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Autologous conditioned serum in equine and human orthopedic therapy: A systematic review

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ABSTRACT

This systematic review aims to compile and present information of studies evaluating the effectiveness of autologous conditioned serum (ACS) in the healing of tendon, ligament and articular lesions in humans and horses. A systematic search of articles using Medline, PubMed, Embase, Bireme and Google Scholar was conducted up to August 2020. Studies regarding ACS' use in human orthopedic lesions were included if classified as RCTs, cohort and case-controls. All studies regarding this therapy in equine medicine were included given their scarcity. Pre-clinical experimental studies were selected if controlled. A total of 1474 results were found; 126 articles were fully accessed, and 28 studies met the inclusion criteria. *In vitro* studies failed to demonstrate consistent positive properties and effects, while most clinical trials and observational studies indicated a beneficial response associated with ACS administration. However, RTCs and observational studies presented together mostly an unclear to high risk of bias, with only a few being considered of low risk. In face of the observed inconsistencies, the use of ACS in the treatment of musculoskeletal lesions, although safe, promising and appealing, still cannot be recommended without due caution. Overcoming these incongruences will demand efforts to construct well-designed studies and to regard ACS as an autologous product that encompass a diverse composition.

1. Introduction

Musculoskeletal lesions are commonly associated with high physical demands. Injuries in joints, tendons or ligaments greatly impact on common daily activities and athletic performance of human and equine subjects. Such tissues have demonstrated an intrinsic poor healing potential with inadequate reorganization and usual relapse of the original lesions. The current scarcity of effective proven therapies for their treatment poses a challenge for clinicians and raise the focus for research in this field (Ziltener et al., 2012; Dehghani and Rodeo, 2019).

The use of blood-derived products as therapy for musculoskeletal lesions has emerged as a type of regenerative medicine that aims to control the degenerative disease process and restore the structural and functional capacity of tissues (Dehghani and Rodeo, 2019). These therapies intend to supply the demand for cost-effective, efficient and safe forms of treatment and although not well established yet, have been explored by clinicians, regardless of conflicting data on their therapeutic potential.

The employment of autologous blood preparations relies in the fundamentals of exploring the beneficial mechanisms of body's natural response to tissue damage. Autologous conditioned serum is a cell-free product harvested after the exposition of blood to activating surfaces, which prompt the production of several anti-inflammatory cytokines and growth factors by mononuclear cells and degranulated platelets (Fjordbakk et al., 2015; Evans et al., 2016; Strümper, 2017; Geburek et al., 2015). Reported as the major anti-inflammatory cytokine in the ACS, the interleukin-1 receptor antagonist (IL-1Ra) is a natural inhibitor of the interleukin-1, which has been implicated as the key pro-inflammatory mediator of several pathologic conditions (Evans et al., 2016). Other anti-inflammatory cytokines, such as IL-10 and IL-4 and growth factors as insulin-like growth factor 1 (IGF-1) and transforming growth factor β (TGF-β), to name a few, are also found in high concentrations in ACS (Evans et al., 2016; Genç et al., 2018). In addition, antioxidant properties regarding this therapy were described by Brossi et al. (2012) where a decrease in the production of free radicals by synovial fluid cells treated with interleukin-1 receptor antagonist protein (IRAP) was demonstrated *in vitro* (Brossi et al., 2012).

Although beneficial effects have been associated with this treatment

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in a considerable amount of studies, results are not unanimous and occasionally, conflicting. A systematic review of RCTs, observational and pre-clinical studies performed in human and equine medicine was conducted to assess the ACS' effectiveness in the treatment of tendon, ligament and joint lesions. Furthermore, evidence was critically evaluated in order to assist clinicians in their choice of treatment for orthopedic disorders.

2. Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement published by the CONSORT group (Moher et al., 2009). A broad literature search was conducted up to August 2020 for all relevant articles addressing the therapeutic use of ACS in articular, tendinous or ligamental lesions in humans and equine. Articles were included if written in English, French, German, Spanish or Portuguese. The database of Embase, Bireme, Medline, PubMed, and Google Scholar were consulted, searching the terms "autologous conditioned serum" or "ACS" AND "horses" OR "equine" OR "humans". The reference lists of the selected articles were also examined for identification of further studies. Articles where ACS was used in conjunction with other blood-derivatives or targeting tissues other than the ones described above or in other medical fields (*e.g.* reproduction) were excluded. Studies testing products with similar function or composition of ACS were not included if not derived from blood.

Pre-clinical studies (*in vivo* and *in vitro)* investigating the ACS' effects in the target tissues were included if controlled. Clinical trials performed in human species were included if double-blinded and randomized (RCTs). Cohort studies with a control group describing ACS therapy in these subjects were also admitted (Fig. 1). Abstracts presented at conferences or oral communications and other study designs not described above were not included.

Due to the paucity of equine clinical research currently available in this area, studies were included regardless of their design or evidence level.

Primarily, all titles generated by the literature search up to August 2020 were analyzed for selection of the articles eligible for full-text review. Studies that did not fit in the inclusion criteria were excluded and after eliminating duplicates, relevant data was extracted from elected articles and organized in tables. Selection was made by P.K.A.T. and R.Y.A.B. If any disagreement was found, a discussion between the authors, including P.M.B. was made. The results were further identified as positive (+), partially positive (±) or negative/impartial (−) regarding its statistically significant findings about ACS effects.

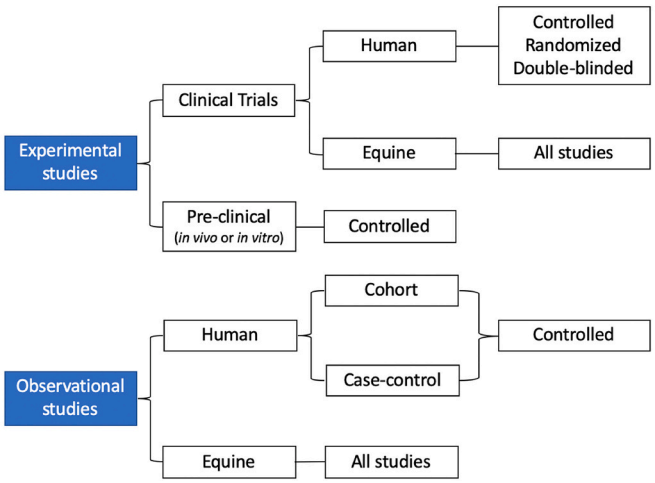

Fig. 1. Inclusion criteria according to study design.

In order to grade the risk of bias, included RCTs were analyzed with the Cochrane Risk of Bias 2.0 tool which classifies its items as "low" (green), "unclear" (yellow) or "high risk" (red). According to the tool's instructions, a final criteria is defined as "low risk" if the risk is considered low at all items. When there is one or some items classified as "unclear", the final risk of bias is considered "unclear". Finally, if the majority of items are classified as "unclear" or if at least one item is classified as "high risk", the final criteria of the study is "high risk of bias" (Sterne et al., 2019). For the included observational studies, risk of bias was evaluated with the MINORS tool, in which topics are graded as 2 = reported and appropriated (green); 1 = reported but unappropriated (red); and 0 = not reported (yellow). In the MINORs tool, total scores of 13–16 for studies that did not include a control or comparative group were considered of low risk, while total scores of 12 or less were considered high risk (Slim et al., 2003; Ajrawat et al., 2019). For studies including control groups, total scores of 19–24 were considered of low risk and scores of 18 or less, considered high risk. Pre-clinical studies were not graded.

Two main tables were constructed in order to accommodate studies investigating ACS therapy for articular tissues and soft tissues (tendon and ligament) (Tables 1 and 2).

3. Results

A total of 1474 titles were found in the systematic search, of which 407 were from PubMed, 190 from Medline, 408 from Google Scholar, 244 from Embase and 225 from Bireme. After the screening of titles and abstracts, 1179 articles were excluded due to lack of correlation with the investigated tissues and therapy and 295 articles were further analyzed. The removal of duplicates led to 126 studies which were evaluated in full text and after applying the inclusion criteria of studies' design, 27 articles were selected. One experimental research investigated the ACS' effects *in vitro* and *in vivo* and therefore, it was considered as two separated studies, leading to a total of 28 studies analyzed in this systematic review (Rutgers et al., 2010) (See Fig. 2).

In order to facilitate results' analysis, studies were gathered according to tissue type in which ACS effectiveness was investigated. There were 20 studies investigating ACS in articular tissues and 9 articles assessing ACS therapy in soft tissues (ligamental and tendinous). One study evaluated articular and soft tissues together and it was included in both analysis (Schneider and Veith, 2013).

3.1. ACS therapy in articular tissues

3.1.1. Studies' characteristics

The effects of ACS therapy in articular tissues and their summarized data are presented in Table 1. Among the selected articles, six were pre-clinical researches (Frisbie et al., 2007; Rutgers et al., 2010; Carlson et al., 2013; Garbin, 2017; Alvarez et al., 2020), seven were randomized clinical trials (RCTs) (Yang et al., 2008; Baltzer et al., 2009; Darabos et al., 2009; Darabos et al., 2011; Lasarzik et al., 2018; Hashemi et al., 2019; Hashemi et al., 2020), and seven were observational studies (Weinberger, 2008; Chiaradia et al., 2012; Schneider and Veith, 2013; Tatarniuk, 2015; Warner et al., 2016; Zarringam et al., 2018; Marques-Smith et al., 2020). There were 11 articles involving horses or articular tissues harvested from this species and 9 studies employing human subjects or cartilage explants from human osteoarthritic joints.

Quality assessment by the Cochrane RoB 2.0 tool for RCTs demonstrated that three RCTs were considered as "low risk" and four were classified as "unclear risk" (Fig. 3). In the observational studies assessed by the MINORs tool, only two were considered as "low risk" while five were considered as "high risk" (Fig. 4).

The ACS acquisition was achieved through the Orthokine® product in 14 studies and the "according to manufacturer's instructions" or 24-h incubation protocol was followed by 10 of them (Frisbie et al., 2007; Yang et al., 2008; Weinberger, 2008; Darabos et al., 2009; Darabos et al.,

Table 1
Studies investigating ACS therapy in articular tissues (1/7).

Author/year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Frisbie et al., 2007	Experimental controlled *in vivo*	Horses; n = 16	OA middle carpal joint - chip fracture (Experim. Induced)	6 ml ACS 4 inj. on days 14, 21, 28 and 35 after surgery	6 ml PBS (placebo) solution 4 inj. on days 14, 21, 28, 35 after surgery	70 days	Outcome assessors	Autologous Orthokine - incubation 24 h - Following manufacturer's directions [] IL-1Ra increased [] of IL-1Ra using mouse ELISA assay - signif. Greater (modestly higher) than unconditioned serum	Clinical and radiologic evaluation; Synovial fluid analysis (TP, cytology, total WBC, aspect, mucin clot, GAG, PGE2, IL-1Ra); Histologic examination (HE, SOFG), GAG, $35SO_4$ incorporation; Gross evaluation post-mortem.	Clinical examination: Signif. ↓ of lameness of ACS group compared to placebo. SF Analysis: signif. ↑ neutrophils in ACS treated group. GAG and PGE2 [] of the ACS treated joints slightly ↓ but not statistically significant. IL-1Ra [] showed signif. ↑ at days 35 and 70 when ELISA mouse antibody was used. Gross pathology: The total score in ACS was better, but this difference was not significant. Histologic examination: signif. ↓ inintimal hyperplasia of synovial membrane of OA joints in ACS group. No signif. Changes btw groups in other topics.	Poor ACS characterization.	±
Yang et al., 2008	RCT	Human *n* = 167	KOA KL I-III	ACS 2 ml 6 inj. On days 0, 3, 7, 10, 14 and 21	Physiological saline 2 ml 6 inj. On days 0, 3, 7, 10, 14 and 21	12 months	Patients and outc. Asses.	Orthokine incubation 24 h	Patient reported outcomes: VAS for pain, the KOOS [pain, stiffness, function, sport and Quality of Life (QoL)] and the KSCRS. At 3, 6, 9 and 12 months after the first injection. Surgeon Physical examination for completion of KSCRS and check for adverse events and changes in NSAIDS.	ACS group resulted in significantly more improvement for KOOS symptomatology and KOOS sport, as compared to placebo. Primary efficacy objective to demonstrate 30% superiority of the Orthokin treatment on the WOMAC was not met, but absolute values of the WOMAC and most other outcome measures showed that ACS treated patients scored consistently better. ACS induced an improved knee function as measured by the surgeon on the KSCRS.	Outcome measures limited; poor ACS characterization	±
Weinberger, 2008	Case series	Horses *n* = 262	OA 110 DIP joints; 87 fetlock joints; 26 carpal joints; 33 hock joints and 6 hip joints	ACS 2–3 inj. 8–12 days interval	no control	12 weeks	x	Orthokine 24 h incubation + centrifugation at 3700 rpm for 10 min	Lameness evaluation (AAEP score system) at 6 and 12 weeks.	199 horses were lame free after 6 weeks. Further 22 showed improvement of lameness at this timepoint. 178 horses were still lame free at week 12 and were back to normal training.		+

Studies investigating ACS therapy in articular tissues (2/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Baltzer et al., 2009	RCT double-blinded	Human *n* = 376	KOA KL II-III	ACS 6 inj. 2 ml Two inj. Per week over 3 weeks *n* = 134 HA inj. 2 ml one inj. Per week	Saline inj. 2 ml one inj. Per week over 3 weeks *n* = 107	6 months and 2-years	Patients and outc. Asses.	Orthogen incubated and centrifuged (no further specifications)	Patient reported outcomes: VAS for pain, WOMAC (dimensions of pain, stiffness and physical function), SF-8 health	ACS group scored signif. Better than either HA or saline on all WOMAC subscales at all time points after the injections. VAS	Differences in protocols;	+

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Table 1 (*continued*)

Studies investigating ACS therapy in articular tissues (2/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
				over 3 weeks *n* = 135					related quality of life, and GPA of treatment efficacy at baseline, and at weeks 7, 13 and 26.	ratings at weeks 7, 13, and 26 were lowest in the ACS group. In all SF-8 HRQL dimensions and component scores, ACS treatment was associated with the largest improvement. Treatment with ACS results in a signif. Better therapeutic effect compared to HA and saline not only at 6 months, but also at 2 years. GPA scores were the only domain where ACS did not show better results compared to saline and HA.		
Darabos et al., 2009	RCT double-blinded	Human *n* = 20	Traumatic rupture of ACL followed by reconst. and knee arthrosis	ACS inj. 2 ml on the day of surgery and post op. Days 1, 6 and 10 n = 10	Physiological solution 2 ml on the day of surgery and post op. Days 1, 6 and 10 *n* = 10	10 days	Patients and outc. Asses.	Autologous ACS - Orthokine according to the manufacturer's instructions.	IL-1β [] in peripheral circulation on day 0, 1, 6 and 10. Intra-articular IL-1β [] circulation on day 1, 6 and 10.	ACS group had a steady ↓ in IL-1β levels over time. At 10 days values were lower than those reported for normal joints and signif. Lower than in control group.	small n;	+
Rutgers et al., 2010	Experimental controlled *in vivo*	Human *n* = 22	KOA KL- III-IV	ACS 6inj. 2 ml At days 0, 3, 7, 10, 14 and 21. n = 22 analysis only performed in *n* = 14 patients	SF cytokine [] before treatment	21 days	x	Autologous Orthokine - Orthogen incubated for 6 h and centrifugated at 1.000 x g for 10 min ACS [] of IL-10 and IL-1ra, TGF-β1, IL-6 IL-1β, OSM and TNF-α were ↑ and OPG ↓ compared to unconditioned serum.	SF cytokines measured were IL-1Ra, IL-1β, TGF-β1, IL-4, IL-6, IL-10, IL-13, IFN-γ, TNF-α, OSM and OPG.	No signif difference in cytokine profile of synovial fluid before and after ACS treatment was noted.	Incubation time differs from the usual recommendations	–

Studies investigating ACS therapy in articular tissues (3/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Rutgers et al., 2010	Experimental controlled *in vitro*	1) KOA cartilage explants *n* = 48 2)KOA cartilage explants n = 24	KOA KL- III-IV	1) ACS 25% n = 24 2) a) ACS 25% n = 8 b) ACS 25% + Ettanercept *n* = 8	1)Unconditioned serum 25% *n* = 24 2) Unconditioned serum 25% n = 8	16 days	x	Allogenic - healthy donors Orthokine - incub. For 6 h and centrif. at 1.000 x g for 10 min. ACS [] of IL-10 and IL-1ra, TGF-β1, IL-6 IL-1β, OSM and TNF-α were ↑ and OPG ↓ compared to unconditioned serum.	1) proteoglycan metabolism 2) 35S incorporation: medium release of proteoglycans and newly synthesized on days 4, 8, 12 and 16. DNA and GAG content on day 16	1) Proteoglycan metabolism of cartilage explants: PG release, PG content at the end of the culture and 35S incorporation showed no difference btw groups at any timepoint. 2) Addition of etanercept to conditioned serum or control serum did not alter PG release, PG incorporation and final PG or DNA content after culture.	Incubation time differs from the usual recommendations	–
Darabos et al., 2011	RCT double-blinded	Human *n* = 62	Tunnel widening after ACL reconstruction	2 ml ACS 4 inj. On days 0, 1, 6 and 10 n = 31	2 ml Physiologic saline 4 inj. On	12 months	Patients and outc. Asses.	ACS - Orthokine according with the manufacturers' recommendations.	CT scans on postop. Day 1, and 6 and 12 months. Intraarticular and serum	CT scans showed that tunnel widening in the ACS group is only half	short follow-up	+

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Table 1 (*continued*)

Studies investigating ACS therapy in articular tissues (3/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
					days 0, 1, 6 and 10 $n = 31$				IL-1β [] on postop. Day 1, 6 and 10. WOMAC and IKDC 2000 at baseline, and 6 and 12 months after surgery. Adverse events and changes in analgesic use.	that in the placebo group after 6 and 12 months. SF [] IL-1β continuously ↓ until day 10. WOMAC and IKDC 2000 in ACS-treated patients were consistently better with the lowest pain scores. ACS resulted in signif. More improvement in WOMAC stiffness scores compared with placebo. Clinical examination IKDC 2000/Surgeon found better results in ACS group with ↓ effusion and better functional tests at 6 months and results were still better at 12 months. ROM signif. Better in ACS group.		
Chiaradia et al., 2012	Case series	Horses $n =$ 10	OA (MCPJ/MTPJ)	3–5 ml ACS 4 inj. 7–10 days intervals	x	40 days	x	ACS – Orthokineincubation for 24 h + centrif. At 2100 ×g for 10 min	SF proteomic analysis before and at last inj.	↓ of acute phase proteins (C4A, CE-D1, α2MG, CP, ST and APO-A1) 7/10 ACS treated horses returned to their athletic activity	Short follow-up; small n;	+
Schneider and Veith, 2013	case series	Horses $n =$ 36 lesions = 37 (one horse bilateral)	Cartilage damage (OCD, OA, Arthritis) $n = 19$ Soft tissue disorders (Sesamoidosis, tendinosis, susp. Lig. Damage) n = 18	GOLDIC 4 inj. Approx. 4 ml On Day 7, the horses began a strenuous exercise regimen five days per week for the remaining 24 weeks of the study.	x	24 weeks	x	Goldic according to manufacturer's guidelines	Lameness evaluation (AAEP score system), swelling and/or effusion graded by 5-point scale (0 = not present and 5 = severe swelling/ effusion) before treatment and at week 1, 2, 3, 12 and 24.	significant reduction of lameness, effusion (osteoarthritis group), and swelling (soft tissue disorders group) within 2 weeks of treatment was found. Up to 3 and 6 months after treatment, all horses were free of symptoms.	lack of control group, different types of lesions included	+

Studies investigating ACS therapy in articular tissues (4/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Carlson et al., 2013	Experimental controlled *in vitro*	Horses n = 6 Chondrocyte pellets $n = 46$	Chondrocyte pellets stimulated with rhIL-1β	ACS n = 23 on days 2 and 5 10% ACS + rhIL-1β 20% ACS + rhIL-1β	AES $n = 23$ on days 2 and 5 10% AES 10% AES + rhIL-1β 20% AES + rhIL-1β	6 days	x	AES: Blood into borosilicate glass tube containing clot activator + centrifugation. ACS - IRAP II - Arthrex according to manufacturer's instructions.	Measurement of pellet GAG synthesis, total GAG content, total DNA content, medium MMP-3 activity, medium IGF-I	ACS lead to an ↑ in culture medium IL-1RA [] but did not lead to a direct effect on *in vitro* proteoglycan cartilage metabolism. ACS had no significant effect on	small n; do not fully replicate OA; limited outcome measurements	−

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Table 1 (*continued*)

Studies investigating ACS therapy in articular tissues (4/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
									and IL-1Ra [], pellet mRNA content.	MMP-3 mRNA expression, compared with AES.		
Tatarniuk, 2015	Case series	Horses $n = 11$	OA of DIP joint unilateral	4 ml ACS 3 inj. Weekly intervals No anti-inflammatory medications or joint supplements were given.	x	21 days	Outcome assessors	Arthrex® IRAP II incub. 18-20 h and centrif. For 10 min at 1.800 xg Comparison of ACS x 1-h and 19-h incub. ACS Serum IL-1ra [] not signif. Different from 19-h control serum; ACS signif. ↓ IL-4; IL-8 and IL-6; Ratios of MMP-1 to TIMP-1, −2, −3, −4 and MMP-9 to TIMP-1, −2, −4 were the only ratios ↑in ACS compared to controls. Marked variability between horses in IL-1ra [] in ACS	Blind graded (by 3 different assessors) lameness videos based on AAEP; SF IL-1β, IL-4, IL-6, IL-8, IL-10 and TNFα; MMP-1, MMP-3, MMP-9, and MMP-13; TIMP-1, TIMP-2, TIMP-3, and TIMP-4	SF cytokine concentrations: SF only had 1% of the IL-1ra present in the ACS, 7 days after the ACS injection. There was no significant change in concentrations of any cytokine, MMP, TIMP or MMP:TIMP ratio when comparing post ACS treatment synovial fluid to baseline. Lameness: no signif. Changes detected	short follow-up	−
Warner et al., 2016	Retrospective cohort study	Horses n = 26	OA DIP joint	2 ml - 6 ml of ACS 2–4 inj. 7–21 days intervals *The number of injections and the amount of ACS used per injection depended on the amount of serum obtained.	x	2 years	x	Irap - orthokine	Success of treatment determined by patient's files and owner survey by telephone	Treatment success: in 8 of 26 horses (31%) the ACS therapy was successful. The therapy was partially successful in four (15%) and unsuccessful in 14 horses (54%).	Limited outcome measurements	+

Studies investigating ACS therapy in articular tissues (5/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Garbin, 2017	Experimental controlled *in vitro*	Horses n = 16 (Art. Cartilage n = 8 Synovium n = 8)	Stimulation of explants with IL-1β	IL-1β + 10% *v*/v IL-1β + 30% *v*/v for each treatment ACS Frozen; FD and FFD *n* = 4 Allogenic CS Frozen; FD and FFD n = 4 Treat. applied at Day 0 and Day 4	Cartilage and synovium explants cultured with ITS or FBS exposed to IL-1β	10 days	x	ACS prod. at their lab. 18–24 h incub. centrif. at 4.000 rpm for 10 min Sample from each horse separated to produce frozen, FD and FFD. Allogenic CS: ACS from 4 horses polled together	Total GAG content in media and in cartilage; 35SO4-labeled proteoglycans quantification in media and in cartilage; Gene expression ADAMTS-5, ADAMTS-4, MMP-1, COX-2, IL-1β in cartilage. Gene expression of COX-2 and IL-1β in synovium	Compared to controls, CS treats. Were not effective in protecting cartilage from the catabolic stimulus induced by IL-1β. Allogenic x Autologous CS showed differences in media but not in cartilage. Author suggests similar effects of Autologous and Allogeneic CS. CS did not signif. Interfere with gene expression of ADAMTS-5 and ADAMTS-4 in cartilage explants. MMP-1 on the other hand, was strongly up-regulated (up to 101-fold). CS did not have a signif. Effect in expression		−

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Table 1 (*continued*)

Studies investigating ACS therapy in articular tissues (5/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Lasarzik et al., 2018	RCT	Horses n = 12	Advanced OA (OCD, Traumatic Arthritis and DJD) Joints included: MCPJ; MTPJ; TCJ; MFTJ.	1) ACS 3 inj. At weekly intervals; n = 6 2) ACS 3 inj. At 2-day intervals; n = 6 Treatments started 14 days after arthroscopy; Volumes of ACS varied according to joint type	x	42 days	x	ACS - ABPS Arthrex prepared following manufacturer's instructions. [] of IL-Ra showed a wide range; and IL-1β consistently lay under detection limit	Daily clinical and orthopedic examination until the end of the ACS treatment period. Synovial Fluid: Group 1: SF collected before each injection, 1 h after each injection and 42 days after the start of the treatment. Group 2: SF collected before each injection, 4 h after each injection and 42 days after the start of the treatment. Quantification of IL-Ra and IL-1β in the SF; Quantification of C12C, CS 846 and CPII in the SF before and 42 days after the start of the treatment in both groups.	of COX-2 and IL-1β in cartilage. No differences were shown in the synovium explants. Increases of SF [] IL-1ra showed positive correlation with the ACS IL-1ra []. Injections of ACS increased [] of IL-1ra in the SF at 1 and 4 h, but these concentrations decreased back to baseline values within 48 h. Authors suggested that half-life of IL-1ra lies between 4 and 48 h after ACS inj. The 2-day treatment interval showed better results with decrease in SF IL-1ra and IL-1β [] as well as C12C, CS 846 and CP II biomarkers 42 days after the first inj. No changes were found in the weekly treatment intervals group at the same time. ACS treatment given at two-day intervals might indicate an improvement in joint inflammation and cartilage degrading processes on a long-term effect.	no control group; different joints included; different etiologies of articular lesions; no info about the orthopedic exams that were cited in methods - selection bias?	±
Zarringam et al., 2018	Prospective cohort study	Human n = 126	KOA KL I-III	Orthokine 6 inj. On days 0, 3, 7, 10, 14 and 21; n = 72	Placebo Saline 6 inj. On days 0, 3, 7, 10, 14 and 21; n = 54	10 years	x	Orthokine 24 h incubation	Patients were approached by phone or letter and provided informed consent. For non-responders the electronic health reports (EHR) were evaluated.	At the end of this follow-up, 46.3% of the placebo and 40.3% of the Orthokin group had been treated surgically - not statistically significant different.	Did not take non-surgical treatment into account	−

Studies investigating ACS therapy in articular tissues (6/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Hashemi et al., 2019	RCT double blinded	Human n = 60	KOA KL II-III;	2 ml ACS 3 inj. 7-day intervals n = 30	2 ml HA 3 inj. 7-day intervals n = 30	6 months	Patients and outc. Asses.	Orthokine incubated at 37 °C and transferred to a laboratory within 24 h.	KOOS and WOMAC to assess pain, symptoms, daily activities, sport-recreational performance, and knee related quality of life. Performed before treatment and 6 months after last injection.	Improvement of KOOS mean scores of symptoms, daily activities, and sport-recreational activities in the ACS group compared to HA. No differences in WOMAC or VAS scores btw groups.	limited outcome measures;	±
		co-cultured cartilage and	co-cultured cartilage and	TA n = 2; ACS 25% n = 2; ACS	IL-1β stimulated control explants	96 h	x	ACS - Orthokine 24 h incub. + centrif. at 3000 RCF 10 min	PGE2 [] in culture media; Gene expression 18 s,	APS 50% v/v was the most effective at ↓ PGE2 [] in media.		±

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Table 1 (*continued*)

Studies investigating ACS therapy in articular tissues (6/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Alvarez et al., 2020	Experimental controlled *in vitro*	synovium explants n = 12	synovium explants stimulated with IL-1β	50% *n* = 2; APS 25% n = 2; APS 50% n = 2;	without treatment n = 1			APS - ProStride APS↑ WBCs, RBCs, PTLs compared to ACS. ACS ↑ TGF-β and sTNF-R1 compared to APS. IL-1ra: IL-1β ratio of ACS ↑, but not signif. Than APS	SCAMP3 (reference genes), IL-1β, MMP-1, MMP-3, MMP-13, IL-6, IL-8, IL-10, and ADAMTS-4 (in synovial membrane) and GAPDH and SCAMP3 (reference genes), type II collagen (COL2A1), aggrecan (ACAN), TNF-α, IL-1β, and ADAMTS-4 (in articular cartilage)	TA was more efficient at ↓ IL-1β expression in the synovial membrane; ACS and APS produced a stronger anti-inflammatory effect, modulating pro inflammatory cytokines (IL-1β and TNF-α) involved in cartilage destruction in OA. ACS and APS, showed a chondroprotective effect by ↑ matrix gene expression, while TA treatment did not modify gene expression. Both ACS and APS significantly ↓ PGE2 in media compared to TA, which could be one of the reasons horses with naturally occurring OA show improvement in lameness after treat. With ACS or APS.		
Hashemi et al., 2020	RCT double blinded	Human n = 60	KOA KL I-III	2 ml ACS 4 injections 7-days intervals n = 30	VAS, KOOS and WOMAC scores before treatment; ozone (30 μg/ml) + 5 ml of lidocaine 1% 3 inj. 1 month intervals n = 30	6 months	Patients and outc. Asses.	Orthokine incubated at 37 °C and transferred to laboratory in 24 h.	KOOS (pain, symptoms, daily activities, athletic and recreational functions, and knee-related quality of life) + WOMAC (pain, stiffness, and physical function) completed by patients before treat. And 6 months after last injection. VAS level of pain by patients before, 1 and 3 months after last injection.	VAS: no differences at 1 and 3 months after initiation treatment but statistically significant improvement in pain levels in the ACS group 6 months after treatment. KOOS scores of symptoms, daily activities, and athletic and recreational functions significant higher in ACS group six months after treatment compared to ozone group. WOMAC: pain scores not signif. Different btw groups but other scores (physical function and joint stiffness) signif. Higher in ACS group No complications reported.	Differences in protocols;	±

Studies investigating ACS therapy in articular tissues (7/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Marques-Smith et al., 2020	Prospective cohort study	Horses *n* = 20	naturally occurring low-grade	ACS 3 inj. 15 days interval (mean); *n* = 20	x	43 days (mean)	x	ARTHREX ABPS Accord. to manuf. Instructions; 22-24 h incub. + centrif. 4000 rpm for 10 min. ACS content of IL-1Ra, IGF-1 and TGF-β. IL-10, IL-1β	AAEP lameness score for lameness evaluation at baseline	58% of included horses responded to ACS treatment, and that these horses had higher ACS levels of IL-1Ra and IGF-1 than non-	small n; inclusion of different joints; not standardized treatment and	±

(*continued on next page*)

Table 1 (*continued*)

Studies investigating ACS therapy in articular tissues (7/7)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations
			OA; multiple joints;					and TNF-α [] ↓below or near the lower limit of detection and were therefore omitted from statistical analyses.	lameness and after flexion test	responders; joint effusion was subjectively improved in 8 horses; unchanged in 10 and more pronounced in 1 horse.	reevaluation intervals; no control group

+ = Positive; ± = Partially positive; – = negative/no changes regarding ACS effects; [] = concentrations; AAEP = American Association of Equine Practitioners; ACAN = aggrecan; ACL = Anterior cruciate ligament; ACS = autologous conditioned serum; ADAMTS = a disintegrin and metalloproteinase with thrombospondin motifs enzyme; AES = Autologous equine serum; APO-A1 = apolipoprotein A-I; APS = autologous protein solution; C12C = Catabolic collagenase-cleaved type II collagen epitope; C12C = Catabolic collagenase-cleaved type II collagen epitope; C4A = Complement component C4A; CE-D1 = carboxylesterase D1; COL2A1 = type II collagen gene; COX = Cyclooxygenase; CP = Ceruloplasmin; CPII = anabolic procollagen II C-propeptide; CS = conditioned serum; CS 846 = Aggrecan chondroitin sulfate 846 epitope; CT = computed tomography; DIP = distal interphalangeal joint; DJD = degenerative joint disease; FBS = Fetal bovine serum; FD = freeze-dried; FFD = filtered freeze-dried; GAG = glycosaminoglycan; GPA = Global patient assessment; HA = Hyaluronic acid; HE = Hematoxylin & eosin staining; HRQL = Health related quality of life; IFN-γ = Interferon gamma; IGF-1 = insulin-like growth factor 1; IKDC 2000 = International Knee Documentation Committee 2000; IL = Interleukin; IL-1ra = interleukin 1 receptor antagonist; IL-1β = interleukin 1 beta; ITS = Insulin transferrin-selenium; KL = Kellgren-Lawrence classification; KOA = knee osteoarthritis; KOOS = Knee and Osteoarthritis Outcome Score; KSCRS = Knee Society Clinical Rating System; MCPJ = Metacarpophalangeal joint; MFTJ = Medial femorotibial joint; MMP = Matrix metalloproteinases; MTPJ = Metatarsophalangeal joint; NSAIDS = Non-steroidal anti-inflammatory drugs; OA = osteoarthritis; OCD = osteochondritis dissecans; OPG = Osteoprotegerin; OSM = Oncostatin M; PBS = phosphate buffered saline; PG = proteoglycan; PGE2 = prostaglandin E2;PLT = platelets; Post op. = postoperative; RBC = red blood cells; RCT = randomized clinical trial; ROM = range of motion; rpm = revolutions per minute; SF = synovial fluid; SF-8 = Short form 8; SOFG = Safranin-O fast green; ST = serotransferrin; sTNF-R1 = soluble tumor necrosis factor R1; Susp. Lig. = suspensory ligament; TA = triamcinolone; TCJ = Tarsocrural joint; TGF- β1 = Transforming growth factor beta 1; TIMP = Tissue inhibitors matrix metalloproteinases; TNF-α = Tumor necrosis factor alpha; TP = total protein; VAS = Visual analogue scale; WBC = white blood cells; WOMAC = Western Ontario and McMaster Universities Osteoarthritis index; xg = G force; α2MG = Alpha2 macroglobulin;

2011; Chiaradia et al., 2012; Zarringam et al., 2018; Hashemi et al., 2019; Alvarez et al., 2020; Hashemi et al., 2020). Incubation period of 6-h was employed only by Rutgers et al. (2010) in both *in vitro* and *in vivo* investigations and two authors did not describe their full protocol (Baltzer et al., 2009; Warner et al., 2016). The Arthrex® product was employed in four studies and protocols were followed according to instructions in two of them (Carlson et al., 2013; Lasarzik et al., 2018), while periods of 18-20 h or 22-24 h of incubation were employed by two authors (Tatarniuk, 2015; Marques-Smith et al., 2020). In one study, ACS was produced in the university's lab in a method similar to commercial products in which 18-24 h of incubation was performed (Garbin, 2017). Finally, the Goldic® was employed in one study and protocol followed manufacturer's instructions (Schneider and Veith, 2013).

Investigated lesions in horses included a range variety of joints (distal interphalangeal, fetlock, carpal, hock, stifle and hip) and etiology (experimentally induced chip fractures, osteochondritis dissecans, osteoarthritis, traumatic arthritis). The grades of lesions were mostly mild and moderate, while advanced osteoarthritis (OA) was only found in one study (Lasarzik et al., 2018). Sample sizes were often limited in comparison with human studies (10 to 26 horses), but one cohort was able to include 262 horses (Weinberger, 2008). Age ranged from 1 to 23 years old and treatments were followed up to a minimum of 21 days and a maximum of two years among studies. Only one author managed to include a control (placebo) group which granted the study the best design (experimental controlled *in vivo*) (Frisbie et al., 2007). This study included horses of similar age and standardized grade and etiology of lesions. The remaining research articles with horses showed higher risk of bias since they were mostly designed as cohort and case series.

In human population, all included studies evaluated the ACS' effects in the knee joint and OA (Kellgren-Lawrence grade I-III) was the major lesion among them. There were only two authors that investigated different type of lesions: knee arthrosis (up to grade 1) with chondral lesion (up to grade 2) caused by anterior cruciate ligament (ACL) rupture; and tunnel widening after ACL reconstruction (Darabos et al., 2009; Darabos et al., 2011). The average age of patients was generally 50–60 years old with only few studies including younger patients (Darabos et al., 2009; Darabos et al., 2011). Sample sizes were considered small in two studies (20 and 22 participants included) (Darabos et al., 2009; Rutgers et al., 2010), and sufficient in six studies (60, 60, 62, 167, 376 and 126 included patients) (Yang et al., 2008; Baltzer et al., 2009; Darabos et al., 2011; Zarringam et al., 2018; Hashemi et al., 2019; Hashemi et al., 2020). The follow-up period ranged from 10 days to 12 months in RCTs, but six months was the most employed interval for investigation. Among other study designs, one *in vivo* study investigated effects of ACS therapy up to 21 days after treatment (Rutgers et al., 2010) and one prospective cohort followed patients up to 10 years (Zarringam et al., 2018). Regarding studies' design, the majority were RCTs and included control (saline; PBS) or comparative groups (hyaluronic acid; ozone injections) (Baltzer et al., 2009; Hashemi et al., 2019; Hashemi et al., 2020) and higher quality in design was found when comparing to studies in equine species.

In the *in vitro* experiments, chondrocyte pellets, cartilage explants, synovium explants and co-culture of cartilage and synovium were employed. Stimulation of tissues with IL-1β was performed in three experiments in order to induce inflammation (Carlson et al., 2013; Garbin, 2017; Alvarez et al., 2020). In one experiment, cartilage tissues were harvested from osteoarthritic joints (KOA, KL III-IV) and therefore, no further stimulation was needed (Rutgers et al., 2010) [13]. Follow-up period among *in vitro* studies varied from 96 h to 16 days.

3.1.2. Outcome measurements

3.1.2.1. Lameness in horses. Lameness was evaluated from grades 0 to 5 (AAEP grading system or similar) in five equine studies and a statistically significant improvement of lameness – of 1 to 3 degrees - in the

majority of the horses was reported in all studies (Frisbie et al., 2007; Weinberger, 2008; Schneider and Veith, 2013; Marques-Smith et al., 2020) except one (Tatarniuk, 2015). Studies showing positive results investigated ACS effects on lameness from 43 days to 24 weeks after treatment, while the study that showed no significant changes employed a shorter follow-up of 21 days (Tatarniuk, 2015). It is important to highlight that four out of these five studies were observational studies of routine clinical cases in which no control group was included and that majority of them were classified as high risk of bias.

3.1.2.2. Patient reported outcome measures in humans. In studies of human populations, patient reported outcome measures (PROMs) were the major tools assessing joint pain and function after ACS treatment and in general, significant or at least mild improvements were found.

The WOMAC (Western Ontario and McMaster Universities Osteoarthritis Index) was applied in five RCTs, however, significant improvement after ACS therapy in all of the WOMAC subscales was found by one author only, Baltzer et al. (2009), who compared ACS with hyaluronic acid (HA) and saline over a six months period (ACS 6 injections, 2 per week). HA and ACS were also compared in another study within the same evaluation period, but with a different treatment protocol (ACS 3 injections, 1 per week) (Hashemi et al., 2019); in this case, no significant changes were found in WOMAC. Significant improvements in isolated subscales of the WOMAC (joint stiffness and/or physical activity) were found by two authors (Darabos et al., 2011; Hashemi et al., 2020); and despite absolute values in all WOMAC subscales displayed enhancement in the study constructed by Yang et al. (2008), results were not statistically significant.

In the Visual Analogue Scale (VAS) of pain, statistically significant improvements after ACS therapy were found in two studies (Baltzer et al., 2009; Hashemi et al., 2020), enhancement of pain was found in one study, but not statistically significant (Yang et al., 2008) and no significant differences were observed by Hashemi et al. (2019). Baltzer et al. (2009) reported that besides showing the lowest scores for VAS, the ACS group also had more patients with at least 50% of pain improvement compared to HA and saline. Again, no difference in this score was found by Hashemi et al. (2019) which also compared ACS to HA.

The evaluation of the Knee injury and Osteoarthritis Outcome Score (KOOS) was employed in three RCTs which compared ACS with placebo, HA or ozone (Yang et al., 2008; Hashemi et al., 2019; Hashemi et al., 2020). Improvements in the ACS groups were detected in the subscores of symptoms and sports in all articles and further enhancement on subscores of daily and recreational activities were also found by two authors (Hashemi et al., 2019; Hashemi et al., 2020). No significant differences regarding the subscore of pain of the KOOS were identified.

The Knee Society Clinical Rating Scale (KSCRS) was only assigned by Yang et al. (2008) which found a better outcome for knee function in ACS treated patients, but no significant differences in the other components of this scale.

Darabos et al. (2011) assessed ACS effects with the International Knee Documentation Committee Score (IKDC) 2000 and identified an enhancement in the ACS treated group, however differences were not statistically significant (Darabos et al., 2011).

The Short Form-8 of Health-related Quality of Life (SF-8 HRQL) and the Global Patient Assessment (GPA) were only employed by Baltzer et al. (2009) which associated ACS treated patients with the largest improvement in the SF-8 HRQL, in all of its dimensions, compared to saline or HA. In addition, the GPA in this study showed that a higher percentage of patients reported to be at least satisfied with the ACS treatment.

3.1.2.3. Effusion. The level of effusion was evaluated in three equine studies but results were not consistent (Frisbie et al., 2007; Schneider and Veith, 2013; Marques-Smith et al., 2020). A significant improvement of effusion in horses was reported 24 weeks after ACS treatment in a case series (Schneider and Veith, 2013), however, in another study, designed as cohort, similar improvements could only be demonstrated in 8/20 horses in a shorter follow-up time (43 days) (Marques-Smith et al., 2020). Furthermore, worsening of effusion was presented in one horse and unchanged effusion level was found in the remaining animals of this study. It is important to highlight that no control groups were included in any of these investigations. Only the study performed by Frisbie et al. (2007) managed to include a control group for comparison, and results showed that there were no significant differences between ACS and saline groups in the level of joint effusion over a 70-day period.

3.1.2.4. Synovial fluid content. The articular synovial fluid (SF) content after ACS therapy was investigated in horses or humans in six studies (Frisbie et al., 2007; Darabos et al., 2009; Rutgers et al., 2010; Darabos et al., 2011; Tatarniuk, 2015; Lasarzik et al., 2018). Measured cytokines and pro or anti-inflammatory biomarkers included: IL-1β, IL-4, IL-6, IL-10, IL-13, IL-1Ra, IFN-α, OMS, OPG, TNF-α, PGE_2, GAG, MMP-1, MMP-9, MMP-13, TIMP-1, TIMP-2, TIMP-3, TIMP-4, CP II, CS 846 and C12C.

IL-1β was the most measured cytokine among studies and a significant reduction of its SF concentrations after ACS treatment at 1-h, 4-h, 1-, 6- and 10-days was found in three studies (Darabos et al., 2009; Darabos et al., 2011; Lasarzik et al., 2018). Lasarzik et al. (2018) also found significant decrease in the IL-1β SF concentrations 42 days after a 2-day interval treatment protocol was applied in horses. This result was not the same when a weekly treatment protocol was employed by the same author. In contrast with these findings, Rutgers et al. (2010) and Tatarniuk (2015) could not detect any significant difference in SF IL-1β concentrations after ACS therapy when measurements were performed at days 0, 3, 7, 10, 14 and 21 (in humans), and 0, 7, 14 and 21, (in horses) respectively.

IL-1Ra SF concentrations were measured in three studies (Frisbie et al., 2007; Tatarniuk, 2015; Lasarzik et al., 2018). Frisbie et al. (2007) analyzed synovial fluid of horses between days 0 and 70 on a weekly interval and found that IL-1Ra concentrations fluctuated between days 0 and 28, and then, significantly ($P = 0.005$) increased at days 35 and 70 compared to controls. Lasarzik et al. (2018) aimed to compare the usual weekly ACS treatment protocol employed in horses with a 2-days interval protocol and found that IL-1Ra concentrations were increased at 1 and 4 h after injections but tend to decrease back to baseline values at 48 h and 7 days after ACS application. For the weekly protocol, the IL-1Ra concentrations showed no changes 42 days after treatment but concentrations at the same timepoint within the 2-day treatment protocol showed a significant decrease of this cytokine comparing to baseline values. Finally, Tatarniuk (2015) did not find any significant increases in SF concentrations of IL-1Ra, at any timepoint up to 21 days after ACS treatment in horses.

Only one study reported SF concentrations of OA biomarkers (CP II, CS 846 and C12C) and no significant differences were found after ACS treatment when the weekly-injection protocol was applied (Lasarzik et al., 2018). Again, better results were found when the two-day interval protocol was employed, which demonstrated a significant reduction of these biomarkers 42 days after ACS injection.

In regard to the remaining proteins, no significant differences were found after ACS therapy in any of the studies included in this systematic review.

3.1.2.5. Proteomic analysis of synovial fluid. Proteomic analysis of the synovial fluid of 10 osteoarthritic horses was performed in one study before and at the last ACS injection (protocol of 4 injections, 7 to 10-days intervals) (Chiaradia et al., 2012). It was found that results varied among horses but ACS therapy showed a trend to decrease concentrations of C4A, CE-D1, α2MG, CP, ST and APO-A1 out of the 17 deregulated proteins found in osteoarthritic joints.

3.1.2.6. Diagnostic imaging. One study compared radiographic images

before and after ACS and placebo were employed to treat experimentally induced OA in horses (Frisbie et al., 2007). No significant changes were found in the images 70 days following ACS therapy compared to controls.

Darabos et al. (2011) evaluated tunnel widening in humans – a common complication of anterior cruciate ligament reconstruction - through CT scans of human patients at 1 day, 6- and 12-months following surgery. Intra articular ACS was applied in these patients because synovial fluid that reaches the bone tunnel increases cytokine content within the knee joint, leading to a hostile environment which could also induce osteolysis and tunnel widening (Darabos et al., 2011). In such study, it was found that the tunnel widening in the ACS group was significantly decreased (half size) compared to patients that received placebo injections.

3.1.2.7. Histologic and macroscopic post-mortem evaluation of joints. Macroscopic post-mortem evaluations of OA joints of horses were also performed by Frisbie et al. (2007) 70 days after ACS therapy. It was found that ACS treated joints had a better total score of cartilage erosion and synovial membrane hemorrhage compared to placebo treated OA joints, although these values were not statistically significant.

Following gross evaluation of joints, articular cartilage, synovial membrane and joint capsule were harvested for histological examination in the same study (Frisbie et al., 2007). Significant improvements were only found in the degree of intimal hyperplasia of synovial membrane following ACS therapy. No significant changes were found in articular cartilage morphologic variables.

3.1.2.8. Long-term effects. Among selected studies, two cohort aimed to investigate the long-term effects of ACS treatment. The first article investigated the ability of horses to return to performance after having their distal interphalangeal joint treated with ACS (Warner et al., 2016). In such study, only 8/26 horses were considered to have a successful outcome and 4/26 had partial success of treatment in a 2-year follow-up. Treatments were considered failures when horses became chronically lame, had neurectomy or were euthanized due to its joint lesion. Information about their performance level two years after treatments was acquired through phone interviews with the owners of treated horses.

The second cohort study followed human patients that had previously participated in a clinical trial performed by Yang et al. (2008) (Zarringam et al., 2018). In this cohort, the ability of ACS therapy in preventing the need for surgical treatment in a 10-year period was investigated. Information was obtained by phone, letters or through the electronic health reports (EHR) and results showed that the ACS had no surgery preventive effect compared to placebo. The authors further suggest that it has no clinically relevant disease modifying effect.

3.1.2.9. Articular cartilage metabolism. In order to investigate the effects of ACS in the articular cartilage metabolism, some *in vitro* studies investigated GAG content and $^{35}SO_4$ labeled GAG in media and cartilage tissues – harvested from humans or horses – and treated with ACS and compared results with controls, but no significant changes were observed (Frisbie et al., 2007; Rutgers et al., 2010; Carlson et al., 2013; Garbin, 2017).

3.1.2.10. Gene expression. Gene expression was measured in equine chondrocytes pellets (Carlson et al., 2013), in cartilage explants (Garbin, 2017), in synovium explants (Garbin, 2017) and in co-cultures of cartilage and synovium explants (Alvarez et al., 2020). Gene expression was measured for IL-1β, IL-6, IL-8, IL-10, MMP-1, MMP-3, MMP-13, ADAMTS-4, ADAMTS-5, COX-2, Collagen II, Aggrecan and TNF-α.

In equine chondrocytes pellets, no significant differences were found for COX-2, collagen II, MMP-3 or Aggrecan expressions (Carlson et al., 2013).

In equine cartilage explants, it was found a mild downregulation of ADAMTS-5 and ADAMTS-4 expression (less then 1-fold) comparing to controls, considered not biologically relevant by the author (Garbin, 2017). Furthermore, the author found a substantial upregulation of MMP-1 (up to 101-fold), a mild upregulation of COX-2 (less than 2-fold) and IL-1β (less than 1-fold) expression in explants treated with ACS compared to controls.

In synovium explants, no differences were found in gene expression of IL-1β or COX-2 when comparing treated and untreated explants (Garbin, 2017).

One study compared controls, triamcinolone (TA), autologous protein solution (APS) and ACS in co-cultures of equine synovium and cartilage (Alvarez et al., 2020). When gene expression was measured, it was found that ACS tend only to upregulated IL-10 in synovium and regarding to cartilage tissue analysis, ACS induced a significant downregulation of IL-1β expression (10-fold greater than TA), reduced TNF-α expression and showed trend to upregulate aggrecan (ACAN) and type II collagen (COL2A1) but also ADAMTS-4 expression. In addition, authors indicated that a treatment dose-effect was found with more concentrated solutions of ACS showing greater effects for TNF-α, ACAN, COL2A1 and ADAMTS-4. This *in vitro* experiment was followed for 96 h and inflammation was induced through IL-1β stimulation.

3.1.2.11. Total DNA content. Total DNA content of equine chondrocytes pellets treated with ACS and control was measured in one study (Carlson et al., 2013), however no significant difference was found between groups.

3.1.2.12. Media content. Concentrations of IGF-I, IL-1Ra and MMP-3 activity in media were also measured by Carlson et al. (2013) for equine chondrocytes pellets and results showed no significant differences between groups for MMP-3 activity, however, significant higher concentrations of IGF-I and IL-1Ra were found in medium of ACS treated pellets.

PGE_2 concentrations in media were measured in co-cultures of equine synovium and cartilage by another author (Alvarez et al., 2020) and results showed that PGE_2 concentrations reduced by 4.13-fold in cultures treated with ACS.

3.1.2.13. Adverse events. The presence or absence of adverse events after ACS intra-articular injections were observed in seven studies (Frisbie et al., 2007; Weinberger, 2008; Yang et al., 2008; Baltzer et al., 2009; Darabos et al., 2011; Schneider and Veith, 2013; Marques-Smith et al., 2020). There were no adverse events in one experimental *in vivo* study with 16 horses (Frisbie et al., 2007) and in one case series with 262 horses (Weinberger, 2008). In three studies, only mild to moderate reactions, as local pain or pressure sensations (receding within 48 h or less) were observed in humans and horses (Baltzer et al., 2009; Darabos et al., 2011; Schneider and Veith, 2013). Yang et al. (2008) reported a total of 159 knee-related adverse events in a RCT with 167 human patients, in which most of them was also due to increase in knee pain. These events included patients treated with both placebo or ACS and distribution of adverse reactions was equal between groups. Only two severe reactions were found in this study, both in the ACS group (one septic arthritis – reaction was attributed to the procedure - and one severe inflammation). One severe adverse effect was also observed by Marques-Smith et al. (2020), where 1 out of the 20 included horses developed septic arthritis.

3.2. ACS therapy in soft tissues

3.2.1. Studies' characteristics

A total of 9 included studies explored the advantages of ACS injections in soft tissues, most of them, specifically in tendons. Only one study included ligamental injuries (Schneider and Veith, 2013). There were five experimental pre-clinical studies (Majewski et al., 2009;

Table 2
Studies investigating ACS therapy in soft tissues (1/3).

Author/year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Majewski et al., 2009	Experimental controlled *in vivo*	Sprague Dawley rats *n* = 80	Tendinopathy of Achilles tendon experim. Induced	ACS 3 inj. 24, 48, and 72 hours post op. n = 40	Untreated *n* = 40	8 weeks	Outcome assessors	Allogenic ACS - Orthokine Pooled rat blood incub. For 9 h + centrifuged ↑ signif. TGF- β1 levels, slightly ↑VEGF levels, no changes in PDGF-BB levels	Immunohistochemistry, biomechanical and histologic evaluation, fluorometric assay (Lysil oxidase activity);	Tendons exposed to ACS had greater expression of COL1AI gene, had greater type 1 collagen content, were thicker and regained stiffness and histologic maturity earlier. Maximum load to failure was not significantly affected by ACS.		±
Heisterbach et al., 2012	Experimental controlled *in vivo*	Sprague Dawley rats n = 60	Tendinopathy of Achilles tendon experim. Induced	BMP-12 n = 15 TGF-β1 *n* = 15 ACS 3 inj 24 h-interval n = 15	Control group untreated n = 15	8 weeks	Outcome assessors	Allogenic ACS - Orthokine Pooled rat blood incub. For 9 h + centrif.	Immunohistochemical analysis: Gene expression for bFGF, BMP-12, VEGF and TGF-β1	ACS ↑bFGF significantly after 8 weeks compared to controls and the BMP-12 and TGF-β1 groups; Overall TGF-β1 expression was ↑ in the ACS group compared to controls and the TGF-β1 group.	Short follow up; limited outcome measurements;	+
Schneider and Veith, 2013	Case series	Horses n = 36 lesions = 37 (one horse bilateral)	Cartilage damage (OCD, OA, Arthritis) n = 19 Soft tissue disorders (Sesamoidosis, tendinosis, susp. Lig. Damage) *n* = 18	GOLDIC 4 inj. Aprox. 4 ml On Day 7, the horses began a strenuous exercise regimen five days per week for the remaining 24 weeks of the study.	x	24 weeks	x	Goldic according to manufacturer's guidelines	Lameness evaluation (AAEP score system), swelling and/or effusion graded by 5-point scale (0 = not present and 5 = severe swelling/ effusion) before treatment and at week 1, 2, 3, 12 and 24.	Significant reduction of lameness, effusion (osteoarthritis group), and swelling (soft tissue disorders group) within 2 weeks of treatment was found. Up to 3 and 6 months after treatment, all horses were free of symptoms.	lack of control group, different types of lesions included	+

Studies investigating ACS therapy in soft tissues (2/3)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Geburek et al., 2015	RCT	Horses n = 15 (17 lesioned tendons)	Naturally occurring forelimb SDFT tendinopathy	Single Intralesional ACS injection 1–3 ml according to size of lesion *n* = 10 limbs	Control: single intralesional inj. Of saline 1-3 ml according to size of lesion; n = 2 or untreated tendons *n* = 5	190 days	Patients and outc. Asses.	Autologous Irap-10 Orthokine Incubation for 6-9 h; centrifugation at 4.000 rpm for 10 min (universal 320 centrifuge, Hettich, Tuttlingen, Germany).	Clinical and B-MODE US examination (D0 D11 D22 D36 D50 D78 D106 D134 D162 and D190); Histologic and Immunohistochemical expression of collagen I and III (D0, D36 and D190)	Lameness: all horses sound by D36 - ACS group showed a faster improvement (D11); Signs of inflammation: Swelling ↓ by D50-D78 and remained (ACS); Sensitivity and surface temperature: ↓ both groups by D22. B-MODE US: mean %T-lesion ↓ and echogenicity ↑ in ACS group. Histology: more spindle shaped tenocytes and more uniform cell density on D36 and improvement of fibers organizations compared to baseline on ACS group. Immunohistochemistry: ↑ collagen-I btw D36 - D190 in ACS group - not changed in Control group	small n	+
							x					+

(*continued on next page*)

Table 2 (*continued*)

Studies investigating ACS therapy in soft tissues (2/3)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Pecin et al., 2017	Experimental controlled *in vivo*	Rabbits n = 26	Tendinopathy of Achilles tendon experim. Induced	3 inj. ACS At 0 h, 24 h and 48 h n = 13	3 inj. PBS At 0 h, 24 h and 48 h *n* = 13	4 weeks		IRAP® incubated for aprox. 6 h and centrifugation at 3000 rpm for 10 min	IL-1β tissue []; histological tendon healing assessment; microscopic evaluation;	[] IL-1β in tissue was 2.5 ↓ in ACS group. ACS treated group showed more visible healing patterns and prevented the progression of inflammation. In histological exam, ACS group appeared better structured, more properly arranged bundles with little ground substance and cellular elements. Collagen fibers were more mature. A small number of tenocytes with less pronounced amount of extracellular matrix mucopolysaccharides in the IRAP group indicated rapid restoration of certain parts of the tendon in relation to the findings in the control group.		
Genç et al., 2018	Experimental controlled *in vivo*	Sprague-Dawley rats *n* = 45	Tendinopathy of Achilles tendon experim. Induced	ACS 3 inj. n = 20 appl. Times not specified	Saline 3 inj. n = 20 appl. Times not specified	30 days	x	Allogenic ACS – Orthokine Blood collected from 5 different rats - donor group Incub. time not mentioned; centrif. At 3500 rpm for 10 min.	Histopathological: Bonar and Movin scales; Immunohistochemical: staining of collagen-III; Biomechanical test: tensile testing.	ACS group: histopathological results significantly better on D15 and D30; Immunohistochemical density of collagen-III reduced at D30. Biomechanical: maximal load to failure values higher at D15.	small n; limited outcome measurements; short follow-up	+
Damjanov et al., 2018	RCT double blinded	Human *n* = 32	Naturally occurring Supraspinatus tendinopathy	2 ml ACS 4 inj. Weekly intervals n = 16	Betamethasone 3 inj. Weekly intervals + Saline 1 inj. At the 4th week *n* = 16	24 weeks	Patients and outc. Asses.	Autologous EOT®II syringe - Orthokine; incubated for 7 h; centrifuged (3000 xg) for 10 min	Shoulder pain - VAS and joint function - CSS at weeks 0, 4 and 24 Safety of ACS over the 24 weeks	After 24 weeks of follow up, the ACS group exhibited a significantly better reduction in pain (VAS) and greater improvement in CSS function compared to the glucocorticoid/ placebo group. No adverse effects in ACS group.	small n;	+

Studies investigating ACS therapy in soft tissues (3/3)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations	
Wehren et al., 2019	Retrospective cohort study	Human *n* = 50	Chronic Achilles Tendinopathy (at least 6 weeks) Confirmed by clinical signs and MRI	ACS 3 inj. 7-days interval n = 25	Eccentric training for 3 months *n* = 25	6 months	MRI evaluation blinded	ACS – Orthokine Did not specified the incubation time or centrifugation protocol	Clinical evaluation: VISA-A questionnaire (pain, functional status and activity) Before treatment, after 6 weeks, 12 weeks and 6 months. MRI: Before and 6 months after treatment.	VISA-A-G scores: ACS group significantly better after 6 months in VISA-A-G domain activity. Signif. More improvement in ACS group between timepoints: - VISA-A-G total score and VISA-A-G domain activity at 12 weeks *vs* baseline and at 6 months *vs* baseline; - VISA-A-G total score and VISA-A-G domain of pain and functional status comparing 12 weeks *vs* 6 weeks. ACS has better outcome compared to	no true control group, short follow-up, ACS inj. Were not USG.	+

(*continued on next page*)

Table 2 (*continued*)

Studies investigating ACS therapy in soft tissues (3/3)

Author/ year	Study Design	Population	Lesion	Intervention	Control/ Comparative	Follow up	Blinding	Hemoderivative characteristics	Outcome Measurements	Results	Observations
Genç et al., 2020	Experimental controlled *in vivo*	Sprague-Dawley rats n = 40	Tendinopathy of Achilles tendon experim. Induced	1) ACS 3 inj. At 24, 48 and 72 h P. O. *n* = 10 2) PRP 3 inj. At 24, 48 and 72 h P.O. n = 10	Untreated n = 10	30 days	Outcome assessors	Pooled blood from 5 rats Orthokine- under a temperature of 37 °C - not specified how long for; centrifuged 3500 rpm for 10 min.	Histopathological: Bonar and Movin scales; Sirius Red staining of collagen-III; Biomechanical test: tensile testing.	eccentric training as demonstrated by the 12 weeks' and 6 months' data analysis. MRI: MRI-findings showed no significant differences between the two groups. Histological evaluation showed signif. Better results for PRP group (Bonar and Movin scales and collagen I/III ratio), with no singnif. Differences between ACS and control groups. No siginif. Differences between groups on biomechanical tests.	–

+ = Positive; ± = Partially positive; – = negative/no changes regarding ACS effects; %T-lesion = Percent of total lesion; AAEP = American Association of Equine Practitioners; ACS = Autologous conditioned serum; appl. = applications; bFGF = Basic fibroblastic growth factor; BMP = Bone morphogenetic proteins; COL1A1 = collagen type I gene; CSS = Constant Shoulder Score; IL-1β = interleukin 1 beta; MRI = magnetic resonance imaging; OA = osteoarthritis; OCD = Osteochondritis dissecans; PBS = phosphate buffered saline; PDGF = Platelet-derived growth factor; PRP = Platelet rich plasma; RCT = randomized clinical trial; rpm = revolutions per minute; SDFT = superficial digital flexor tendon; TGF- β1 = Transforming growth factor β1; USG = ultrasound guided; VAS = visual analogue scale; VEGF = Vascular endothelial growth factor; VISA-A-G = German Victorian Institute of Sports Assessment – Achilles tendon.

Heisterbach et al., 2012; Pecin et al., 2017; Genç et al., 2018; Genç et al., 2020), all of them performed *in vivo;* two RCTs (Geburek et al., 2015; Damjanov et al., 2018) and two observational studies (Schneider and Veith, 2013; Wehren et al., 2019). Four experimental *in vivo* studies were performed with Sprague-Dawley rats (Majewski et al., 2009; Heisterbach et al., 2012; Genç et al., 2018; Genç et al., 2020) and one with rabbits (Pecin et al., 2017). In human species there was only one RCT (Damjanov et al., 2018) and one retrospective cohort meeting the inclusion criteria (Wehren et al., 2019) and among equine investigations, there was one included RCT (Geburek et al., 2015) and one case series (Schneider and Veith, 2013).

The employed tools to assess the risk of bias of RCTs and observational studies demonstrated that a high risk was found in two studies, one RCT was considered to have an unclear risk and one cohort was of low risk of bias (Fig. 5).

As in studies investigating ACS effects in articular tissues, the most employed acquisition method of ACS in the studies exploring its advantages in soft tissue healing was the Orthokine®. It was used in eight articles but incubation periods followed by authors were usually 6–9 h, differing from the employed protocols for articular tissues (Majewski et al., 2009; Heisterbach et al., 2012; Geburek et al., 2015; Pecin et al., 2017; Damjanov et al., 2018; Genç et al., 2018; Wehren et al., 2019; Genç et al., 2020). As described earlier, one study evaluating ACS effects in both articular and soft tissues disorders used the Goldic® commercial kit (Schneider and Veith, 2013).

The majority of studies – six out of nine – investigated ACS therapy for lesions in the Achilles tendons (Majewski et al., 2009; Heisterbach et al., 2012; Pecin et al., 2017; Genç et al., 2018; Wehren et al., 2019; Genç et al., 2020). This lesions were experimentally induced in five *in vivo* researches and naturally occurring in one retrospective cohort enrolling humans (Wehren et al., 2019). Among the remaining articles, one study investigated ACS treatment for supraspinatus tendinopathy in humans (Damjanov et al., 2018), one explored its advantages in acute superficial digital flexor tendinitis of horses (Geburek et al., 2015) and the last one investigated general soft tissue disorders (defined by the author as tendinosis, suspensory ligament damage and sesamoidosis) (Schneider and Veith, 2013).

The range of age of horses included in the RCT and in the case series varied from 2 to 19 years old and the sample sizes were relatively small (*n* = 15 and 18 – for soft tissues). The follow up period ranged from 24 to 27 weeks approximately and a control group was included only in the RCT performed by Geburek et al. (2015).

In the studies enrolling humans, mean age ranged from 54 to 65 years old; the number of participants were 32 and 50 (Damjanov et al., 2018; Wehren et al., 2019 – respectively) and in both studies, the effects of the ACS therapy were evaluated for 24 weeks. Both studies included comparative groups, where ACS was compared to betamethasone injections for supraspinatus tendinopathies and eccentric training was compared to ACS injections for treatment of lesions in the Achilles tendon (Damjanov et al., 2018; Wehren et al., 2019).

Among experimental *in vivo* studies, Sprague-Dawley rats were employed in four trials (Majewski et al., 2009; Heisterbach et al., 2012; Genç et al., 2018; Genç et al., 2020) and rabbits were chosen for one experiment (Pecin et al., 2017). All lesions within the Achilles tendons of these animals were experimentally induced and sample sizes varied from 26 to 80 animals. The evaluation period ranged from 30 days to 8 weeks.

3.2.2. *Outcome measurements*

3.2.2.1. Lameness in horses. One RCT demonstrated that horses with superficial digital flexor tendon (SDFT) injuries, treated with ACS, showed a faster reduction of lameness compared to controls (Geburek et al., 2015). In agreement, significant reduction of lameness was also found in horses with several soft tissue disorders 24 weeks after ACS

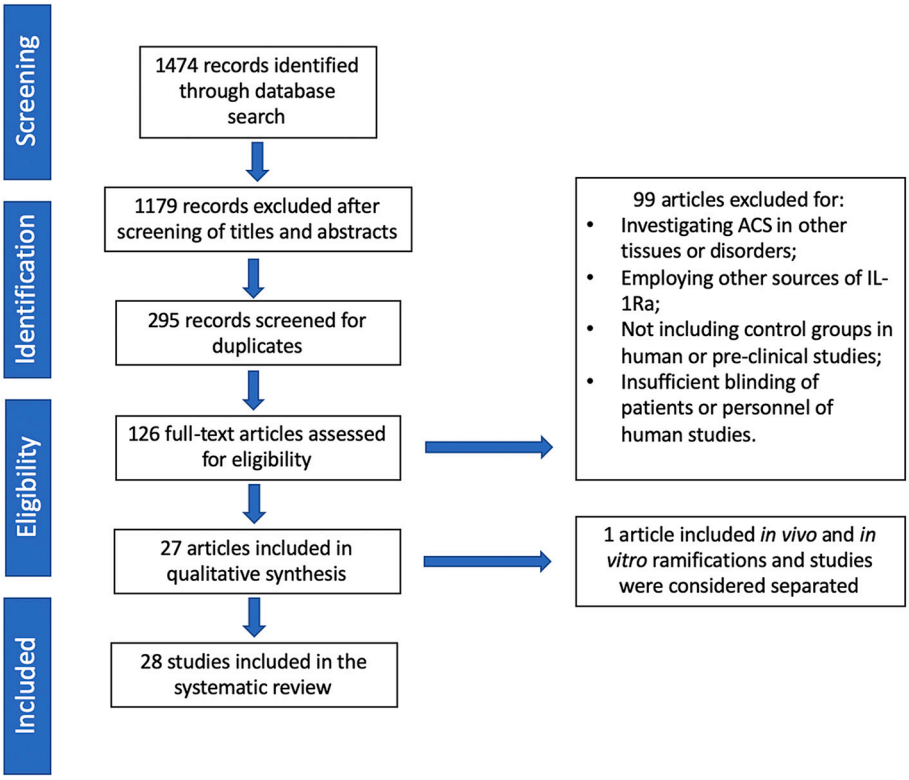

Fig. 2. Flow diagram for identification of published studies (PRISMA 2009).

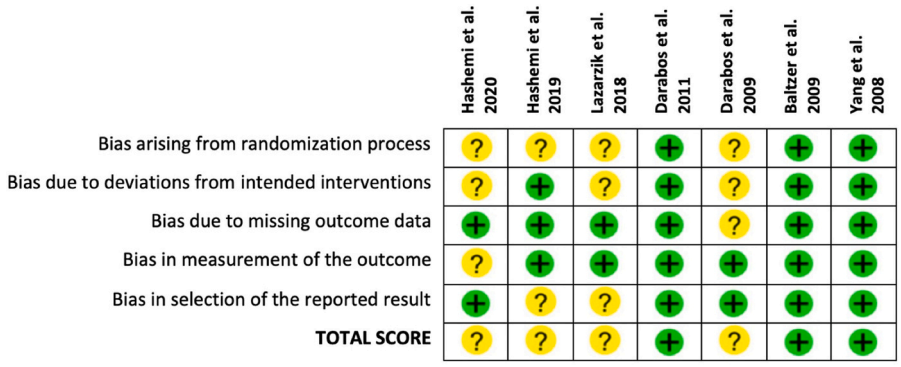

Fig. 3. Risk of bias for included RCTs – articular tissue.

therapy, however, in this study no control group was included (Schneider and Veith, 2013).

3.2.2.2. Patient reported outcome measurements (PROMs) in humans. Employed PROMs in included human studies were specific to the investigated lesions. Damjanov et al. (2018) found a significant greater improvement in the VAS (Visual Analogue Scale) and in the CSS (Constant Shoulder Score) compare to the usual treatment – betamethasone – for supraspinatus tendinopathy, at 24 weeks after treatment. Accordingly, significant superior results were found in the VISA-A-G score (Victorian Institute of Sport Assessment-Achilles – German version) when injuries in Achilles tendons were treated with ACS, in comparison with eccentric training at 12 weeks and 6 months (Wehren et al., 2019).

3.2.2.3. Physical exam. Signs of inflammation such as elevated skin temperature, local sensitivity to palpation and swelling of horses' tendons were evaluated by Geburek et al. (2015) and despite both treatment and control groups showed significant decrease of skin temperature and tendon sensitivity, ACS treated horses showed further significant decrease of swelling while no changes were observed in controls.

3.2.2.4. Diagnostic imaging. When evaluated with ultrasound imaging, SDFTs of horses showed a significant decrease in percentage of the lesion in the tendon by 190 days; and faster improvement in the total echo score compared to controls by 22 to 78 days; however, no significant changes in size of total cross-sectional area or total fiber alignment score were found (Geburek et al., 2015).

MRI was employed to evaluate lesions in Achilles tendons of humans in one study and no significant changes were found when comparing groups treated with ACS and eccentric training on a 6 months follow-up (Wehren et al., 2019).

3.2.2.5. Histological examination. Histological evaluation of treated tendons was performed in one equine RCT and four *in vivo* experiments. Overall, positive results were found in tendons treated with ACS,

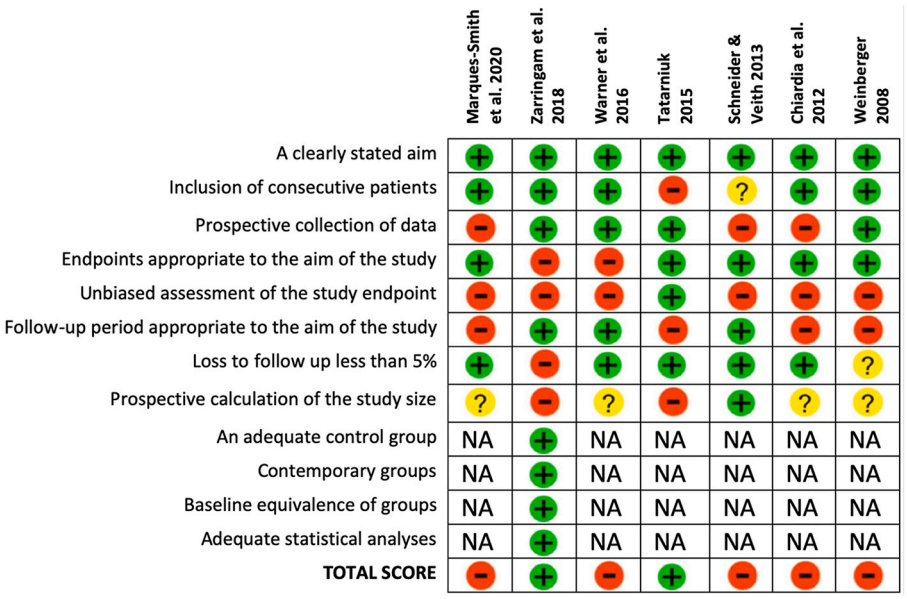

Fig. 4. Risk of bias for included observational studies – articular tissue.

showing faster healing process and better organization of tissue when compared to controls (saline injections or untreated tendons) (Majewski et al., 2009; Geburek et al., 2015; Pecin et al., 2017; Genç et al., 2018). Conversely, one recent published study comparing ACS with controls and PRP showed that no significant differences were found between the control and ACS groups while PRP demonstrated consistently superior scores of histological examination (Genç et al., 2020).

3.2.2.6. Immunohistochemistry. Immunohistochemical analysis was performed by Majewski et al. (2009) to detect collagen III content. The study showed that in the ACS treated rats, there was a decrease in its content by approximately one third compared to controls. In study performed by Heisterbach et al. (2012), ACS treated tendons showed an increase in gene expression of bFGF, BMP-12 and TGF-β1 and Geburek et al. (2015) found increased collagen-I content in tendons of horses treated with ACS injections.

3.2.2.7. Fluorometric assay. No changes were observed by Majewski et al. (2009) when fluorometric assay was employed to measure lysyl oxidase activity in tendons of rats.

3.2.2.8. IL-1β content in tissue. A significant reduction in IL-1β concentration (2.5 times lower) was found in tendons of rabbits treated with ACS compared to control (Pecin et al., 2017).

3.2.2.9. Biomechanical analysis. Analysis of maximum load to failure were performed in three studies (Majewski et al., 2009; Genç et al., 2018; Genç et al., 2020). The study of Genç et al. (2018) improvements in treated rats compared to controls were only found at day 15, with no changes on day 30 of the follow-up. The same follow-up time was employed by Genç et al. (2020) with no changes being observed at any timepoint. In agreement, Majewski et al. (2009) investigated maximum load to failure through 8 weeks and no changes were found in ACS treated group compared to control group.

3.2.2.10. Adverse events. Adverse events were described in humans, horses, or rats as not present or only mild (local pain for few hours) (Schneider and Veith, 2013; Geburek et al., 2015; Damjanov et al., 2018; Wehren et al., 2019).

4. Discussion

Regenerative therapies and the use of blood-derived products have become popular treatment options for musculoskeletal disorders in human and equine species. The ACS therapy has earned its place within the range of available options for tissue healing in orthopedic lesions despite the diversity of results in the available literature (Chevalier, 2010; Fox and Stephens, 2010; Malemud, 2010; Burnouf et al., 2013; Gross and Hoffmann, 2013; Wehling et al., 2017; Bogers, 2018; Ortved, 2018).

This review aimed to present available information of ACS effectiveness in joints, tendons and ligaments. It encompassed 28 pieces and different study designs were included due to the scarce published data on the subject - mainly among equine studies - and aimed to gather a more comprehensive amount of information about the topic. The heterogenicity in studies' designs was taken into consideration in the appraisal of each paper and studies were rated accordingly, as having high, unclear or low risk of bias. No correlation between the studies' risk of bias and their results regarding ACS effects was observed in overall results. The RoB Cochrane tool showed that most of the RCTs included in this review were classified as unclear risk with bias arising manly from randomization process or due to deviations from the intended intervention, while the MINORs tool demonstrated a high risk of bias of included observational studies, in which fails could arise mainly due to biased assessment of the study endpoint.

The analysis of studies' characteristics demonstrated that investigations in horses involved usually only small samples compared to included studies with humans, different etiologies of lesions and a range variety of follow-up time, hampering comparisons of results. In addition, these studies usually did not include control groups. In humans, characteristics were more homogeneous which is explained by the stricter criteria applied when selecting studies of this species as a greater amount of higher quality evidence was available.

ACS was usually acquired through similar commercial kits or methods. However, changes in the usual 24 h of incubation period could be noted. In studies investigating ACS in articular tissues, employed time for incubation was mostly 24 h or "according to manufactures instructions" with only four studies presenting shorter periods. Three of them, were not able to identify changes in results when ACS was employed (Rutgers et al., 2010; Tatarniuk, 2015; Garbin, 2017) while the one which described the most appropriate time (22-24 h)

demonstrated partially positive results (Marques-Smith et al., 2020). In studies investigating ACS in soft tissues, on the other hand, 6-9 h was the most employed period for incubation and all of the studies that described this period for ACS preparation managed to acquire positive results. Incubation time was proven to be one of the key factors in the induction of IL-1ra synthesis, and the increase in IL-1ra concentration occurred in a time-dependent manner, even in the absence of the glass beads, in commercial serum glass tubes or plastic tubes with Z Serum Clot Activator (Hraha et al., 2011; Ascurra et al., 2019). The preconized incubation period of blood to effectively produce maximal amounts of the IL-1ra in the ACS is 24-h (Meijer et al., 2003). However, incubation periods ranging from 6 to 9 h are also recommended by the manufacturer depending on the chosen product (EOT®II-syringe and Orthokine®vet IRAP10) (Arbel, 2017), nonetheless, there are no available studies that explain the recommendation of this shorter period for incubation.

Clinical effects of ACS were mostly measured through subjective, yet well accepted methods, such as subjective scoring of lameness or local effusion/swelling in horses or employment of PROMs in humans. In horses, majority of studies agreed that improvements in lameness could be observed after ACS treatment (Frisbie et al., 2007; Weinberger, 2008; Schneider and Veith, 2013; Geburek et al., 2015; Marques-Smith et al., 2020). Most of them were rated as high risk of bias, however, in a better designed *in vivo* controlled study performed by Frisbie et al. (2007), equivalent results were found. Only one study could not demonstrate improvements in lameness of horses but this finding could be attributed to the shorter follow-up time of the investigation or the shorter incubation period for ACS preparation (Tatarniuk, 2015). Conclusions about articular effusion, on the other hand, are difficult to be made as results were not unanimous (Frisbie et al., 2007; Schneider and Veith, 2013; Marques-Smith et al., 2020), and despite inflammation of tendons treated with ACS seems to present faster improvement in sensibility and temperature along with decrease in swelling, there are not enough studies performing such evaluations to allow a definitive verdict (Geburek et al., 2015). In humans, PROMs mostly indicate – at low risk of bias - some improvements in pain and function after ACS therapy. Enhancements, at least in the absolute values, or displays of significant superiority within at least of some of the subscores of employed PROMs were observed in all studies, agreeing with clinical improvements found in horses.

The results of SF analysis were conflicting regarding the concentrations of IL-1β and its antagonist after ACS treatment. Concentrations of IL-1β seems to decrease shortly after injections but not all studies could demonstrate this effect (Darabos et al., 2009; Rutgers et al., 2010; Darabos et al., 2011; Tatarniuk, 2015; Lasarzik et al., 2018). In addition, although elevated levels of IL-1Ra are thought to play the major role in the ACS mechanism of action, increases in its concentrations in the SF after ACS injection were not consistently detected in all included articles (Rutgers et al., 2010; Tatarniuk, 2015). A significant increase in IL-1Ra levels in the SF of horses was found by two authors only, in different timepoints (Frisbie et al., 2007; Lasarzik et al. (2018). Lasarzik et al. (2018), have found increases in concentrations of this protein in the SF of horses only a few hours after each ACS application despite the follow-up of 42 days. The authors suggested that the half-life of the induced increase of this cytokine by the ACS would last less than 48 h in the articular environment. This could explain the unchanged levels of SF IL-1Ra concentrations found in other experiments in which different timepoints for SF analysis were employed. On the other hand, Frisbie et al. (2007) found increases in the IL-1Ra concentrations at the 4th injection of ACS at day 35, which remained high weeks later (at day 70), suggesting that ACS treatment could have some effect on the stimulation of the endogenous production of IL-1Ra. The findings of Frisbie et al. (2007) and Lasarzik et al. (2018) reflect the diversity of results that are available in the literature and cast doubt about the actual mechanism of action and the role of the IL-1Ra in ACS therapy.

The eventual participation of molecules other than IL-1ra in the observed effects of ACS has been described. Conditioning of blood for ACS acquisition also results in synthesis of other anti-inflammatory cytokines that may be beneficial for tissues, such as IL-4 and IL-10 (Meijer et al., 2003; Hraha et al., 2011; Textor et al., 2011). Interleukin-10, for example, is considered an antioxidant and a potent anti-inflammatory cytokine (Dokka et al., 2001; Haddad and Fahlman, 2002; Adib-Conquy and Cavaillon, 2009) with several potential therapeutic indications (Opal and DePalo, 2000). These cytokines can contribute to the positive results observed when ACS is administered but adopted outcome measures are not specifically selected to detect their presence or effects.

Positive effects of ACS administration on protein profile regarding inflammatory state, coagulation pathways, oxidative stress, and matrix damage were revealed by proteomic analysis of SF from OA equine joints (Chiaradia et al., 2012). The reduction of oxidative burst generated in inflammatory processes could, therefore, contribute to the clinical improvements described in clinical trials. Brossi et al. (2012) investigated the antioxidant effects of blood-products on equine synovial fluid cells *in vitro*. In such study, the authors were able to demonstrate a markedly reduction of free radicals generated by synovial leukocytes, restoring the redox equilibrium of these cells. The deleterious effects of reactive oxygen species on cartilage homeostasis and joint inflammation have already been demonstrated (Henrotin et al., 2003).

Effects of ACS were also investigated through diagnostic imaging tools in some studies (Frisbie et al., 2007; Darabos et al., 2011; Geburek et al., 2015; Wehren et al., 2019) but conclusions could not be drawn as each of them employed a different type of equipment for different lesions, and so further studies including these evaluations should be conducted in the future to allow proper analysis.

Likewise, post-mortem macroscopic evaluation and histology of joint tissues were only performed by one author, which found a tendency of improvement in cartilage and synovial membrane macroscopic scores and a significant decrease of synovial membrane intimal hyperplasia in ACS treated joints. However, once again, no conclusions can be drawn due to scarcity of studies evaluating such characteristics in joints (Frisbie et al., 2007).

Histologic evaluation of tendons, on the other hand, were much employed in studies investigating ACS effects. Four out of five studies – most of them designed as controlled *in vivo* experiments - could demonstrate that ACS treated subjects showed significant improvements in quality of tissue healing and a faster evolution compared to controls (Majewski et al., 2009; Geburek et al., 2015; Pecin et al., 2017; Genç et al., 2018). Only one study could not demonstrate equal findings (Genç et al., 2020). In such study, a comparison was also made with a PRP treated group which showed superior ($p < 0.05$) results over both ACS ($p > 0.05$) and controls at Bonar's and Movin's Scales which evaluate respectively: abnormalities in tenocytes, ground substance, collagen and vascularity; and abnormalities in fiber structure, fiber arrangement, rounding of the nuclei, regional variations in cellularity, increased vascularity, decreased collagen stainability, hyalinization and GAG content. PRP has been widely used as treatment for tendinopathies and its superiority over control groups was recently shown in a systematic review (Miller et al., 2017). However, ACS has also shown encouraging results, with improvements in histologic and immunohistochemistry tests and reduction of IL-1β content in treated tendons, therefore, further studies comparing these therapies should be performed in the future (Majewski et al., 2009; Heisterbach et al., 2012; Geburek et al., 2015; Pecin et al., 2017).

In vitro studies were mainly performed with articular tissues, investigating articular cartilage metabolism, gene expression, DNA content and media content of biomarkers (Rutgers et al., 2010; Carlson et al., 2013; Garbin, 2017; Alvarez et al., 2020). The results were not able to consistently demonstrate beneficial properties of ACS as shown by outcomes from clinical trials, case series or *in vivo* studies. This may suggest that the *in vitro* scenario has, so far, failed in reproducing the pathways responsible for ACS' effects observed clinically and that it may

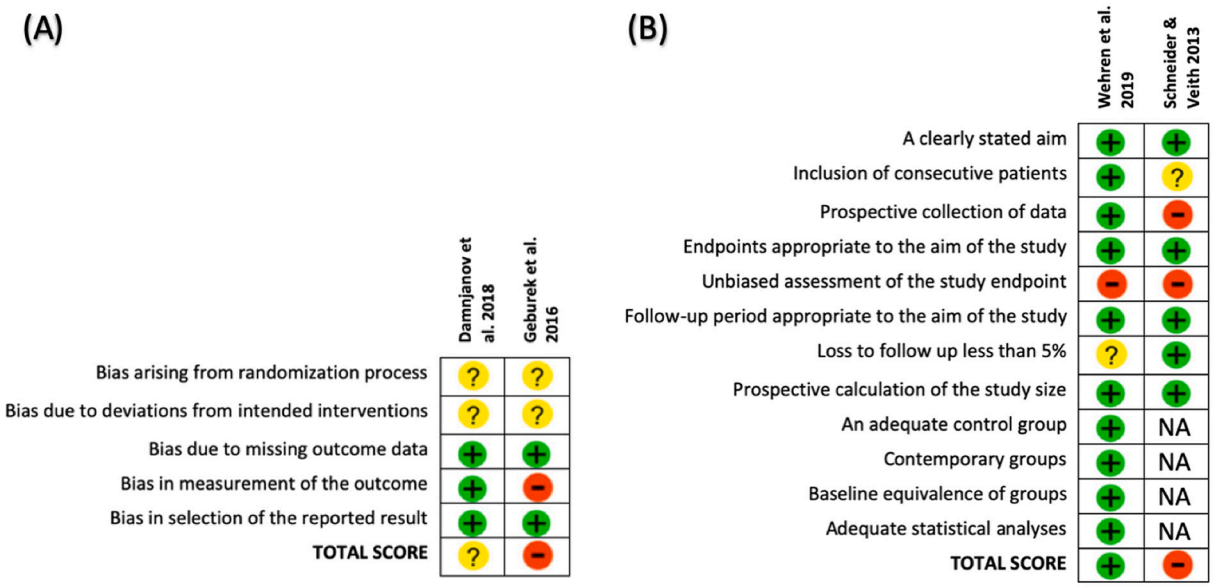

Fig. 5. Risk of bias for RCTs (A) and observational studies (B) – soft tissues.

acts through mechanisms or in tissues different from the ones investigated in these particular experiments. For instance, ACS had no effects on cartilage metabolism (Rutgers et al., 2010; Carlson et al., 2013; Garbin, 2017) which lead us to speculate that other tissues could be the primary target for ACS actions in articular lesions. The synovial membrane, for instance, has been recognized as a potent source on inflammatory mediators and proteolytic enzymes that fuel the cycle of deleterious intra-articular events leading to cartilage damage (Sutton et al., 2009). Reduction in synovial membrane intimal hyperplasia was found by Frisbie et al. (2007) in ACS treated OA joints and as synovitis has been implicated to play an important role in osteoarthritis, synovial tissues could be important targets for the treatment of this condition (Attur et al., 2010; Sellam and Berenbaum, 2010). Another hypothesis is that failure to observe positive effects on cartilage after ACS treatment can be explained by the short follow-up period employed in *in vitro* studies. Given the slow metabolic rate of this tissue, maybe a longer-term evaluation would be necessary to demonstrate beneficial effects on cartilage structure.

Studies that aimed to investigate the clinical effects at a long-term basis were much limited. One study with human subjects concluded that the term of "disease modifying drug" couldn't be employed for ACS therapy, as it wasn't able to prevent the need for future surgery in KOA patients on a 10-year basis (Zarringam et al., 2018). Their results and graphs, however, also demonstrate that the ACS group could indeed show a better cumulative survival in a shorter follow-up time (5 years), but this was not discussed by the authors. In addition, patients were aware of their treatment (placebo x control) which could lead placebo treated patients to search for other IA treatments during the follow-up period leading to bias on the results. In horses, a long-term investigation was conducted to verify if treated patients had a successful outcome up to 2 years after treatment. The conclusion was that no long-term positive effects were present in the majority of horses (Warner et al., 2016).

Regarding ACS safety, adverse events after its injection were mostly temporary and considered as mild and moderated. Severe reactions, such as infections, were scarce and in general, associated with the procedure of the injection and not with the ACS product, meaning that it is safe to use it in joints or tendons (Frisbie et al., 2007; Weinberger, 2008; Yang et al., 2008; Baltzer et al., 2009; Darabos et al., 2011; Schneider and Veith, 2013; Geburek et al., 2015; Damjanov et al., 2018; Wehren et al., 2019; Marques-Smith et al., 2020).

5. Conclusion

Clinical improvements were observed in the majority of studies in both humans and horses, but not in the *in vitro* scenario. However, available information from RCTs and observational studies presented together mostly an unclear to high risk of bias, with only a few being considered of low risk.

The use of ACS in the treatment of musculoskeletal lesions, although safe, promising and appealing, still cannot be recommended without due caution in face of the observed inconsistencies. This review highlights the need for well-designed studies, which would prevent a high risk of bias in its construction and avoid the association of results to a low evidence level.

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References

Adib-Conquy, M., Cavaillon, J.M., 2009. Compensatory anti-inflammatory response syndrome. Thromb. Haemost. 101 (01), 36–47.
Ajrawat, P., Dwyer, T., Chahal, J., 2019. Autologous interleukin 1 receptor antagonist blood-derived products for knee osteoarthritis: a systematic review. Arthroscopy 35 (7), 2211–2221.
Alvarez, A.V., Boone, L.H., Pondugula, S.R., Caldwell, F., Wooldridge, A.A., 2020. Effects of autologous conditioned serum, autologous protein solution, and triamcinolone on inflammatory and catabolic gene expression in equine cartilage and synovial explants treated with IL-1β in co-culture. Front. Vet. Sci. 7.
Arbel, R., 2017. Orthokine. In: Gobbi, A., Espregueira-Mendes, J., Lane, J., Karahan, M. (Eds.), Bio-Orthopaedics: A New Approach, 1st ed. Springer, Berlin, Heidelberg, pp. 561–569.
Ascurra, J.L., Ehrle, A., Einspanier, R., Lischer, C., 2019. Influence of incubation time and incubation tube on the cytokine and growth factor concentrations of autologous conditioned serum in horses. J. Equine Vet. Sci. 75, 30–34.
Attur, M., Samuels, J., Krasnokutsky, S., Abramson, S.B., 2010. Targeting the synovial tissue for treating osteoarthritis (OA): where is the evidence? Best Pract. Res. Clin. Rheumatol. 24 (1), 71–79.
Baltzer, A.W.A., Moser, C., Jansen, S.A., Krauspe, R., 2009. Autologous conditioned serum (Orthokine) is an effective treatment for knee osteoarthritis. Osteoarthr. Cartil. 17 (2), 152–160.
Bogers, S.H., 2018. Cell-based therapies for joint disease in veterinary medicine: what we have learned and what we need to know. Front. Vet. Sci. 5, 70.
Brossi, P.M., Baccarin, R.Y., Massoco, C.O., 2012. Do blood components affect the production of reactive oxygen species (ROS) by equine synovial cells in vitro? Pesq. Vet. Brasileira. 32 (12), 1355–1360.

Burnouf, T., Goubran, H.A., Chen, T.M., Ou, K.L., El-Ekiaby, M., Radosevic, M., 2013. Blood-derived biomaterials and platelet growth factors in regenerative medicine. Blood Rev. 27 (2), 77–89.
Carlson, E.R., Stewart, A.A., Carlson, K.L., Durgam, S.S., Pondenis, H.C., 2013. Effects of serum and autologous conditioned serum on equine articular chondrocytes treated with interleukin-1 β. Am. J. Vet. Res. 74 (5), 700–705.
Chevalier, X., 2010. Intraarticular treatments for osteoarthritis: new perspectives. Curr. Drug Targets 11 (5), 546–560.
Chiaradia, E., Pepe, M., Tartaglia, M., Scoppetta, F., D'Ambrosio, C., Renzone, G., Avellini, L., Moriconi, F., Gaiti, A., Bertuglia, A., Beccati, F., Scaloni, A., 2012. Gambling on putative biomarkers of osteoarthritis and osteochondrosis by equine synovial fluid proteomics. J. Proteomics. 75 (14), 4478–4493.
Damjanov, N., Barac, B., Colic, J., Stevanovic, V., Zekovic, A., Tulic, G., 2018. The efficacy and safety of autologous conditioned serum (ACS) injections compared with betamethasone and placebo injections in the treatment of chronic shoulder joint pain due to supraspinatus tendinopathy: a prospective, randomized, double-blind, controlled study. Med. Ultrason. 20 (3), 335–341.
Darabos, N., Hundric-Haspl, Z., Haspl, M., Markotic, A., Darabos, A., Moser, C., 2009. Correlation between synovial fluid and serum IL-1β levels after ACL surgery–preliminary report. Int. Orthop. 33 (2), 413–418.
Darabos, N., Haspl, M., Moser, C., Darabos, A., Bartolek, D., Groenemeyer, D., 2011. Intraarticular application of autologous conditioned serum (ACS) reduces bone tunnel widening after ACL reconstructive surgery in a randomized controlled trial. Knee Surg. Sports Traumatol. Arthrosc. 19 (1), 36–46.
Dehghani, B., Rodeo, S., 2019. Cell therapy—a basic science primer for the sports medicine clinician. Curr. Rev. Musculosk. Med. 12 (4), 436–445.
Dokka, S., Shi, X., Leonard, S., Wang, L., Castranova, V., Rojanasakul, Y., 2001. Interleukin-10-mediated inhibition of free radical generation in macrophages. Am. J. Physiol. Lung Cell. Mol. Physiol. 280 (6), L1196–L1202.
Evans, C.H., Chevalier, X., Wehling, P., 2016. Autologous conditioned serum. Phys. Med. Rehabil. Clin. 27 (4), 893–908.
Fjordbakk, C.T., Johansen, G.M., Løvås, A.C., Oppegård, K.L., Storset, A.K., 2015. Surgical stress influences cytokine content in autologous conditioned serum. Equine Vet. J. 47 (2), 212–217.
Fox, B.A., Stephens, M.M., 2010. Treatment of knee osteoarthritis with Orthokine®-derived autologous conditioned serum. Expert. Rev. Clin. Immunol. 6 (3), 335–345.
Frisbie, D.D., Kawcak, C.E., Werpy, N.M., Park, R.D., McIlwraith, C.W., 2007. Clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum in horses with experimentally induced osteoarthritis. Am. J. Vet. Res. 68 (3), 290–296.
Garbin, L.C., 2017. Evaluation of Allogeneic Freeze-Dried Platelet Lysate and Conditioned Serum in Joint Tissues under Inflammatory Condition In Vitro. Doctoral dissertation. Colorado State University.
Geburek, F., Lietzau, M., Beineke, A., Rohn, K., Stadler, P.M., 2015. Effect of a single injection of autologous conditioned serum (ACS) on tendon healing in equine naturally occurring tendinopathies. Stem Cell Res. Therapy. 6 (1), 126.
Genç, E., Beytemur, O., Yuksel, S., Eren, Y., Çağlar, A., Küçükyıldırım, B.O., Güleç, M.A., 2018. Investigation of the biomechanical and histopathological effects of autologous conditioned serum on healing of Achilles tendon. Acta Orthop. Traumatol. Turc. 52 (3), 226–231.
Genç, E., Yüksel, S., Çağlar, A., Beytemur, O., Güleç, M.A., 2020. Comparison on effects of platelet-rich plasma versus autologous conditioned serum on Achilles tendon healing in a rat model. Acta Orthop. Traumatol. Turc. 54 (4), 438.
Gross, G., Hoffmann, A., 2013. Therapeutic strategies for tendon healing based on novel biomaterials, factors and cells. Pathobiology. 80 (4), 203–210.
Haddad, J.J., Fahlman, C.S., 2002. Redox-and oxidant-mediated regulation of interleukin-10: an anti-inflammatory, antioxidant cytokine? Biochem. Biophys. Res. Commun. 297 (2), 163–176.
Hashemi, M., Taheri, M., Adlkhoo, H., Dadkhah, P., Abbasian, M.R., 2019. Comparison of the effect of intra-articular injection of autologous (Orthokine) Interleukin-1 receptor antagonist (IL-1Ra) and hyaluronic acid in pain control of knee osteoarthritis. Novelty Biomed. 7 (4), 210–217.
Hashemi, M., Adlkhoo, H., Dadkhah, P., Rohanifar, R., Taheri, M., 2020. A comparative assessment of autologous conditioned serum and ozone for knee osteoarthritis treatment: mid-term follow up. Novelty Biomed. 8 (1), 45–52.
Heisterbach, P.E., Todorov, A., Flückiger, R., Evans, C.H., Majewski, M., 2012. Effect of BMP-12, TGF-β1 and autologous conditioned serum on growth factor expression in Achilles tendon healing. Knee Surg. Sports Traumatol. Arthrosc. 20 (10), 1907–1914.
Henrotin, Y.E., Bruckner, P., Pujol, J.P., 2003. The role of reactive oxygen species in homeostasis and degradation of cartilage. Osteoarthr. Cartil. 11 (10), 747–755.
Hraha, T.H., Doremus, K.M., McIlwraith, C.W., Frisbie, D.D., 2011. Autologous conditioned serum: the comparative cytokine profiles of two commercial methods (IRAP and IRAP II) using equine blood. Equine Vet. J. 43 (5), 516–521.
Lasarzik, J., Bondzio, A., Rettig, M., Estrada, R., Klaus, C., Ehrle, A., Einspanier, R., Lischer, C.J., 2018. Evaluation of two protocols using autologous conditioned serum for intra-articular therapy of equine osteoarthritis— a pilot study monitoring cytokines and cartilage-specific biomarkers. J. Equine Vet. Sci. 60, 35–42.
Majewski, M., Ochsner, P.E., Liu, F., Flückiger, R., Evans, C.H., 2009. Accelerated healing of the rat Achilles tendon in response to autologous conditioned serum. Am. J. Sports Med. 37 (11), 2117–2125.
Malemud, C.J., 2010. Anticytokine therapy for osteoarthritis. Drugs Aging 27 (2), 95–115.
Marques-Smith, P., Kallerud, A.S., Johansen, G.M., Boysen, P., Jacobsen, A.M., Reitan, K. M., Henriksen, M.M., Löfgren, M., Fjordbakk, C.T., 2020. Is clinical effect of autologous conditioned serum in spontaneously occurring equine articular lameness related to ACS cytokine profile? BMC Vet. Res. 16 (1), 1–9.
Meijer, H., Reinecke, J., Becker, C., Tholen, G., Wehling, P., 2003. The production of anti-inflammatory cytokines in whole blood by physico-chemical induction. Inflamm. Res. 52 (10), 404–407.
Miller, L.E., Parrish, W.R., Roides, B., Bhattacharyya, S., 2017. Efficacy of platelet-rich plasma injections for symptomatic tendinopathy: systematic review and meta-analysis of randomized injection-controlled trials. BMJ Open Sport Exerc. Med. 3 (1).
Moher, D., Liberati, A., Tetzlaff, J., Altman, D.G., 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ. 339, b2535.
Opal, S.M., DePalo, V.A., 2000. Anti-inflammatory cytokines. Chest. 117 (4), 1162–1172.
Ortved, K.F., 2018. Regenerative medicine and rehabilitation for tendinous and ligamentous injuries in sport horses. Vet. Clin. 34 (2), 359–373.
Pecin, M., Kreszinger, M., Vukovic, S., Lipar, M., Smolec, O., Radisic, B., Kos, J., 2017. Accelerated Achilles tendon healing with interleukin-1 receptor antagonist protein in rabbits. Turk. J. Vet. Anim. Sci. 41 (1).
Rutgers, M., Saris, D.B., Dhert, W.J., Creemers, L.B., 2010. Cytokine profile of autologous conditioned serum for treatment of osteoarthritis, in vitro effects on cartilage metabolism and intra-articular levels after injection. Arthritis Res. Therapy. 12 (3), R114.
Schneider, U., Veith, G., 2013. First results on the outcome of gold-induced, autologous-conditioned serum (GOLDIC) in the treatment of different lameness-associated equine diseases. J. Cell Sci. Ther. 5 (1), 151.
Sellam, J., Berenbaum, F., 2010. The role of synovitis in pathophysiology and clinical symptoms of osteoarthritis. Nat. Rev. Rheumatol. 6 (11), 625.
Slim, K., Nini, E., Forestier, D., Kwiatkowski, F., Panis, Y., Chipponi, J., 2003. Methodological index for non-randomized studies (MINORS): development and validation of a new instrument. ANZ J. Surg. 73 (9), 712–716.
Sterne, J.A.C., Savović, J., Page, M.J., Elbers, R.G., Blencowe, N.S., Boutron, I., Cates, C. J., Cheng, H.Y., Corbett, M.S., Eldridge, S.M., Hernán, M.A., Hopewell, S., Hróbjartsson, A., Junqueira, D.R., Jüni, P., Kirkham, J.J., Lasserson, T., Li, T., McAleenan, A., Reeves, B.C., Shepperd, S., Shrier, I., Stewart, L.A., Tilling, K., White, I.R., Whiting, P.F., Higgins, J.P.T., 2019. RoB 2: a revised tool for assessing risk of bias in randomized trials. BMJ. 366, l4898.
Strümper, R., 2017. Intra-articular injections of autologous conditioned serum to treat pain from meniscal lesions. Sports Med. Int. Open. 1 (6), E200–E205.
Sutton, S., Clutterbuck, A., Harris, P., Gent, T., Freeman, S., Foster, N., Barrett-Jolley, R., Mobasheri, A., 2009. The contribution of the synovium, synovial derived inflammatory cytokines and neuropeptides to the pathogenesis of osteoarthritis. Vet. J. 179 (1), 10–24.
Tatarniuk, D., 2015. Autologous Conditioned Serum and Subsequent Synovial Fluid Concentrations of Cytokines, Matrix Metalloproteinases, and Tissue Inhibitors of Metalloproteinases after Injection into Equine Osteoarthritic Distal Interphalangeal Joints. Master of Science Thesis. University of Minnesota.
Textor, J.A., Norris, J.W., Tablin, F., 2011. Effects of preparation method, shear force, and exposure to collagen on release of growth factors from equine platelet-rich plasma. Am. J. Vet. Res. 72 (2), 271–278.
Warner, K., Schulze, T., Lischer, C.J., 2016. Behandlung von Osteoarthritis mit ACS (IRAP®) bei 26 Pferden – Retrospektive Studie. Pferdeheilkunde. 32 (341–248).
Wehling, P., Evans, C., Wehling, J., Maixner, W., 2017. Effectiveness of intra-articular therapies in osteoarthritis: a literature review. Ther. Adv. Musculoskelet. Dis. 9 (8), 183–196.
Wehren, L.V., Pokorny, K., Blanke, F., Sailer, J., Majewski, M., 2019. Injection with autologous conditioned serum has better clinical results than eccentric training for chronic Achilles tendinopathy. Knee Surg. Sports Traumatol. Arthrosc. 27 (9), 2744–2753.
Weinberger, T., 2008. Klinische Erfahrumgen mit der anwendung von ACS/Orthokin/IRAP beim pferd. Pferdespiegel. 11 (3), 111–114.
Yang, K.A., Raijmakers, N.J.H., Van Arkel, E.R.A., Caron, J.J., Rijk, P.C., Willems, W.J., Zijl, J.A.C., Verbout, A.J., Dhert, W.J.A., Saris, D.B.F., 2008. Autologous interleukin-1 receptor antagonist improves function and symptoms in osteoarthritis when compared to placebo in a prospective randomized controlled trial. Osteoarthr. Cartil. 16 (4), 498–505.
Zarringam, D., Bekkers, J.E., Saris, D.B., 2018. Long-term effect of injection treatment for osteoarthritis in the knee by orthokin autologous conditioned serum. Cartilage. 9 (2), 140–145.
Ziltener, J.L., Allet, L., Sclison, P., Grosclaude, M., 2012. How effective are injections of platelet-rich plasma (PRP) for the treatment of sports injuries: a critical review of the literature. J. Sport Med. Doping Stud. 2 (2), 1–6.