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Evaluation of an intra-articular carboxymethylcellulose crosslinked hydrogel in horses with osteoarthritis

This is the final peer-reviewed author's accepted manuscript (postprint) of the following publication:

Published Version:

Rinnovati, R., Spadari, A., Malpighi, A., Meistro, F., Ralletti, M.V., Marcucci, E., et al. (2025). Evaluation of an intra-articular carboxymethylcellulose crosslinked hydrogel in horses with osteoarthritis. JOURNAL OF EQUINE VETERINARY SCIENCE, 150, 1-4 [10.1016/j.jevs.2025.105611].

Availability:

This version is available at: <https://hdl.handle.net/11585/1018343> since: 2025-06-24

Published:

DOI: <http://doi.org/10.1016/j.jevs.2025.105611>

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(Article begins on next page)

1 **EVALUATION OF AN INTRA-ARTICULAR CARBOXYMETHYLCELLULOSE**
2 **CROSSLINKED HYDROGEL IN HORSES WITH OSTEOARTHRITIS**

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12

13 **Abstract**

14 Numerous treatment strategies have been proposed to prevent osteoarthritis or to minimize its
15 clinical signs in sport horses. Carboxymethylcellulose (CMC), a derivative of cellulose, that
16 belongs to the class of cellulose ethers, is utilized in veterinary medicine for diverse applications.
17 This study aimed to test the efficacy of a commercial CMC hydrogel in reducing lameness due to
18 osteoarthritis. Sixteen horses were included in the treated group and fourteen in the control group.
19 Horses in the treated group received an intra articular injection of CMC, while those in the control
20 group received an intra articular injection of hyaluronic acid and 12 mg/joint of triamcinolone
21 acetone (TA). The horses were re-evaluated after 15, 30 and 90 days.

22 At 90 days post-baseline, horses of treated group presented a statistically significant treatment
23 success rate (75%), while control group did not exhibit any statistically significant improvement
24 (8%). The improvements of clinical lameness manifestation in horses treated intra-articularly with
25 CMC pointed out a possible alternative in effectively and durably treating equine osteoarthritis.

26 Keywords: Intra-articular treatment, fetlock joint, lameness

27

28 **1. Introduction**

29 Osteoarthritis (OA) represents one of the most widespread and debilitating condition affecting horses,
30 significantly impacting the economic viability of the equine industry [1]. A variety of treatment
31 modalities including physical, biological and pharmaceutical interventions have been proposed to
32 either prevent the onset of OA or to minimize clinical signs of pain (lameness), reduce joint
33 deterioration, and extend the competitive career of equine athletes [2].

34 CMC is an anionic polymer compound with a molecular weight ranging from thousands to millions
35 Da and is one of the derivatives of cellulose belongs to the class of cellulose ethers. Nowadays, it is
36 utilized in veterinary medicine for diverse applications, such as lubricant, wound healing agent, and
37 barrier material to prevent postoperative adhesions[3]. Due to its low toxicity and immunogenicity,
38 alongside favorable biodegradability and biocompatibility [4], CMC gels have garnered extensive
39 research attention, emerging as one of the most promising materials for use as drug carriers in clinical
40 settings [5]. In human orthopedics, in vitro studies indicate that CMC enhances chondrocyte
41 differentiation and promotes extracellular matrix synthesis [6].

42 This prospective open-label comparative clinical efficacy study was design to investigate the efficacy
43 of a CMC crosslinked hydrogel compared with TA 12 mg/joint and sodium hyaluronate (HA) 20
44 mg/joint for managing naturally occurring fetlock joint lameness in horses. The underlying
45 hypothesis was that there would be a significant difference between treatments throughout the
46 duration of the study.

47

48 **2. Materials and Methods**

49 2.1 Ethical approval

50 The study obtained favorable approval from the ethics committee of the University of Bologna
51 Prot. n. 99874/2024.

52

53 2.2 Materials, tested product group (TPG)

54 The sterile, isotonic CMCgel for intra articular use was supplied by Innate s.r.l (Italy). The gel
55 consists in an association of cellulose gum and croscarmellose (crosslinked) in a physiologic buffer.

56 The composition is: cellulose gum, sodium croscarmellose, sodium chloride, sodium phosphate
57 monobasic dihydrate, dibasic sodium phosphate dodecahydrate, sodium hydrate 30% solution.

58 The pre-filled syringe contains 3 ml of product.

59 2.3 Materials, control group (CG)

60 12 mg/joint TA (Kenacort, Bristol-Myers Squibb S.r.l.) in combination with 20 mg/joint of high
61 molecular weight HA (Alien, ACME S.r.l.). These two products are intended to be administered
62 together in a single injection; however, their physicochemical stability has not been evaluated.

63

64 2.4 Methods

65 To be included in the study, horses were required to be at least 2 years old and weigh less than 650
66 kg. Horses should exhibit lameness originating from osteoarthritis (OA) affecting the forelimb fetlock
67 joint. The lameness had to persist for a minimum duration of four weeks, in addition flexion test must
68 be interpreted as positive as well as intra-articular diagnostic analgesia. Other inclusion criteria were
69 the presence of radiological signs of OA of the affect joint, besides radiological confirmation that no
70 osteochondral fragments or fractures were present.

71 Exclusion criteria comprised several conditions: horses displaying lameness attributed to joint disease
72 affecting multiple joints, or presenting fractures or osteochondral fragments; those subjected to local
73 or systemic administration of nonsteroidal anti-inflammatory drugs, glycosaminoglycans, hyaluronic
74 acid, corticosteroids, or other antiarthritic drugs, shock wave therapy, acupuncture, or any
75 homeopathic or oral supplements in the 30 days prior to the study. Additionally excluded were
76 lactating or pregnant mares, as well as horses with signs of systemic diseases or infectious septic
77 arthritis.

78 Post-inclusion removal from the study cohort could occur if any horse failed to adhere to inclusion
79 criteria during the study period, specifically concerning compliance with prescribed treatments and
80 durations or if serious adverse effects attributable to the drug manifested; or if the owner withdrew
81 the horse from participation without necessitating justification.

82 Horses that met enrollment criteria received a lameness score according to the American Association
83 of Equine Practitioners (AAEP) scoring system.

84

85 2.4.1 Patient management and welfare

86 The horse owner was required to consent to the study protocol and sign an informed consent
87 agreement. All veterinarians involved were instructed on study protocol and standard operating

88 procedures by Quality Control group of Department of Veterinary Medical Sciences, University of
89 Bologna (Italy). The horses involved in the trial remained under the care of their respective owners.

90 2.4.2 Visit protocol and interventions

91 Patients were screened for eligibility based on previously mentioned criteria. The veterinary
92 investigators performed the examinations as follows: each horse was trotted on a loose lead both in a
93 straight line and a circle. Upon identifying the affected limb, further examination for swelling, heat,
94 and pain was performed to localize and score the lameness. A flexion test was performed. The
95 examination proceeded if the investigator considered the findings consistent with fetlock joint
96 disease. After this, mepivacaine 100 mg/joint was aseptically injected into the articular space of the
97 affected joint. The horse was then reevaluated to assess any change in lameness severity. X-ray
98 examination of the joint was then conducted.

99 The researchers randomly assigned patients to one of two groups using a mobile phone application
100 system. Veterinarians involved in the study were not blinded to the treatment.

101 Depending on their group assignment, horses received either 12 mg/joint TA combined with 20
102 mg/joint of high molecular weight HA or CMC prefilled syringe as a single intra-articular injection
103 via an 18–21 gauge needle after aspiration of 2–3 ml of synovial fluid.

104 Post injection, horses were hand-walk for days 1 to 5, up to 20 minutes on the walker for days 6 to
105 15, then from 16 to 20 if serviceably sound were allowed to jog up to a mile a day before returning
106 to full work. The horses were re-evaluated after 15, 30 and 90 days at identical locations and surfaces
107 to assess lameness degree and reaction during fetlock flexion tests.

108

109 2.5 Data analysis

110 No pre-study power calculations were used. Admission scores for lameness and reaction to fetlock
111 flexion were compared between groups using non-parametric Kruskal-Wallis test. Success was
112 defined as a complete resolution of the variable over time. Data were analyzed using Chi-Squared
113 tests for statistical comparisons with significance set at $P < 0.05$. and, point estimates for the
114 difference in percentage of successful treatments and associated 95% Confidence Intervals.

115

116 3. Results

117

118 3.1 Recruitment

119 Horses were recruited from April 2024 to September 2024. Re-examination by the veterinarian was
120 at a 15, 30 and 90 days after baseline.

121

122 3.2 Baseline data

123 Relevant baseline data for participating horses are provided in Table 1. Table 1 summarizes the
124 baseline characteristics of the study population, including age, sex, body weight, affected joint, and
125 initial AAEP lameness grade. No significant differences were observed between groups at baseline

126 There were no statistically significant differences observed in any of the baseline parameters between
127 groups.

128 A total of 30 horses were enrolled in the study, all of which met the inclusion criteria, with an age
129 range of 3 to 12 years and a mean age of 7 years. No horses were excluded throughout the course of
130 the study.

131 Only one horse in the TPG experienced an adverse reaction, specifically severe joint effusion was
132 observed for a few days before regressing spontaneously.

	TPG	CG	
Number	16	14	P values (95% CI)
Age range (mean)	3-12 (6.7)	3-12 (7)	P>0.05
Thoroughbred (%)	7 (43,75%)	8 (57,14%)	P>0.05
Standardbred (%)	3 (18,75%)	0 (0 %)	P>0.05
Pleasure horses	6 (37,50%)	6 (42,86%)	P>0.05
Lameness range (mean)	1-3 (1,5)	1-3 (1,5)	P> 0.05
Reaction to fetlock flexion (mean)	0-2 (1,5)	0-2 (1)	P>0.05

133

134 **Table 1.** Pre admission variables per group

135

136 3.3 Outcomes

137 At 15 days, a significant higher proportion of horses in the CG were classified as treatment success
138 rate for the considered variables (90%), whereas 20% of horses in the TPG group met the criteria for
139 treatment success; however, this difference was not statistically significant when compared to the
140 control group.

141 At 30 days, both groups show statistically significant treatment success rate (75%).

142 At 90 days post-baseline, TPG presented a statistically significant treatment success rate (75%), while
143 CG did not exhibit any statistically significant improvement (8%).

144 To improve the clarity of treatment outcomes, clinical success rates at each evaluation point are
145 summarized in Table 2.

Timepoint	TPG	CG
Day 15	20% (3/15)	90% (13/14)
Day 30	75% (12/16)	75% (10/14)
Day 90	75% (12/16)	8% (1/13)

146

147 **Table 2.** Clinical success rates at each follow-up point in the treated group (TPG) and control group
148 (CG)

149

150 3.4 Adverse events

151 Only one horse in the TPG develop an adverse reaction that appeared to be possibly product-related,
152 based on the timing of the event and clinical manifestations observed (severe joint effusion of the
153 injected joint was observed for a few days), which subsequently regressed spontaneously. The
154 incidence of adverse drug-related events was not statistically different between groups.

155

156 4. Discussion

157 This report describes the results of a randomized clinical trial involving 30 lame horses, aimed at
158 evaluating the comparative clinical efficacy of CMC compared to 12 mg/jointTA combined with 20
159 mg/joint HA.

160 A significant proportion of the CG exhibited an immediate improvement in lameness and fetlock
161 flexion, which was maintained for about 30 days before gradually diminishing. These results suggest
162 a favorable initial clinical response within this patient population. It is likely that corticosteroids
163 provide their potent anti-inflammatory and analgesic effects for a limited period of time following
164 intra-articular injection; however, the incorporation of hyaluronic acid may confer additional
165 advantages [7].

166 Conversely, the TPG horses displayed minimal improvement for the considered variables after 15
167 days, but then substantially improve after 30 days, with this improvement remaining stable for up to
168 90 days.

169 A possible explanation for this result is due to the fact that CMC serves as an effective filler for
170 cartilage defects [6], but is subject to thixotropic behavior. Hydrogels composed of CMC can alter
171 their mechanical properties temporarily under appropriate mechanical stimuli [8]. It is likely that
172 factors such as load, body temperature and controlled exercise facilitate changes in viscosity,
173 contributing to the progressive improvement of the lameness. CMC acts as a mechanical barrier,
174 isolating the tissues from one another [9], and probably the key to improving this mechanical barrier
175 lies in the thixotropic behavior. However, the fact that certain viscosupplementation devices required
176 a period of time to exert their effects is not novel; in fact, this has already been demonstrated in horses
177 for polyacrylamide gel [10,11].

178 Additionally, while the antibacterial properties of CMC have been proved [12], its anti-inflammatory
179 effect, assessed by monitoring the release of IL-1, at 14 days, appears to be limited [6].

180 The observed delayed clinical response in the treatment group receiving CMC is consistent with the
181 proposed mechanical and viscoelastic behavior of the hydrogel. Unlike corticosteroids, which exert
182 a rapid anti-inflammatory effect through direct modulation of inflammatory mediators, CMC appears
183 to act primarily as a mechanical barrier and joint lubricant, modifying the intra-articular environment
184 gradually. This interpretation aligns with the temporal pattern observed in the present study, where
185 horses in the CMC group showed minimal response at day 15, but significant and sustained
186 improvement by day 90. Understanding this mechanism is crucial for guiding clinical expectations
187 and suggests that when using CMC, follow-up periods should be sufficient to capture its full
188 therapeutic potential.

189 The horses included in the TPG received a sterile, isotonic crosslinked CMC gel for intra-articular
190 use. A study conducted by human medical researchers on in-vitro and in-vivo (rabbit knees) effects
191 of a synthetic amidic derivative of CMC on chondrocyte regeneration offered promising outcomes
192 [6]. In vitro results suggested that CMC ameliorated the chondrocyte differentiation as well as the
193 synthesis of extracellular matrix components. In-vivo results led to the conclusion that CMC hydrogel
194 stimulates cartilage healing [6].

195 Moreover, despite the rapid degradation rate typically observed with common hydrogel visco-
196 supplementation when compared to cartilage regeneration, a cellulose derivative appears to mitigate
197 these limitations due to its elevated polysaccharide molecular weight, which ensures reduced

198 degradability and consequently prolongs its intra-articular effectiveness [6]. While long-term studies
199 to exclude further tissue degeneration are not available, a human surgical study [13] demonstrates
200 that the use of intra-articular CMC -polysaccharide B following anterior cruciate ligament
201 reconstruction showed superior results for pain control, hemarthrosis, and knee movement gain in the
202 postoperative period when compared to patients from the non-treated group.

203 In the present study, the reason for using cross-linked CMC and not a polymerized CMC gel is
204 predicated on the potential adverse effects of polymerization, which may destroy certain
205 physiological characteristics of the hydrogel and suffer from several uncontrollable factors like
206 temperature, pH and reaction time. In contrast, hydrogels prepared via cross-linking with a natural
207 polymer exhibit good biodegradability and mild and controllable reaction conditions, which render
208 this approach the most ideal and promising synthesis method for CMC gels [14].

209 The joint selected in this study is the fetlock joint, that is characterized as a highly loaded motion
210 joint that frequently sustains injuries in athletic horses; thus, it serves as a representative model for
211 common joint disorders in equines [15].

212 Radiographic imaging was employed exclusively to exclude cases involving fetlock joint diseases
213 associated with osteochondral fragments or fracture-related complications from this study. Although
214 other diagnostic methods (like CT or MRI) could enhance the classification of joint pathologies, the
215 authors considered radiography sufficient to exclude from the study cases of OA clearly related or
216 complicated by fractures or osteochondral fragment and therefore include just cases of OA mainly
217 caused by inflammatory and degenerative joint diseases alone.

218 The selection of cases with a minimum lameness duration of four weeks helped empirically in
219 excluding horses that might have suffered from an acute joint inflammation or mild synovitis.
220 Consequently, this selection process focused on those cases that are more indicative of chronic
221 conditions, consistent with some degenerative intra-article changing, confirmed by radiographic
222 images. One of the main limitations of this study is the relatively small sample size (30 horses), which
223 may limit the generalizability of the results. However, conducting clinical trials in equine medicine,
224 especially with strict inclusion and exclusion criteria, and involving performance horses, poses
225 logistical, ethical, and economic challenges that frequently restrict large-scale recruitment. Despite
226 this limitation, the consistent findings across multiple time points strengthen the validity of the
227 observations and suggest that even with a limited sample size, the treatment effect was robust and not
228 likely due to random variation.

229 Another limitation of the present study is the relatively short follow-up period of 90 days. However,
230 90 days represents a commonly used endpoint in equine lameness studies to detect meaningful
231 improvements or treatment failures, and it is relevant for informing decisions in clinical practice,
232 particularly in performance horses.

233 An additional limitation is the lack of blinding for the treating veterinarians, which may introduce
234 observer bias, despite the use of standardized clinical evaluation methods.

235 According to the findings of this study at 90 days post-initial treatment, horses in the TPG showed a
236 significant clinical improvement, while the CG horses didn't demonstrate a clinically significant
237 improvement. This observation raises the hypothesis that the previously observed benefits from intra-
238 articular administration TA and HA may have diminished in efficacy within this latter cohort.

239 Further research should aim to validate these findings in larger, multicenter populations and to explore
240 the long-term effects of CMC on joint preservation and function. Studies investigating synovial fluid
241 biomarkers, imaging-based assessment of cartilage condition, and comparison with other emerging
242 viscosupplementation agents would provide valuable insights.

243 **5. Conclusions**

244 While the data derived from this study are based on a limited number of horses and do not provide
245 specific information about the effect of CMC hydrogel on equine joint biological components, nor its
246 interactions and specific effect on inflammatory response and joint degeneration, they offer promising
247 clinical results. The improvements of clinical lameness manifestation in horses treated intra-
248 articularly with CMC pointed out a possible alternative in effectively and durably treating equine OA,
249 encouraging further dedicated research and investigation.

250

251 **6. Conflict of interest**

252 No competing interests have been declared.

253 **7. Source of funding**

254 This work was supported by Innate s.r.l. (Italy) who supplied the tested product

255

256 **8. Acknowledgements**

257 We would like to thank Dr. Pierluigi Capodagli and Dr. Gabriele Rosati for the active patient
258 recruitment.

259

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