



Orthobiologics and Orthogen's autologous blood products

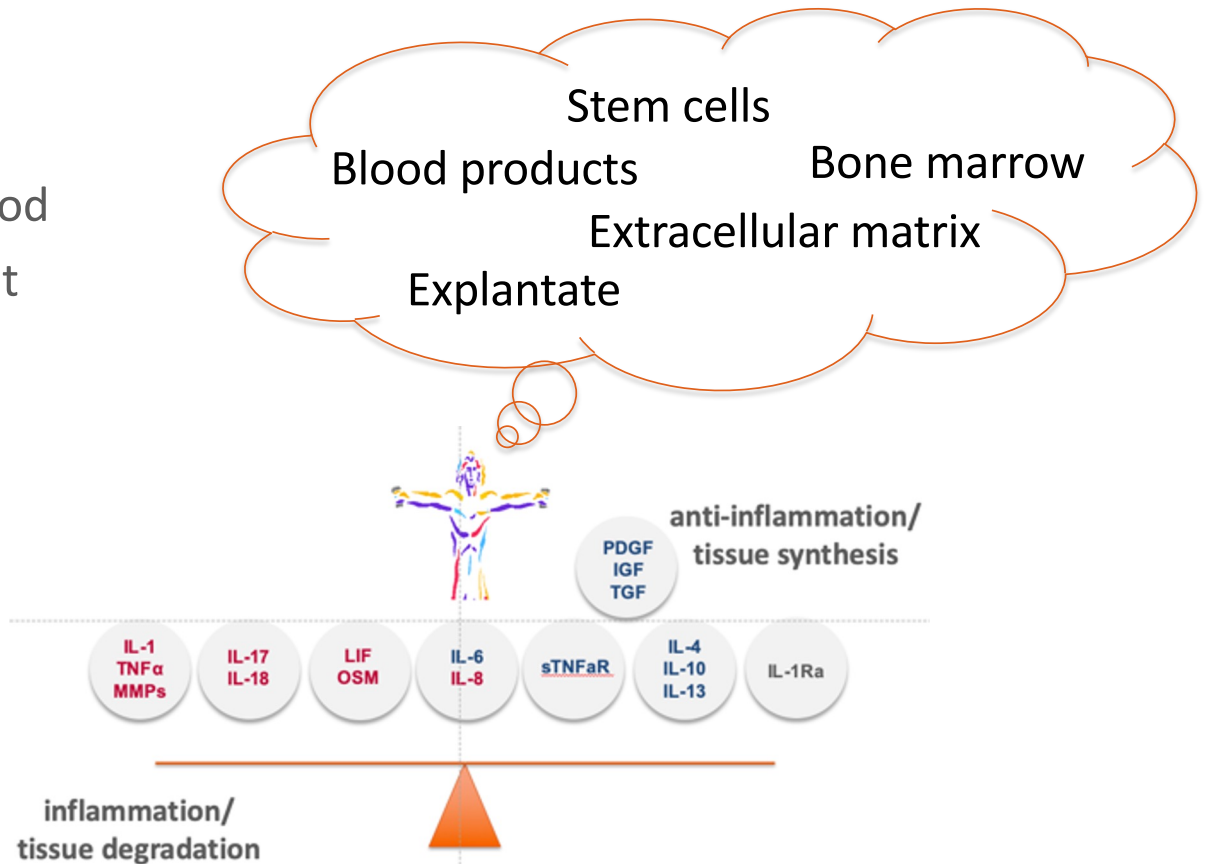
10.02.2023

Dr. Julien P. Troillet
Orthogen Veterinary GmbH Germany



Orthobiologics

- They are not all the same
 - Variation from method to method
 - Variation from patient to patient
 - Variation from time to time
- Pursue the same goal
 - Restitution
 - Prevention
 - **Regeneration ?**



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Regenerative medicine

Process of replacing, engineering or regenerating human or animal **cells, tissues or organs** to restore or **establish normal function** (Mason 2008*)



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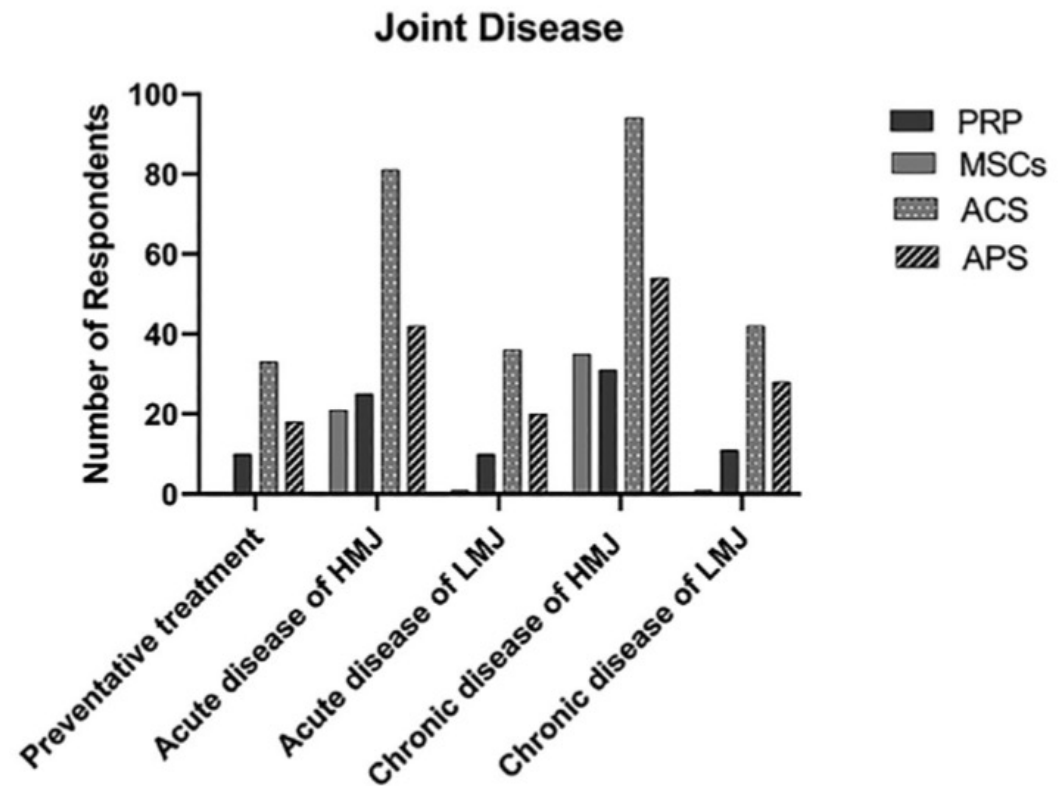
- Joints
 - Cartilage ?
 - Synovial membrane
 - Synovial Fluid
- Tendon
 - Collagen composition



Orthobiologics

- Commonly used Orthobiologics (US) (Knott 2022*)

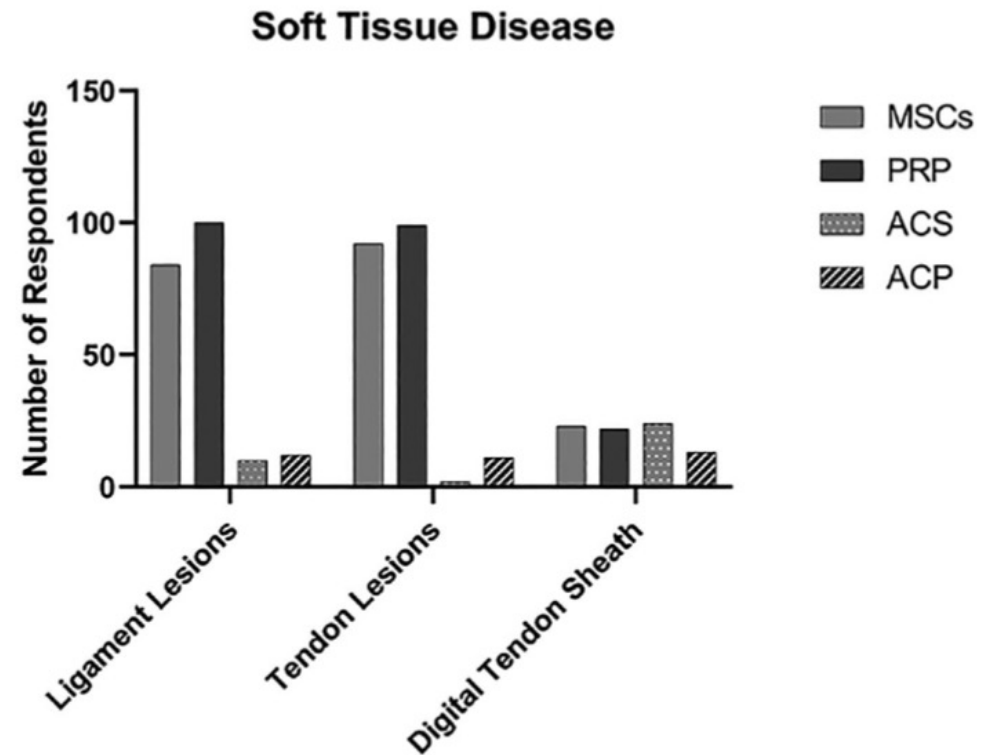
- PRP/ACP 88%
- ACS 80%
- BDMSCs 72%
- APS 46%
- ADMSCs 20%



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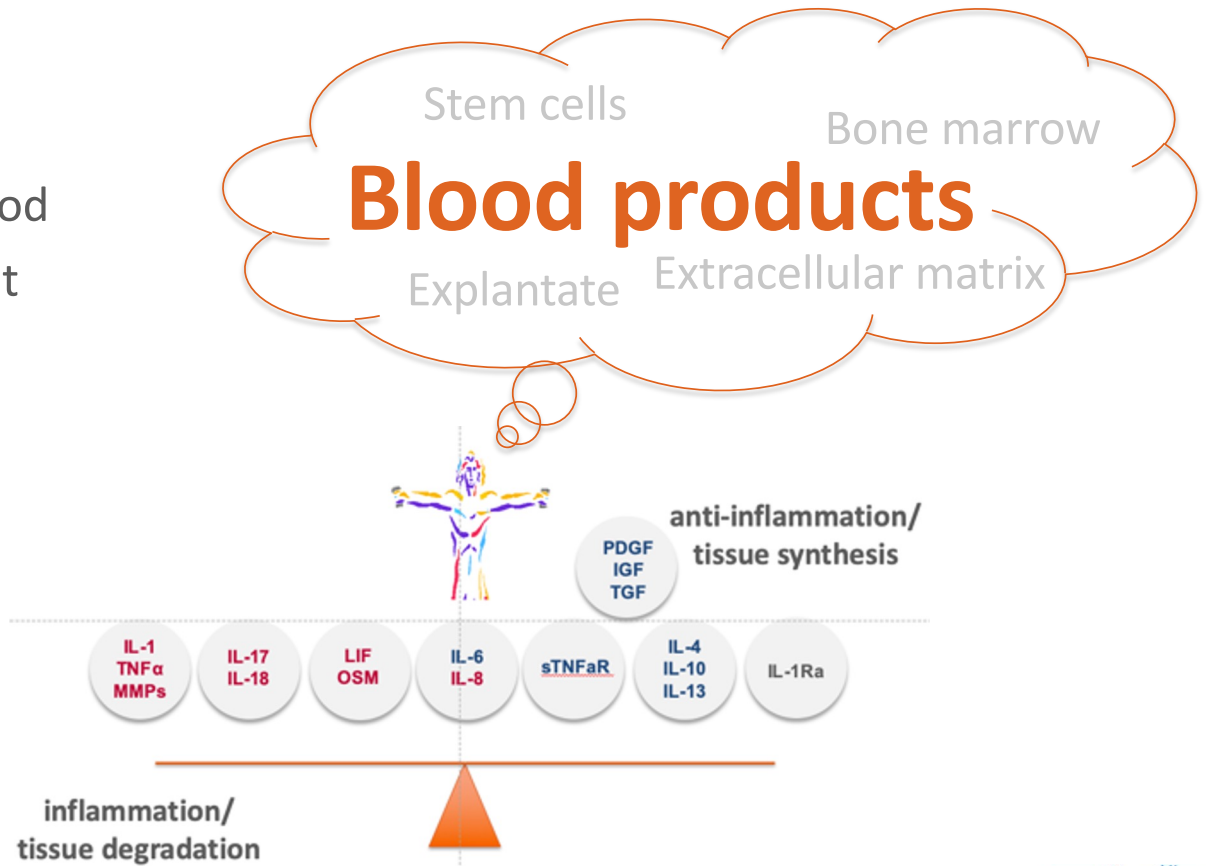
*Current use of biologic therapies for musculoskeletal disease: A survey of board-certified equine specialists ; Lindsay E. Knott et al. Veterinary Surgery. 2022;1-11



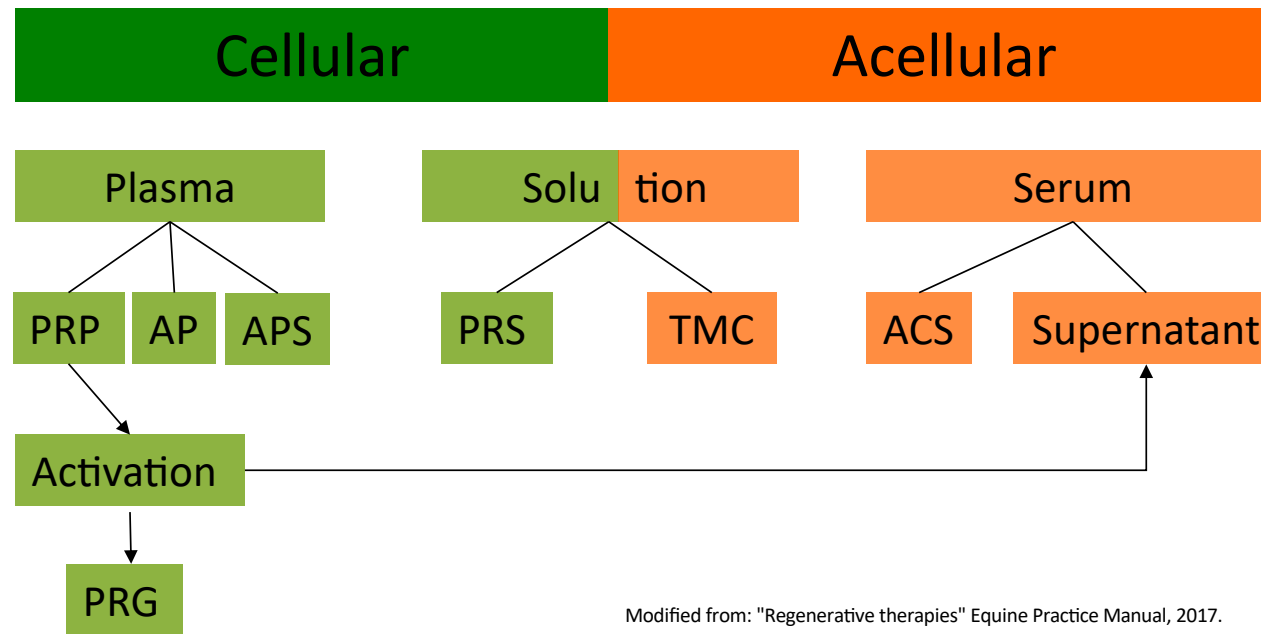
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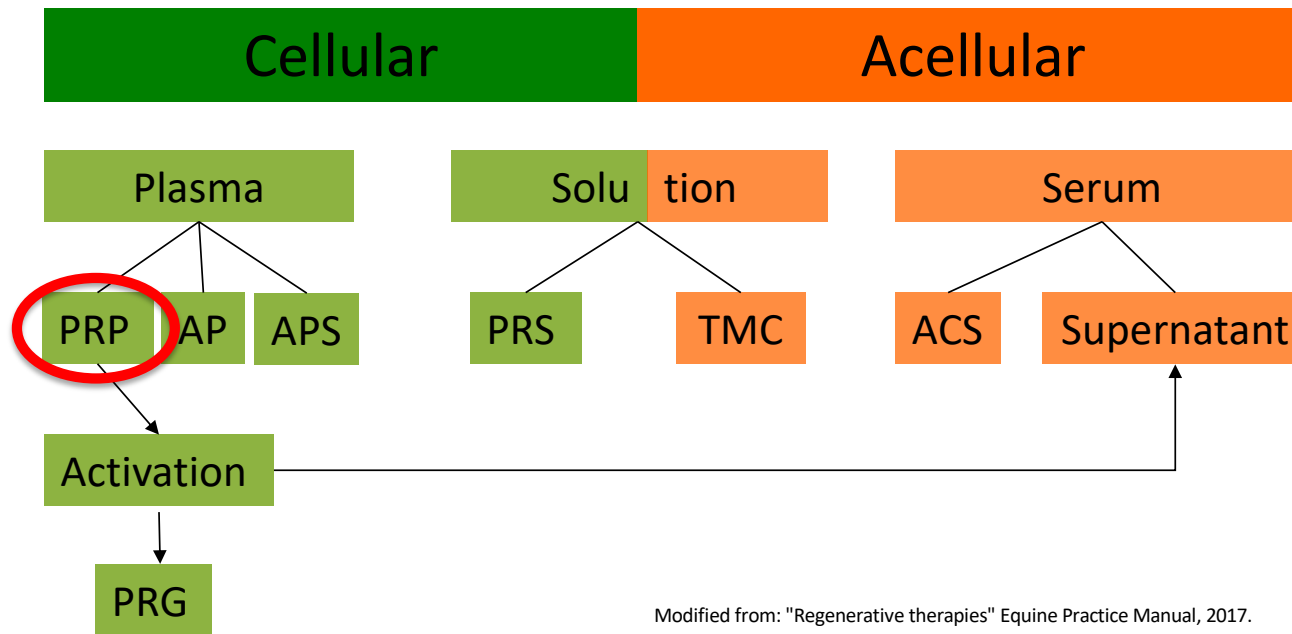


Autologous Blood Products ABPs



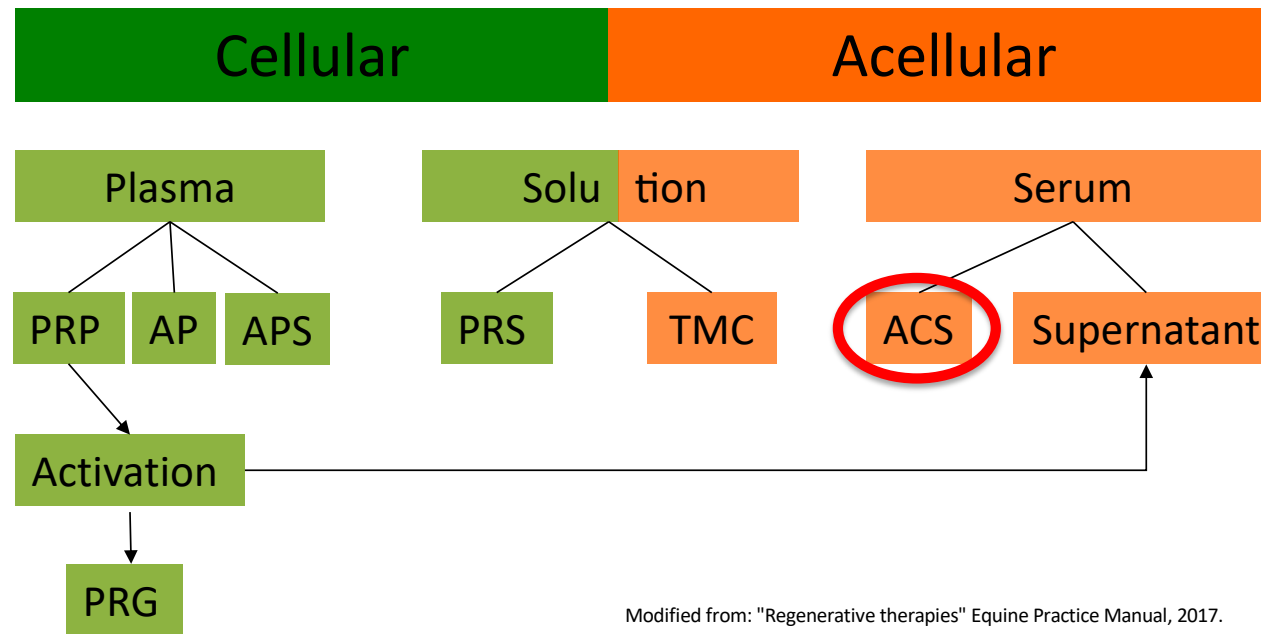
Modified from: "Regenerative therapies" Equine Practice Manual, 2017.

Autologous Blood Products ABPs



Platelet Rich Plasma = Cellular plasma based (non coagulated)

Autologous Blood Products ABPs



Modified from: "Regenerative therapies" Equine Practice Manual, 2017.

Autologous Conditioned Serum = Acellular serum based (fully coagulated)

Production of ABPs with medical devices

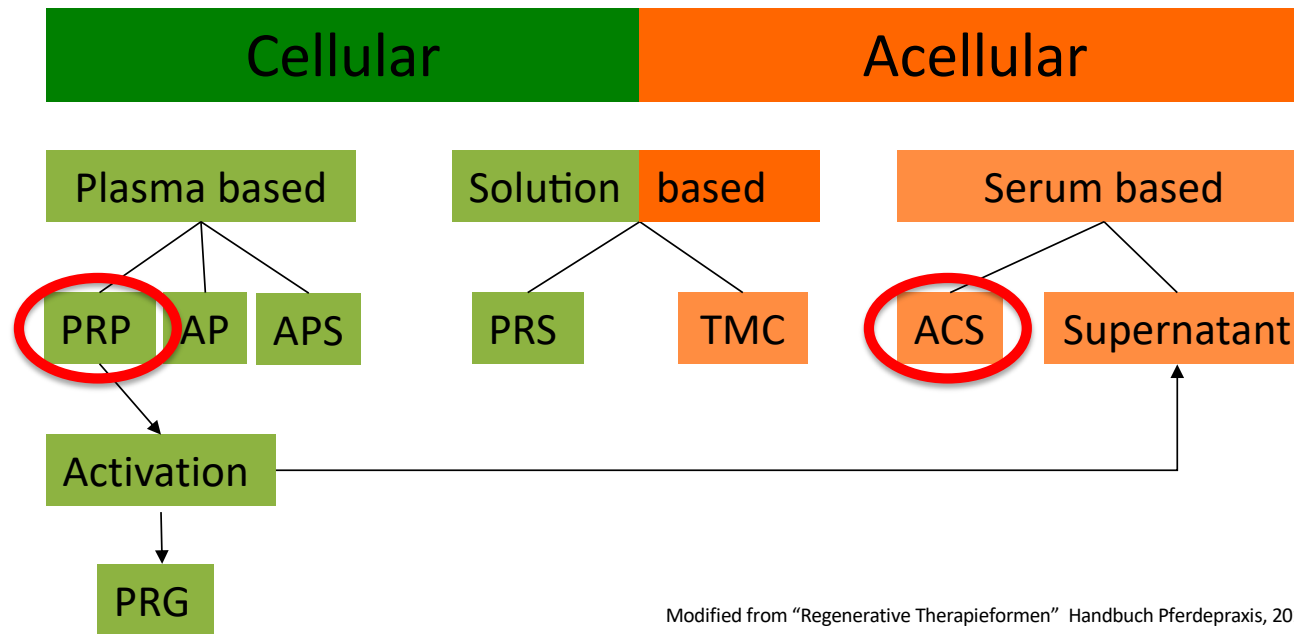
- Production of therapeutic (by veterinarian)
 - For his own individual patient
 - Storage (only autologous)
 - No distribution (B to B)
- Production of device (by Orthogen)
 - Manufacturing according to human medical device directive (MDR)
 - QM: biocompatibility, sterility, mechanical parameters, panels of cytokine ELISAs



Production of ABPs with medical devices

- Production of therapeutic (by veterinarian)
 - **For his own individual patient**
 - Storage (only autologous)
 - No distribution (B to B)
 - Only approved **off the shelf** in the EU
 - Blood derived expanded MSCs
 - Umbilical Cord derived exopanded MSCs
- CAVE with non approved therapeutics !!!**

Autologous Blood Products

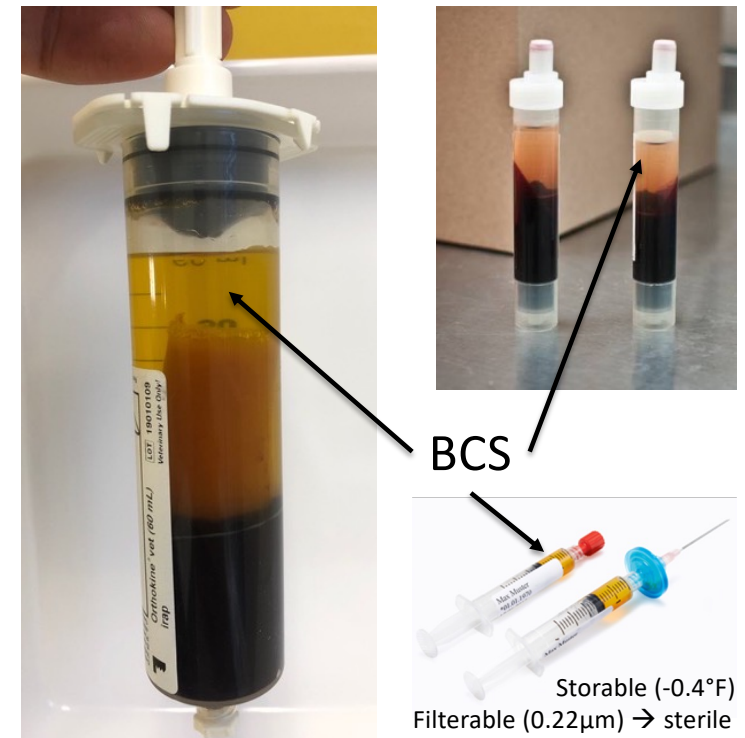


Modified from "Regenerative Therapieformen" Handbuch Pferdepraxis, 2017

PRP = Cellular plasma based (non coagulated)
ACS = Acellular serum based (fully coagulated)

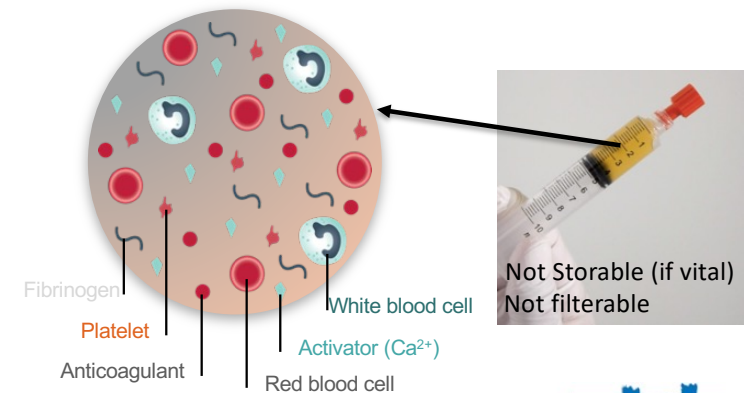
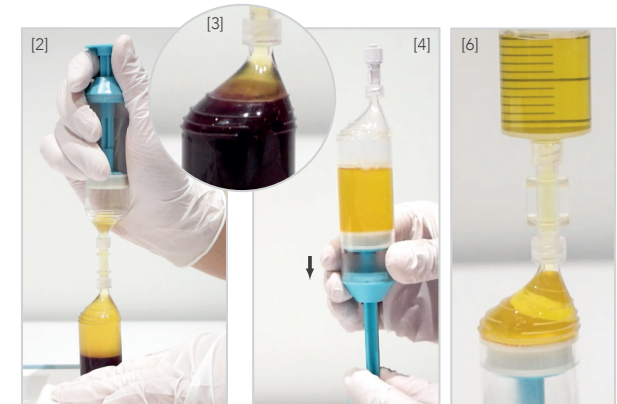
Autologous Conditioned Serum incl. Blood Cell Secretome

- Biophysical stimulation of whole blood
 - Activation/secretion of/by WBCs: IL-1Ra, IL-10...
(*Tatarniuk 2015, Ionita 2016, Sawyere 2016, Marques-Smith 2020*)
 - Secretion by Thrombocytes: PDGF, TGF- β ...
(*Ionita 2016, , Sawyere 2016*)
 - Humoral dissolved proteins: IGF-1...
(*Ionita 2016, Sawyere 2016, Marques-Smith 2020*)
- Extracellular vesicles (EVs)
(*Yang 2012, Orthogen*)
- Lipid mediators (SPMs)
(*Norris 2017*)



Platelet Rich Plasma

- Mechanical separation of whole blood
 - Delivery of Growth Factors by Thrombocytes (vehicle)
 - PDGF, TGF- β ... (*Hessel 2015, Ionita 2016*)
 - Delivery of cytokines by White Blood Cells (standardized?)
 - IL-1Ra, IL-10 (*Ionita 2016*)
 - Delivery of Humoral dissolved proteins (in plasma)
 - IGF-1... (*Ionita 2016*)
 - Formation of fibrin by fibrinogen and prothrombine



ACS with Orthokine®vet 10mL

- One component device
 - Multiple blood-draw with adapter
 - 6-9h incubation
 - Normal 10mL benchtop centrifuge
 - 3-4mL yield (1-2 injections) ACS
-
- Same day treatment
 - Low volume treatment
 - Desmitis e.g. suspensory ligament
 - Tendonitis e.g. bowed tendon
 - Canine



ACS with Orthokine[®]vet 60mL

- One component device production
 - 24h incubation
 - Special fixed-angle rotor (or adapter)
 - Ø 26mL Yield
 - Next day treatment
-
- Multiple dose/large vol treatment
 - Synovial pathologies e.g. chronic OA
 - Tendonitis e.g. bowed tendon
 - Other inflammatory processes



PRP with Osteokine® ProGen

- All in one box
- Filled with anticoagulant
- 50mL swing out rotor
- Special shape
- Same day treatment
- 4mL PRP ($\approx 5 \times$ baseline PLTs)



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PRP with Osteokine® ProGen in comparison

Medical device	PLT x vs Baseline	WBC x vs Baseline	RBC x vs Baseline	PRP/AP Volume cc
O® ProGen (n=13)	4.9	3.1	0.03	4
Harvest SmartPReP N.Jarlov Denmark	5.9	3.6	Yes/red	
V-PET Hessel et al. 2015	3.8	1.8	Yes/red	8
ACP Hessel et al. 2015	1.3	0.1		6
ProTec PRP (PulseVet) Regenlab, other single spins	As ACP			4
ProStride Bertone et al. 2014	1.6	12.1	6.8	3
Ostekine® Geburek et al. 2017	5.7	1.8		4

Clinical use of PRP

Regenerative stimulation

- Cell migration, cell division, tissue synthesis

Pathologies with tissue loss

- Tendon lesions, fractures, implants, wounds....
- Documented for canine, equine and human patients
- Ex. corelesions with intact paratenon ([Geburek 2016](#)).

Joint pathologies

- For OA, **cave**: coagulation, WBCs, RBCs.
- Weak clinical evidence

Application

- Single to multiple injections
- Freshly made
- No (sterile) filtration

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Clinical use of PRP

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Joint pathologies

- For OA, **cave**: coagulation, WBCs, RBCs.
- Weak clinical evidence

- Not filterable

- Coagulation Fibrin Formation^{1,2}

- High levels of IL-1 incl mRNA^{3,4}

- Fast pro-inflammatory action^{5,6}

- Side effects of i.a. injections of PRP⁷

Application

- Single to multiple injections
- Freshly made
- No (sterile) filtration

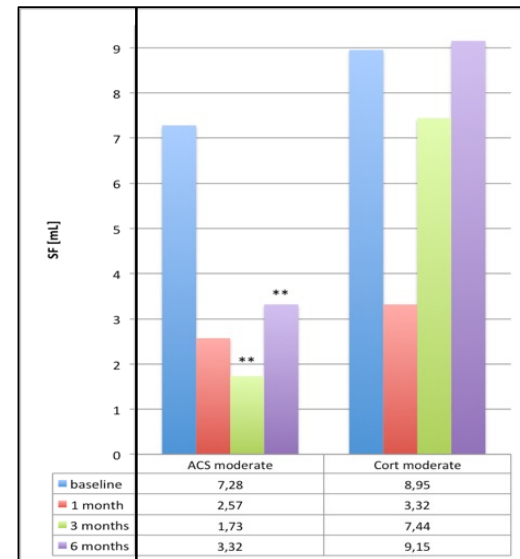
Clinical use of ACS

- Suppression of inflammation and regenerative stimulation
- Joint diseases (*Frisbie 2007, Jöstingmeier 2010*).
 - 2 - 4 inj. (intra articular) / 7 - 10d (post op)
- Synovitis of bursae and tendon sheaths
 - 2 - 4 inj. (intra thecal) / 7 - 10d (post op)
- Tendon and ligament pathologies (*Easter 2014, Geburek 2015, vWehren 2019*).
 - 1 - 2 inj. (intra lesional or peri lesional) / 7 - 10d
- Back pathologies (*Becker 2007, Goni 2015, anecdotal*).
 - 1 - 3 in. (peri-radicular, epidural) / 7 - 10d



MOA of ACS Primary effects

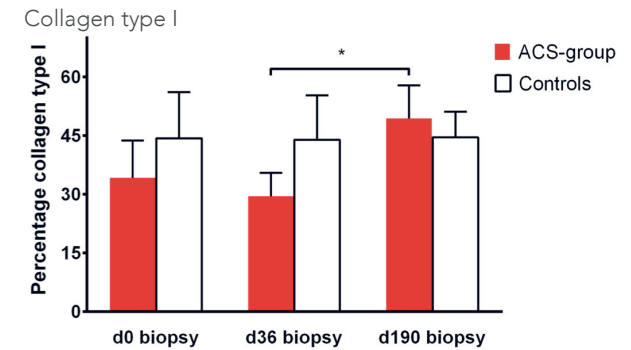
- Anti-inflammatory
 - Pain reduction (Frisbie 2007, Jöstingmeier 2010, Geburek 2015)
 - Hyperplasia reduction (Frisbie 2007, Geburek 2015)



Shirokova 2020, Vol. sig. reduced at 1, 3, 6mo ACS vs 1mo Triam

MOA of ACS Primary effects

- Regenerative / Protective
 - Less bone absorption (Darabos 2011)
 - Stem Cell activation (Wright-Carpenter 2004, Blázquez 2019)
 - Less cartilage Fibrillation (n.s.) (Frisbie 2007)
 - Coll I / Coll III Ratio (Geburek 2015)



ACS-group: significant increase of Coll type I

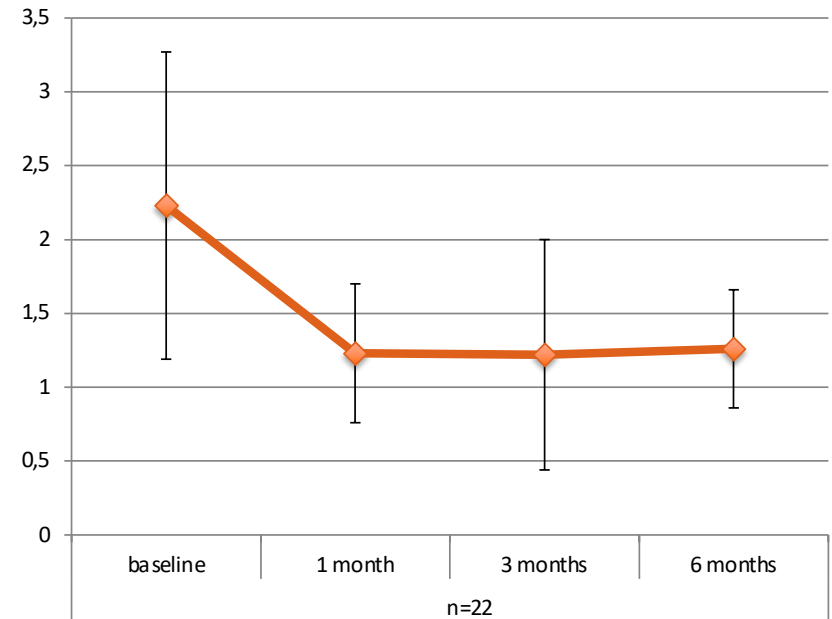
Control-group: no significant increase

MOA of ACS


Biochemical effects

- Synovial fluid content
 - ↑ IL-1Ra ↓ IL-1 β (*Frisbie 2007, Shirokova 2020*)
 - ↓ Nitrate (NO₃) (*Shirokova 2020*)
 - footprint of Nitric Oxide (NO)
 - ↓ Dienes (*Shirokova 2020*)
 - footprint of Radical Oxygen Species
 - ↑ synovial fluid viscosity (*Shirokova 2020*)

Nitrate in synovial fluid of human knee OA with moderate synovitis as a function of time after i.a. ACS therapy.



ACS MOA - Biochemical effects

- Synovial fluid content
 - ↑ IL-1Ra ↓ IL-1 β (*Frisbie 2007, Shirokova 2020*)
 - Upregulated endogenous production IL-1Ra
 - ↓ Nitrate (NO₃), ↓ Dienes (*Shirokova 2020*) 
 - Protection from, less production of radical oxygen species
 - ↑ synovial fluid viscosity (*Shirokova 2020*)
 - Upregulating HA production via GFs (PDGF, EGF,...)
 - Protection from radical oxygen species
- Recovering homeostasis
- Macrophage Shift M1 M2
 - Type A Synoviocytes
- IL-1Ra (irap) can not do this alone
 - see Anakinra[®]

ACS and Stem Cells

- Stem Cell cultivation with ACS

frontiers
in Pharmacology

ORIGINAL RESEARCH
published: 28 June 2019
doi: 10.3389/fphar.2019.00699



Conditioned Serum Enhances the Chondrogenic and Immunomodulatory Behavior of Mesenchymal Stem Cells

Rebeca Blázquez^{1,2*}, Francisco Miguel Sánchez-Margallo^{1,2†}, Julio Reinecke³, Verónica Álvarez¹, Esther López¹, Federica Marinaro¹ and Javier G. Casado^{1,2}

- Proliferative capacity and viability of MSCs may be considered as delivery solution
- Chondrogenic differentiation of MSCs enhanced may induce differentiation of resident MSCs
- MSCs pre-sensitized with Orthokine[®] significantly reduced lymphocyte proliferation induced T-cell differentiation → memory cells. may improve MSCs-mediated anti-inflammatory effect .

ACS and other treatments

- **Blood draw**

- Potential contamination?

> [Vet J.](#) 2017 Dec;230:20–23. doi: 10.1016/j.tvjl.2017.11.005. Epub 2017 Nov 20.

Plasma firocoxib concentrations after intra-articular injection of autologous conditioned serum prepared from firocoxib positive horses

[K F Orved](#)¹, [M B Goodale](#)², [C Ober](#)³, [G A Maylin](#)⁴, [L A Fortier](#)²

This study demonstrated that ACS generated from horses receiving two oral doses of firocoxib at a 24 h interval does not lead to a positive drug test following IA administration, using current analytical methods. Although the ACS contained a similar concentration of firocoxib as plasma collected 4 h post-firocoxib dosing, the amount of firocoxib in the ACS sample did not appear to be large enough to result in detectable systemic concentrations of the medication. Orthobiologics, such as ACS, are...

- Concomitant injection (Velloso 2020*)

- 74% no combination
- 30% Abs
- 9% HA
- 4% other biologics
- 2% GCs

- Mechanical stimulation

- Laser therapy
- Shock wave therapy



Conclusions

- Wide use of Orthobiologics
 - Use quicker than science
- Differences lead to different ideas about indications
- MOA of ACS shows influence on cellular polarization
 - Research (translational human → equine)

- Vet →
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- Slide 22 →
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“Blut ist ein ganz besonderer Saft“

Goethe 1808

“Blood is a very special juice“

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