to re-injury, often at sites adjacent to the original injury.

Exogenous growth factors

- Protein signalling molecules that regulate cell metabolism
- Complex combinations involved in tendon formation and growth
  - TGFβ, GDF5, etc.
- Still unproven
- Single growth factors - IGF1, eST, TGFβ
- Combinations – Platelet Rich Plasma
- Dosage and treatment intervals unknown

Autologous Platelet Concentrates (APC or PRP)

- Used to promote tissue healing
- Rheological properties of equine thrombocytes are different than human!
- Biological behaviour of equine thrombocytes is different than human!
- No preliminary process validation and adequate in vitro biochemical characterization performed in horses!

Efficacy – human studies

- Limited evidence of efficacy of PRP for Achilles tendinopathy
  - ?But adequately powered

Am J Sports Med. 2011
Autologous Platelets Have No Effect on the Healing of Human Achilles Tendon Ruptures: A Randomized Single-Blind Study.
Schepull T, Kvist J, Norrman H, Trinks M, Berlin G, Aspenberg P

n=16!!!!

Br J Sports Med. 2011
No effects of PRP on ultrasonographic tendon structure and neovascularisation in chronic midportion Achilles tendinopathy.
de Vos RJ, Weir A, Tol JL, Verhaar JA, Weinans H, van Schie HT.

n=54

Clin J Sport Med. 2011 Jul;21(4):344-52.
A systematic review of the use of platelet-rich plasma in sports medicine as a new treatment for tendon and ligament injuries.
Taylor DW, Petrera M, Hendry M, Theodoropoulos JS.

'Presently, PRP use in tendon and ligament injuries has several potential advantages, including faster recovery and, possibly, a reduction in recurrence, with no adverse reactions described. However, only 3 randomized clinical trials have been conducted.'

BJR

FOOT & ANKLE

Achilles tendinopathy management

A PILOT RANDOMISED CONTROLLED TRIAL COMPARING PLATELET-RICH PLASMA INJECTION WITH AN ECCENTRIC LOADING PROGRAMME

R. S. Kearney,
N. Parsons,
M. L. Costa

From University of Warwick, Coventry, United Kingdom

Bone Joint Res 2013;2:227–32.

Methods

Two groups of patients with mid-substance Achilles tendinopathy were randomised to receive a PRP injection or an eccentric loading programme. A total of 20 patients were randomised, with a mean age of 49 years (35 to 66). All outcome measures were recorded at baseline, six weeks, three months and six months.

Results

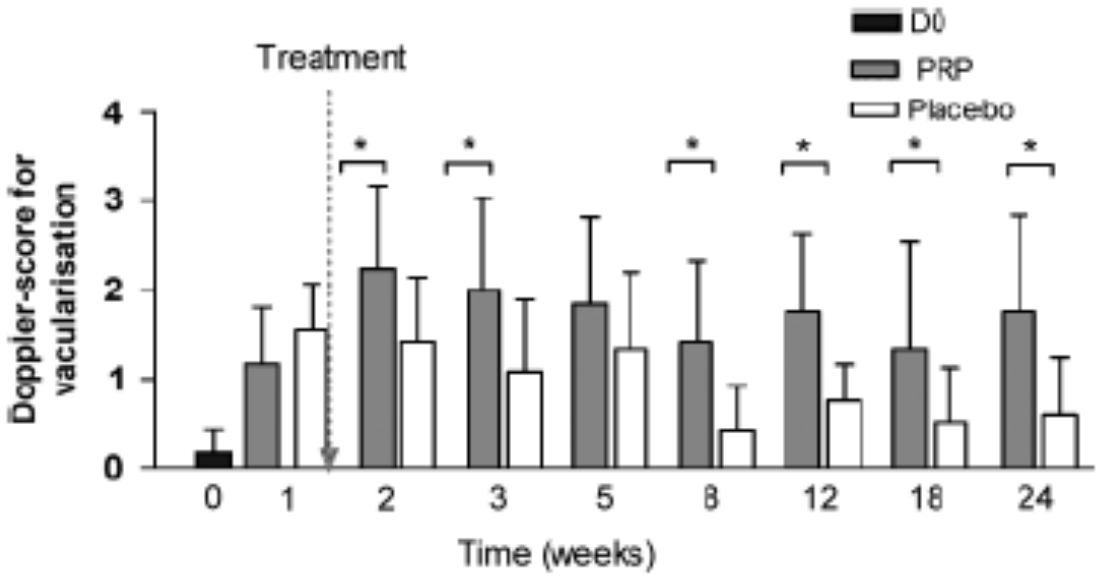
The mean VISA-A score for the injection group at the primary endpoint of six months was 76.0 (95% confidence interval (CI) 58.3 to 93.7) and for the exercise group was 57.4 (95% CI 38.1 to 76.7). There was no statistically significant difference between these scores ($p = 0.171$), which was expected from such a pilot study.

The effect of platelet-rich plasma on the neovascularization of surgically created equine superficial digital flexor tendon lesions

Scand J Med Sci Sports 2010
doi: 10.1111/j.1600-0838.2009.01070.x

G. Bosch[1], M. Moleman[1], A. Barneveld[1], P. R. van Weeren[1], H. T. M. van Schie[1,2]

- Ultrasound significantly better tissue organisation in PRP treated horses
- PRP induced significantly more neovascularization
- VEGF-induced neovascularisation may explain some of the beneficial effects of PRP



PLATELET RICH PLASMA (PRP) ENHANCES ANABOLIC GENE EXPRESSION PATTERNS IN EQUINE SUPERFICIAL DIGITAL FLEXOR TENDONS, BUT NOT SUSPENSORY LIGAMENTS, IN VITRO..

LV Schnabel[1], LA Fortier[1], HO Moahammed[1], BJ Miller[1], MS Jacobson[2]. [1] Cornell University, Ithaca, NY, United States, [2] Children's Hospital, Boston, MA, United States.

ACVS 2009

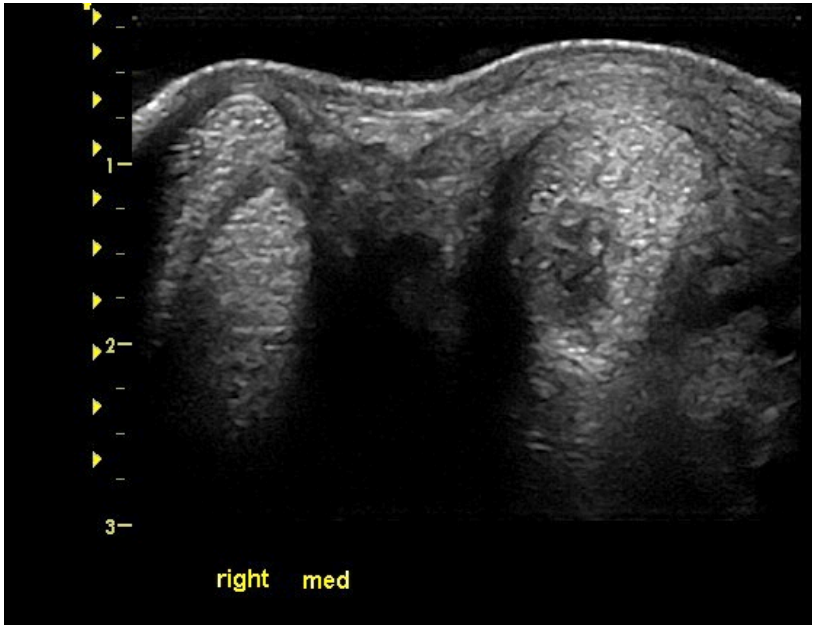
Platelet rich plasma (PRP) has recently been investigated for use in tendon and ligament regeneration due to the growth factors released from platelet alpha-granules. This study examined mRNA expression patterns and DNA content of superficial digital flexor tendon (SDFT) and suspensory ligament (SL) explants cultured with PRP or other blood products.

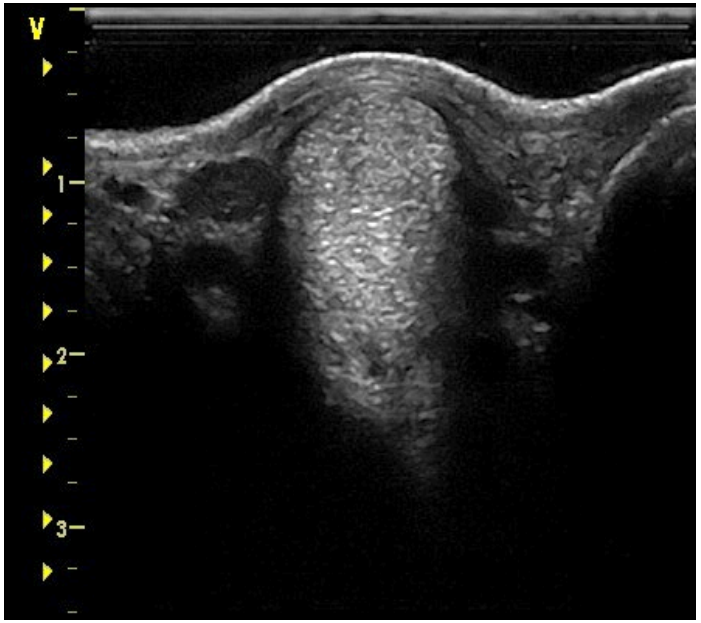
Blood and bone marrow aspirate (BMA) were processed to obtain plasma, PRP, and platelet poor plasma (PPP). IGF-I, TGF-ß1, and PDGF-BB ELISAs were performed on blood products. SDFTs and SLs were harvested and cultured in blood, plasma, PRP, PPP, or BMA at concentrations of 100%, 50%, and 10% in DMEM. Media for control cultures consisted of DMEM with 10% FBS and 10% ACD. RT-PCR for collagen types I and III, COMP, and MMP-13 was performed as were DNA and collagen assays.

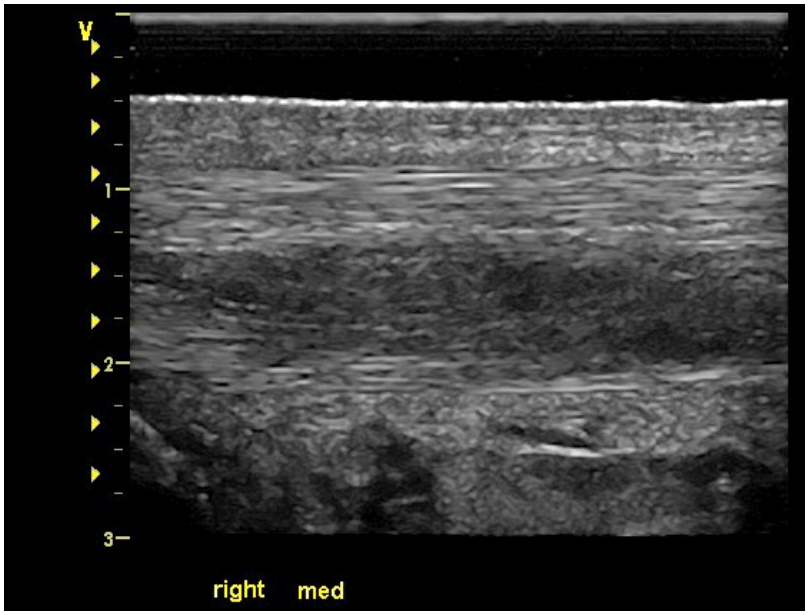
TGF and PDGF concentrations were highest in PRP. A dose-dependent increase in mRNA expression of collagen types I and III as well as COMP above the control was observed for SDFTs cultured in PRP and for SLs cultured in BMA. For both SDFTs and SLs, MMP-13 gene expression was not increased above the control when cultured in 100% PRP and 100% BMA, respectively.

These findings support that PRP is a promising new treatment for tendonitis, but not for suspensory ligament desmitis. Although no deleterious side effects of 100% PRP were detected, in vivo studies are still necessary prior to implementing PRP in routine clinical management of tendonitis.

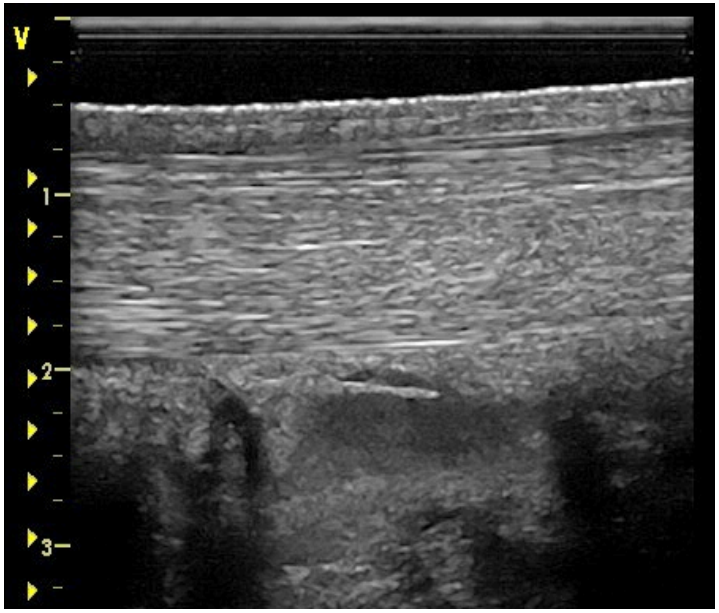
7yr old National Hunt Racehorse







Original injury



Injury 8 weeks post treatment with PRP

PRACTICAL USE OF PRP

Fortier 2012

- Harvest PRP in 3-5 ml aliquots and freeze at -20°C for up to 6 months.
- Day 1-10: routine care 'RICE' + NSAIDS
- Do not inject PRP until day 14 after injury to avoid inflammatory flares.
- Do not use NSAIDs concurrently.
- Inject 3cc thawed PRP (eliminates leukocytes; degranulates platelets): clots in contact with basement membrane
- Stall rest with 10 min handwalking, increase by 5 min each week
- Ultrasound recheck at 30, 60, 90 days: 2nd, 3rd or 4th injections if not 50% improved in lameness, tendon CSA and lesions CSA
- Slowly progressive rehab.

PRACTICAL USE OF PRP

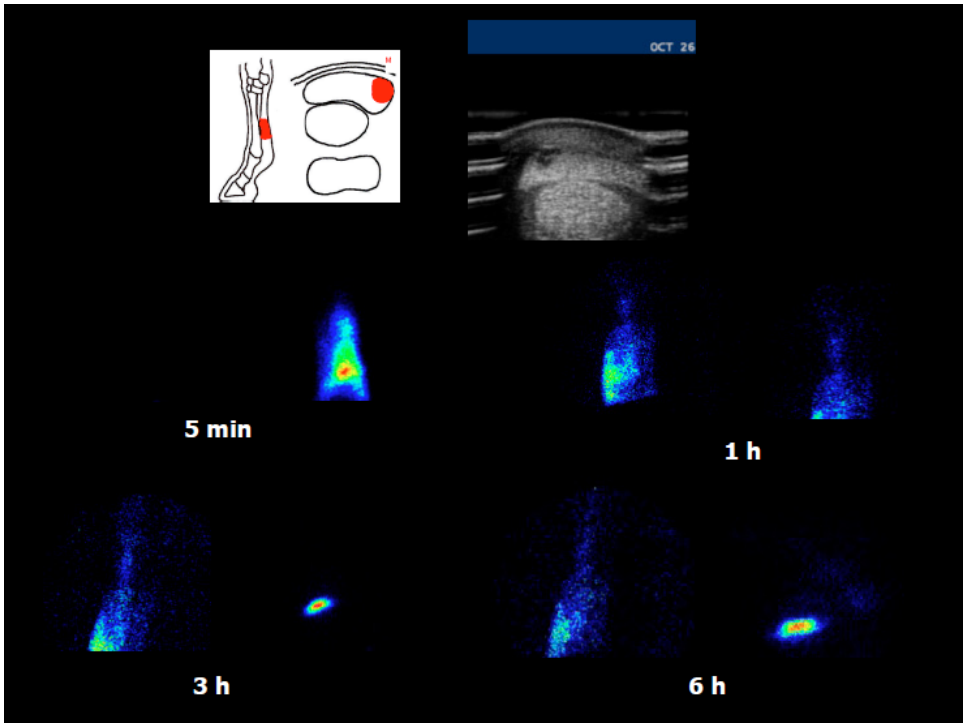
Fortier 2012

REHAB

- Day 0-14: Stall rest, PRP injection.
- Day 14-30: Stall rest with walking.
- Recheck ultrasound at day 30.
- Day 30-90: Ponying, swimming or extra walking. Recheck ultrasound at 60, 90 days.
- Day 90-120: Walk under saddle; 1 or 2 trot periods weekly. Recheck ultrasound at day 120.
- Day 120-150: Add periods of canter every week.
- Day 150-180: Increase periods of canter; add slow gallop. Recheck ultrasound at day 180.
- Day 180- 240: Conditioning gallops

Fortier 2012

How many cells stay in the lesion after IVRP?



Royal Veterinary College
University of London

How many cells stay in the lesion after IVRP?

ECVS proceedings 2012

Homing of Stem Cells

P. Becerra, M.A. Valdés, A.R. Fiske-Jackson[1], J. Dudhia[1], F. Neves, R.K.W. Smith[1]

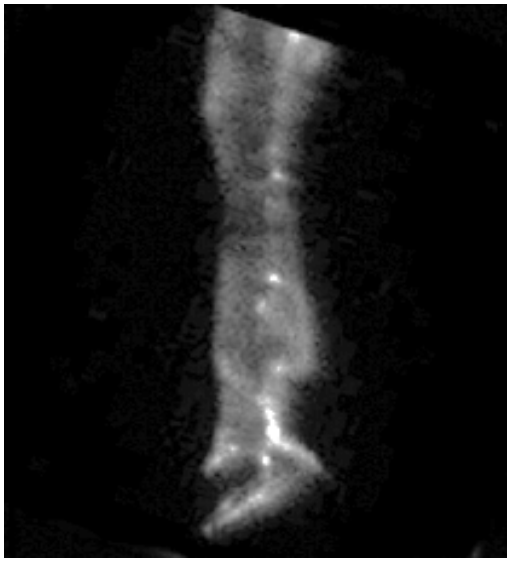
Hospital de Referencia La Equina, Manilva, Malaga, Spain. [1] Department of Veterinary Clinical Sciences, The Royal Veterinary College, North Mymms, Hatfield, Hertfordshire, UK.

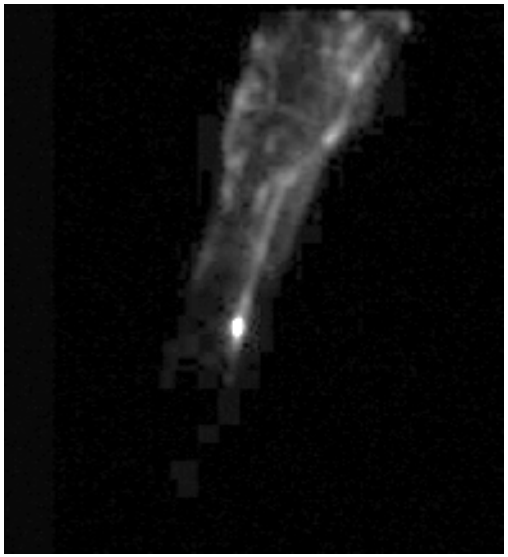
Cell persistence	12 Hrs	24 Hrs	10 Days
Intralesional	32%	24%	< 5%
IVRP	9%	-	-

How many cells stay in the lesion after IVRP?

Scintigraphic evaluation of intra-arterial and intravenous regional limb perfusion of allogeneic bone marrow-derived mesenchymal stem cells in the normal equine distal limb using ^{99m}Tc-HMPAO

A. SOLE, M. SPRIET†*, L. D. GALUPPO†, K. A. PADGETT†, D. L. BORJESSON‡, E. R. WISNER†, R. J. BROSNAN† and M. A. VIDAL†





- Excellent distribution MSCs:
  - 6/6 IA RLP; 3/6 IV RLP
- Poor distribution MSCs:
  - 3/6 IV RLP

- Efficiency of delivery: IVRP or IARP = 5-10 % of intralesional

How many cells stay in the lesion after IVRP?

Scintigraphic evaluation of intra-arterial and intravenous regional limb perfusion of allogeneic bone marrow-derived mesenchymal stem cells in the normal equine distal limb using ^{99m}Tc-HMPAO

A. SOLE, M. SPRIET†*, L. D. GALUPPO†, K. A. PADGETT†, D. L. BORJESSON‡, E. R. WISNER†, R. J. BROSNAN† and M. A. VIDAL†

Equine Veterinary Journal ISSN 0425-1644
DOI: 10.1111/j.2042-3306.2011.00530.x

Cell persistence at 6 hours	Median	Minimum	Maximum
Intra-arterial	39%	30%	60%
Intra-venous	28%	14%	50%

- Severe arterial thrombosis in one horse following IARP

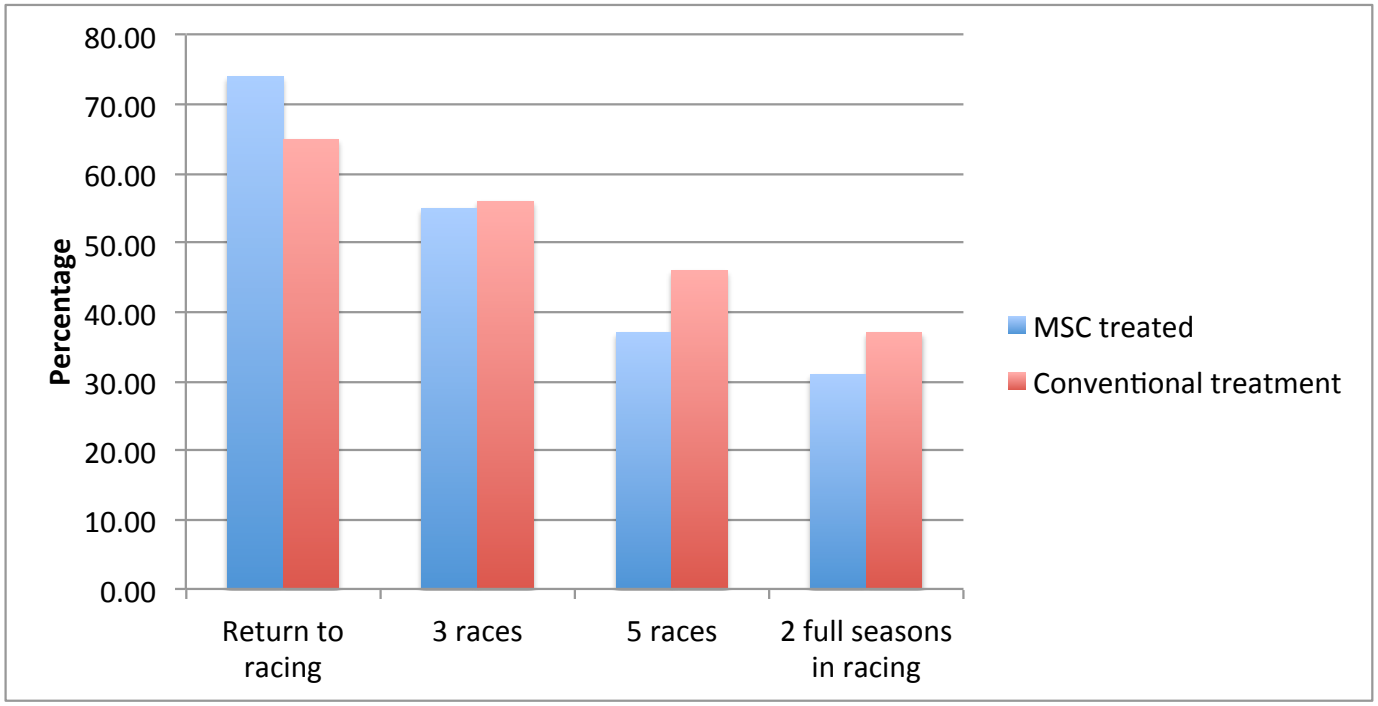
REGENERATIVE MEDICINE: REVOLUTION OR HYPE?

A personal view

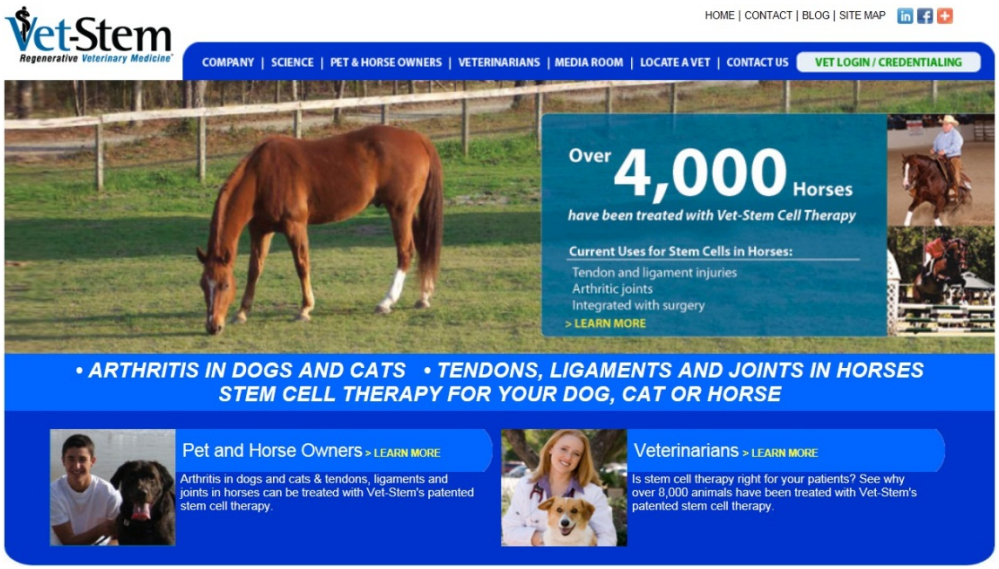
- Biggest problems are those of quality control of the final product:
  - Is FDA approval as a medical device sufficient?
  - What are we using on the patient?
  - Is it what the company says it is?
  - Are academic labs reporting their failures as well as their 'successes'?
  - Should research labs have commercial interests?

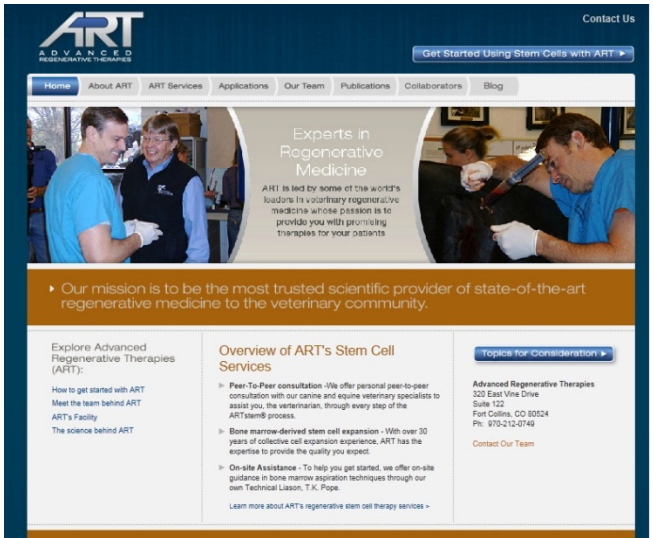
Performance - racehorses

- No indication that treated horses being managed differently
- O'Meara et al. 2010 – no difference in post-injury performance if return to racing



The battle for equine Stem Cell Therapy





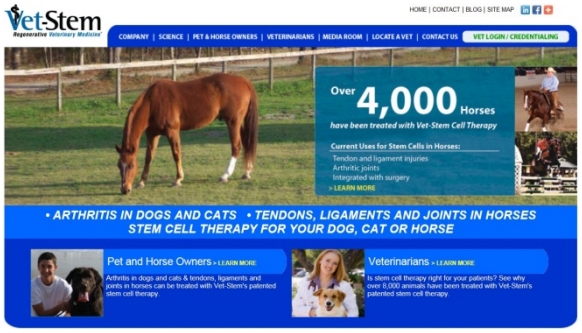
Evaluation of Adipose-Derived Stromal Vascular Fraction or Bone Marrow-Derived Mesenchymal Stem Cells for Treatment of Osteoarthritis

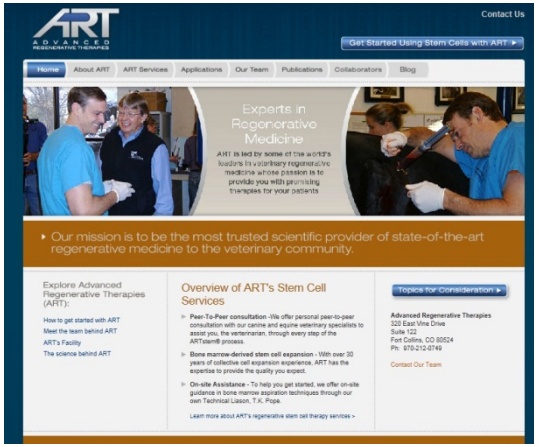
JOURNAL OF ORTHOPAEDIC RESEARCH *DOI 10.1002/jor.20933*

David D. Frisbie, John D. Kisiday, Chris E. Kawcak, Natasha M. Werpy, C. Wayne McIlwraith

Equine Orthopaedic Research Center, Department of Clinical Sciences, Colorado State University, 300 West Drake Road, Fort Collins, Colorado 80523

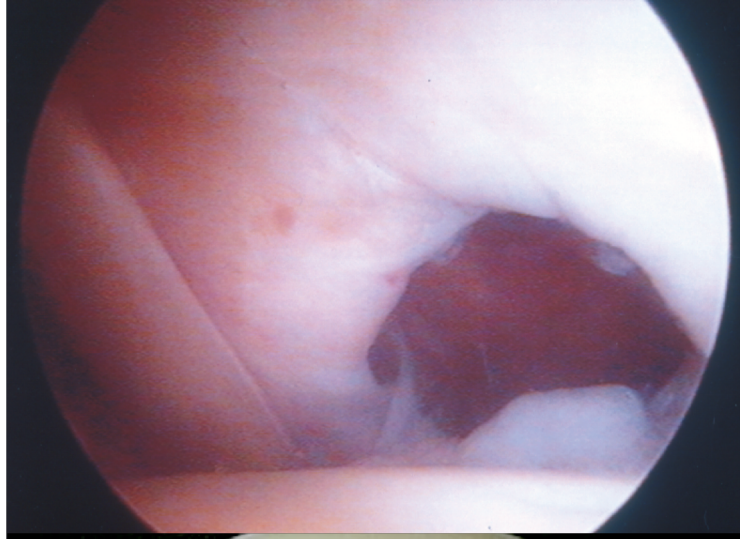
- Bone marrow derived cells significantly reduced PGE2 in arthritic joints.
- Fat-derived cells significantly increased TNFα in arthritic joints
- A greater improvement was seen with bone-marrow derived cells!

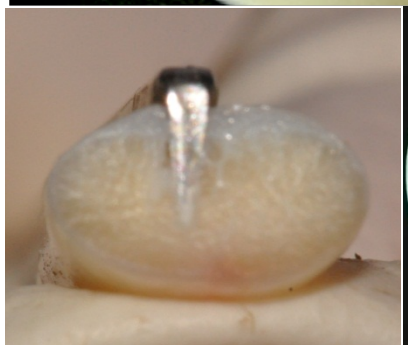


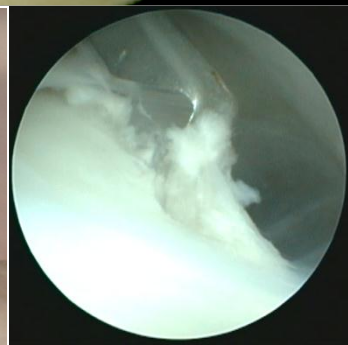


Conclusions

- Supporting evidence for efficacy in experimental and clinical outcomes
- Technique is safe (several thousands treated to date)
- The horse is a relevant translational model
  - A proving ground for new therapies
    - Achilles (Human trial - Goldberg, Smith et al.; UK Stem Cell Foundation funded)
  - Intra-synovial tendon disease
    - Rotator cuff = Equine tendon tears
    - Sheep model (MRC-funded)







What is the mechanism of action behind stem cell therapy?

Action = Orchestra

- 'Musician'
  - Stem cells differentiate into tenocytes and synthesise tendon-like matrix
  - 'Tendon regeneration'
- 'Conductor'
  - Stem cells orchestrate formation of tendon-like matrix
    - Trophic or paracrine effect "recruiting other cells"
    - Anti-inflammatory
    - 'Modulation of repair'

?

Royal Veterinary College
University of London

INTRATENDINOUS DELIVERY BY INTRAVENOUS REGIONAL PERFUSION

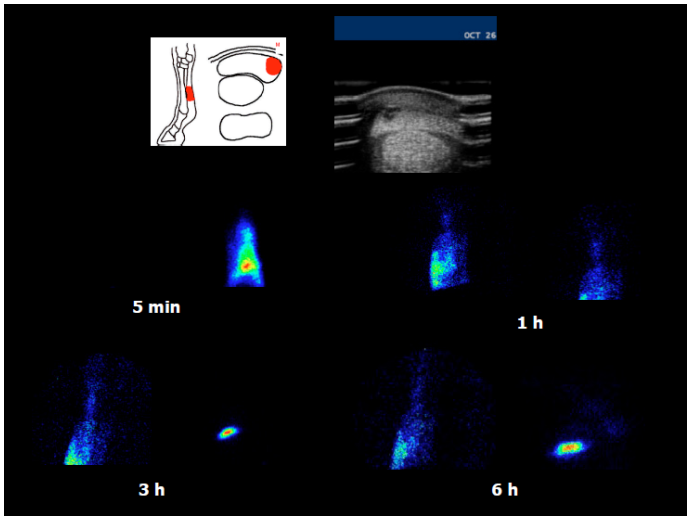
ECVS proceedings 2012

Homing of Stem Cells

P. Becerra, M.A. Valdés, A.R. Fiske-Jackson[1], J. Dudhia[1], F. Neves, R.K.W. Smith[1]

Hospital de Referencia La Equina, Manilva, Malaga, Spain. [1] Department of Veterinary Clinical Sciences, The Royal Veterinary College, North Mymms, Hatfield, Hertfordshire, UK.

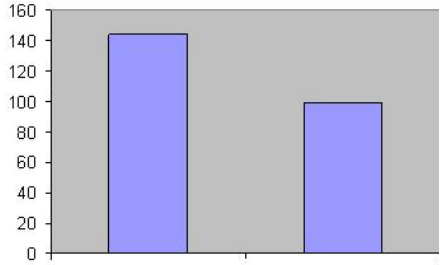
- 18 horses with natural SDFT or ALDDFT injury
- Intralesional injection vs. IVRP of 10 x 10^6 radiolabelled MSCs (Tc99m-HMPAO).
- Cell uptake in tendon lesions?
  - ✓ 18/18 of intralesional injections
  - ✓ 11/17 of IRVP injections;
  - ✓ Efficiency of delivery: IRVP = 2.6 % of intralesional

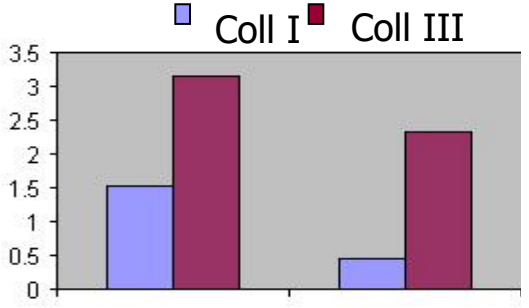


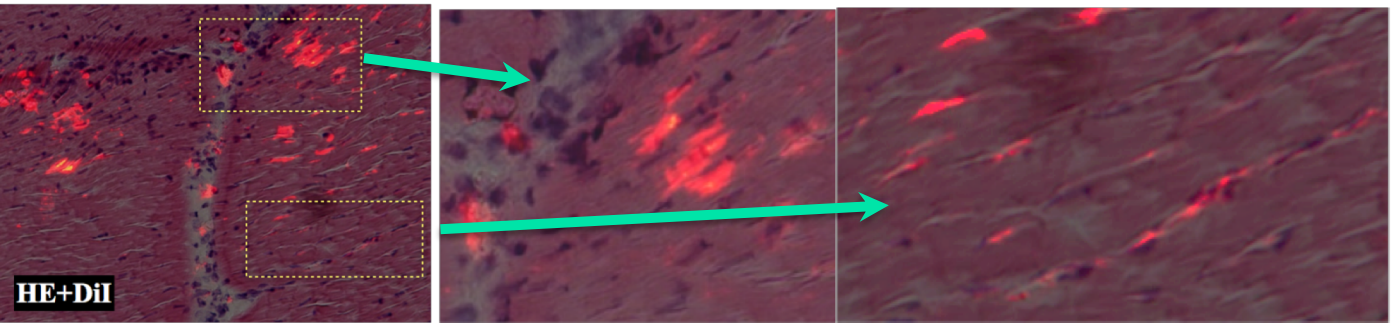
What is the mechanism of action?

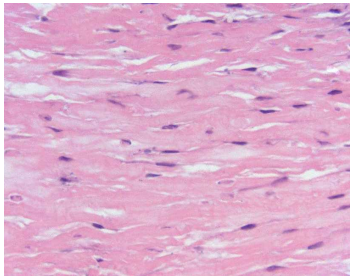
- 'Musician'- True tendon regeneration
  - Stem cells differentiate into tenocytes and synthesise new tendon matrix
  - Hampered by limited markers for true tendon regeneration versus fibrosis
- 'Conductor' - Modulation of repair
  - Stem cells orchestrate formation of tendon-like matrix
  - Trophic or paracrine effect
  - Anti-inflammatory

COMP expression



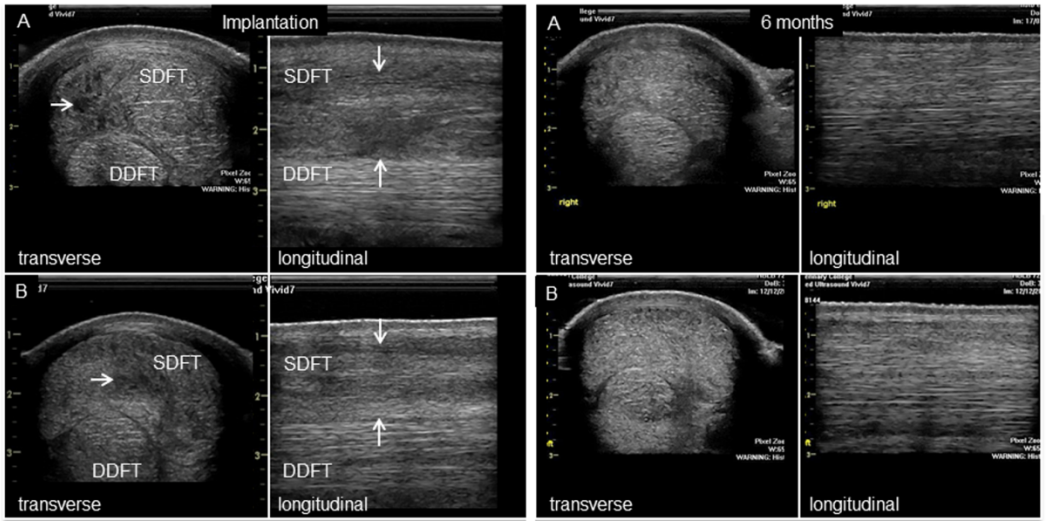






What is the mechanism of action?

- Smith et al. 2013 – Controlled study - MSCs vs saline in naturally-occurring injuries
  - No significant differences in ultrasonographic healing
  - Improved mechanics, organization, cellularity, and composition
    - BUT not normal
- Caniglia et al. 2012 – Controlled study in surgically created lesion – MSCs vs supernatant alone
  - No significant differences in ultrastructure



- Reduction of inflammation and fibrosis remains one of the most attractive mechanisms of action for MSCs
  - Consistent with immunomodulation as the main mechanism of action

Royal Veterinary College
University of London

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PLOS ONE

Beneficial Effects of Autologous Bone Marrow-Derived Mesenchymal Stem Cells in Naturally Occurring Tendinopathy

Roger Kenneth Whealands Smith[1], Natalie Jayne Werling[2], Stephanie Georgina Dakin[1], Rafiqul Alam[1], Allen E. Goodship[3], Jayesh Dudhia[1*]

- "Treatment with autologous MSCs in marrow supernatant therefore provides significant benefits compared to untreated tendon repair in enhancing normalisation of biomechanical, morphological, and compositional parameters."

PLATELET-RICH PLASMA

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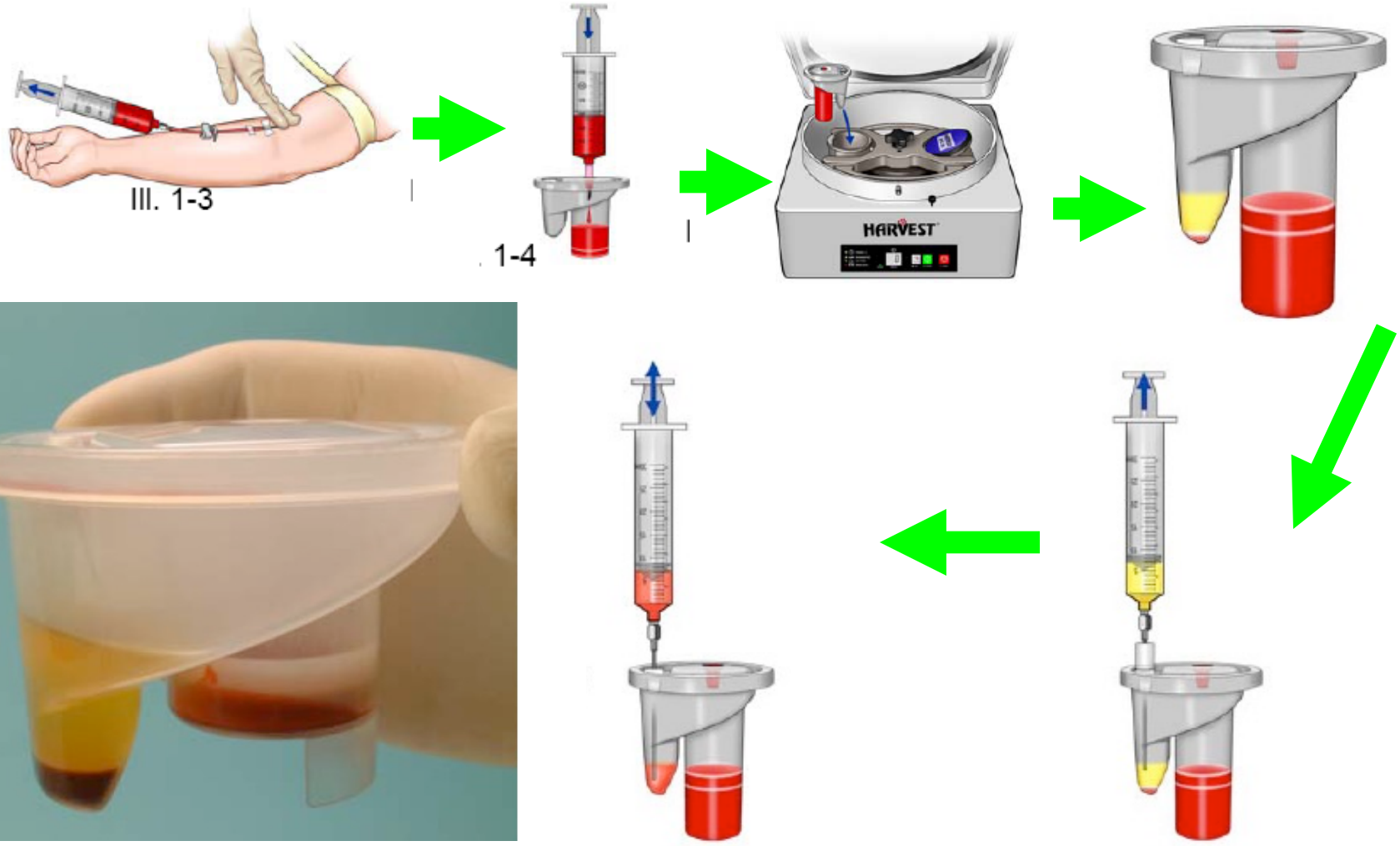

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Arthrex ACP soft centrifugation

- Double syringe – single centrifugation