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Evaluation of the effects of hydroxyethyl starch (130/0.4) administration as a constant rate infusion on plasma colloid osmotic pressure in hypoalbuminemic dogs

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1 **Abstract**

2 **Objective** – Evaluate the effects of two constant rate infusions of hydroxyethyl starch (HES)
3 130/0.4 on plasma colloid osmotic pressure (COP), in hypoalbuminemic dogs.

4 **Design** – Cohort prospective study.

5 **Animals** – 24 client-owned dogs.

6 **Interventions** – Hypoalbuminemic euvolemic dogs [albumin <20 g/L (< 2 g/dl)] with normal
7 perfusion parameters requiring intravenous fluid therapy, were enrolled. In addition to
8 crystalloid, HES 130/0.4 was administered as a constant rate infusion over 24 hours at 1 ml/kg/h
9 (group 1, n=15) or at 2 ml/kg/h (group 2, n=9), in order to support plasma COP. Before infusion,
10 a blood sample was collected to perform cell blood count, serum electrophoresis and serologic
11 tests for some infective diseases. Plasma COP, albumin, packed cell volume and total protein
12 were evaluated serially at baseline (T0) and then at 6, 12 and 24 hours after the start of infusion,
13 and a multilevel model was performed for these parameters to detect statistically significant
14 differences between the two groups.

15 **Measurement and Main Results** – Twenty-four dogs were included. No statistically significant
16 differences in COP were found between the two groups; however, a high level of variability has
17 been identified within the single individual. Among the other laboratory analyses, packed cell
18 volume was significantly decreased in group 1 at T12 and T24 compared with T0 ($p<0.001$) and
19 total protein were significantly increased in group 2 at T12 and T24 compared with T0
20 ($p<0.008$).

21 **Conclusion** - No significant effect on plasma COP was found following infusion with HES
22 130/0.4 at doses of 1 ml/kg/h and 2 ml/kg/h for 24 hours to hypoalbuminemic dogs. The

23 administered concomitant dose of crystalloids, underlying disease and small sample size were all
24 potential confounding factors.

25

26 **ABBREVIATIONS**

27 COP: colloid osmotic pressure

28 CRI: constant rate infusion

29 HES: hydroxyethyl starch

30 ICC: intraclass correlation coefficient

31 PLN: protein losing nephropathy

32 SIRS: systemic inflammatory response syndrome

33

34

35 **Introduction**

36 Colloid osmotic pressure is the pressure exerted by macromolecules across a semipermeable
37 membrane, and it is proportional to the number of molecules present, irrespective of their size.¹

38 Albumin is the most plentiful protein in the body and accounts for about 60 to 70% of plasma
39 COP, whereas globulins and fibrinogen have a limited effect.^{2,3} Conventionally, the Starling

40 equation is used to describe the distribution of fluids from the capillary into the interstitial space
41 as the result of the equilibrium between hydrostatic pressure and plasma COP.^{1,4}

42 Accordingly, any condition that increases the hydrostatic pressure or decreases the COP could
43 cause fluid movement into the interstitial space [given a normal filtration coefficient (K_f) and
44 reflection coefficient (σ)]. Some 50 years ago, a protein layer on the luminal surface of the
45 endothelium was discovered: the endothelial glycocalyx layer is a web of glycoprotein and

46 proteoglycans that covers the luminal side of endothelial cells, separating plasma and
47 erythrocytes from the subglycocalyx space.^{5,6} The introduction of the concept of subglycocalyx
48 COP, in addition to plasmatic COP, **has** led to a revision of the Starling equation.^{5,6}

49 Measurement of COP is indicated in dogs treated with colloid solutions or which have low
50 serum albumin, to **monitor the** efficacy of therapy and reduce complication.⁷ In patients with a
51 decrease in intravascular COP fluid therapy poses challenges, because of the risk of further
52 decreasing COP and increasing fluid filtration into the interstitial space, with formation of
53 peripheral edema and effusion. Moreover, the glycocalyx is semipermeable to certain
54 macromolecules such as albumin, and increases of fluid movement to the interstitium may
55 increase the loss of these proteins.^{8,9} The diseases associated with a decrease in COP are
56 primarily those that cause a reduction in plasma proteins, particularly albumin. Several equations
57 have been derived from plasma protein concentration to monitor the plasma COP, but because a
58 poor correlation exists between calculated and measured COP, especially in critically ill patients,
59 COP needs to be determined by direct measurement.^{10,11}

60 Artificial colloids are fluids characterized by molecules retained within the vasculature, in the
61 absence of increase vascular permeability, exerting an oncotic pressure.¹² In normal conditions
62 colloid molecules are retained within the vessels longer than crystalloids, resulting in longer-
63 lasting plasma volume expansion, and should contribute to support plasma COP.¹²⁻¹⁴
64 Hydroxyethyl starches (HES) are the most widely used synthetic colloids, and their
65 pharmacological properties (oncotic effect, excretion and half-life) depend on their mean
66 molecular weight, molar substitution, and C2/C6 ratio.¹⁵ The intravascular retention time of the
67 molecules and the oncotic effect, are related to molar substitution and the C2/C6 ratio, which
68 regulate the rate of HES degradation by plasma α -amylase.¹⁶ In human and canine studies, HES

69 administration has been associated with some side effects such as tissue accumulation,
70 anaphylactic reaction, hypocoagulability and acute kidney injury.^{2,17-25}

71 Few in vivo studies have assessed COP after HES administration in canine patients.²⁶⁻²⁹ Two,
72 in particular, have evaluated the effectiveness of HES to **increase COP** and duration of action in
73 hypoalbuminemic dogs.^{26,27} Smiley et al. (1994) noted a significant increase in mean COP after
74 **HES 450/0.7** administration (9 to 26 ml/Kg of HES over 6-8 hours), but no relationship between
75 dose and magnitude of increase was found.²⁶ Moore et al. (1996) found a significant increase in
76 mean COP after a single dose of HES 450/0.7 (7.7 to 43.9 ml/Kg over 6 hours), but the effect
77 disappeared within 12 hours.²⁷ Other recent studies have obtained contrasting results: Gauthier et
78 al. (2014) have observed an increase in COP after the administration of a bolus of HES 130/0.4,
79 in both healthy dogs and dogs with induced SIRS, whereas Chohan et al. (2011) found no similar
80 increase in healthy anesthetized dogs.^{28,29} Administration **of a single dose of old generation HES**
81 for several hours has resulted in a transient increase in COP, indicating that multiple doses or
82 continuous administration could be necessary to maintain the rise in COP, although these
83 previous studies used different doses of HES and no relationship was found between dose and
84 effects.

85 In veterinary medicine, the administration of HES 130/0.4 as a CRI, at a rate of 1-2 ml/Kg/h
86 has been reported, but no studies to date have evaluated the efficacy of this protocol to increase
87 COP.^{2,17} The aim of the present study was to evaluate the effect of HES 130/0.4 administered as
88 a CRI on plasma COP in hypoalbuminemic dogs. The hypothesis was that administered HES
89 130/0.4 as a CRI in these dogs would increase the plasma COP.

90

91 **Materials and Methods**

92 This randomized, clinical prospective study involved client-owned dogs. The protocol was
93 approved by the Bioethics Committee of the author's University, and the owners of all dogs
94 recruited for participation in the study were informed about the study protocol and gave their
95 written consent.

96 **Animals**

97 Among patients admitted to our Veterinary Teaching Hospital for hospitalization, adult dogs
98 (>1 year) with hypoalbuminemia [albumin <20 g/L (2 g/dl)] were recruited if they required
99 intravenous fluid therapy to restore ongoing fluid losses and/or treat dehydration due to their
100 underlying disease (e.g., increased losses, anorexia, and dehydration). Exclusion criteria were:
101 administration of artificial colloid or blood products in the 4 weeks before; history, clinical signs
102 or biochemistry abnormalities indicating the presence of cardiac, pulmonary, renal or liver
103 failure; abnormal perfusion parameters [e.g. heart rate >130 bpm, poor pulse quality, capillary
104 refill time > 2 s or < 1 s, systolic blood pressure <90 mmHg and venous lactate >2 mmol/L
105 (18.02 mg/dl)]; diseases which can change the ratio between albumin and globulin, as the
106 suspicion of multiple myeloma and positive serology for *Ehrlichia canis* and/or *Leishmania*
107 *infantum*. If further treatments (blood products and/or surgery) were added after T0, the dog was
108 removed from the study.

109 **Study Design**

110 After placement of a catheter in a peripheral vein, fluid therapy with a crystalloid solution
111 (Lactated Ringer's) was calculated based on the percentage of dehydration, ongoing losses and
112 maintenance daily requirement, and the amount was administered in 24 hours. In addition, HES
113 130/0.4^a was administered as a CRI (T0) and the dogs were randomly assigned to receive 1
114 ml/Kg/h or 2 ml/Kg/h of HES for at least 24 hours, by a computer-generated program.^{b,2,17} The

115 pharmacological treatment, **prescribed at the start of fluid therapy**, remained unchanged during
116 colloid CRI.

117 Respiratory rate, heart rate, capillary refill time, systolic blood pressure (using Doppler),
118 metatarsal pulse quality and hydration status were evaluated every 4 hours; body weight was
119 obtained every 12 hours.

120 **Samples Collection and Analysis**

121 Before infusion, a blood sample was collected to perform CBC^c analysis with blood smear
122 evaluation, serum electrophoresis, creatinine,^d urea,^d glucose,^d alkaline phosphatase,^d aspartate
123 aminotransferase,^d alanine aminotransferase,^d γ -glutamyl transpeptidase,^d fibrinogen level^e and
124 serological tests for Ehrlichia canis,^f Borrelia burgdorferi,^f Anaplasma phagocytophilum,^f
125 Anaplasma platys,^f Dirofilaria immitis and Leishmania infantum.^g After initiation of infusion,
126 plasma COP, albumin,^d venous blood gas analysis and electrolytes,^h PCV and total protein were
127 also evaluated at 6 (T6), 12 (T12) and 24 hours (T24). For COP evaluation, whole blood was
128 collected in tubes with lyophilized heparin, and plasma was separated by centrifugation. The
129 plasma COP was measured by means of Osmomat 050,ⁱ according to manual of instructions. The
130 instrument delivers two consecutive measurements for each sample analyzed; the comparison of
131 both values allows the evaluation of the quality of the measurement.^l

132 **Statistical analysis**

133 Data were collected and entered in an *ad hoc* database.

134 The study design estimated an overall sample size of 26 dogs (13 per group), according to the
135 following criteria: Power=80%, **Standardized** range= 1, Significance level=0.05 and an equal
136 variance between the two groups.

137 As the Osmomat 050 returns two measurements of the same sample, the ICC was calculated
138 to estimate the reliability of the two measurements. A test for Normality based on skewness, one
139 based on kurtosis and another combining the two tests into an overall test statistic were
140 performed. Levene's robust test statistic was applied to verify the equality of variances. To verify
141 the homogeneity of the two groups at T0, Student's T-test for normally distributed data was
142 performed; otherwise the Wilcoxon rank-sum test was used.

143 To compare the results obtained from samples collected at the four-time points (T0, T6, T12,
144 T24), a multilevel linear mixed model was used and adjusted for repeated measures, where the
145 random effect was given by the individual and the fixed effect by the time (T0, T6, T12, T24). A
146 similar model was performed to adjust for the disease. Bonferroni adjustment was applied as
147 needed. The Residual Intraclass Correlation for pairs of responses at the individual level of the
148 model was also calculated to verify variability among individuals. The Wilcoxon matched-pairs
149 signed-ranks test was applied to not normally distributed parameters.

150 All statistics were performed using Stata 14.1.^m The level of significance was set at $p < 0.05$.

151 **Results**

152 **Dogs**

153 A total of 30 dogs were enrolled, but only 24 were included to evaluate changes in plasma
154 COP during a constant rate infusion of HES at two dosages: 15 in group 1 (1 ml/kg/h) and 9 in
155 group 2 (2 ml/kg/h). Six dogs were excluded: 2 dogs needed transfusion (albumin or packed red
156 blood cells), 1 dog underwent surgery before protocol completion, 2 samples had technical
157 problems related to the COP measurement, and 1 dog died before protocol completion. Group 1
158 was composed of 7 males (1 castrated and 6 intact males), and 8 females (2 spayed and 6 intact
159 females), with a median age of 7 years (range 1-12); median body weight was 26.7 Kg (range 5-

160 39) and the breeds included crossbred (n=8), German Shepherd (n=2), and one each of Miniature
161 Pinscher, Rottweiler, Australian shepherd, Boxer and Border collie.

162 Group 2 was composed of 3 intact males, and 6 females (2 spayed and 4 intact females), with
163 a median age of 7 years (range 2–10); median body weight was 17.8 Kg (range 5–44) and the
164 breeds included Jack Russell (n=2), crossbred (n=2) and one each of Rottweiler, Labrador
165 retriever, Dachshund, Segugio Italiano, and English bulldog.

166 In group 1, likely causes of hypoalbuminaemia were: diarrhea in 12/15 (80%) dogs (5/12
167 (41.6%) with acute diarrhea and 7/12 (58%) with chronic diarrhea), chylothorax in 2/15 (13%)
168 and PLN in 1/15 (6.6%). In group 2, likely causes of hypoalbuminaemia were: diarrhea in 6/9
169 (66.6%) dogs (1/6 (16.6%) with acute diarrhea and 5/6 (83%) with chronic diarrhea), septic
170 peritonitis in 2/9 (22%) and hypoadrenocorticism in 1/9 (11%). Effusion (excluding a specific
171 cause of effusion such as chylothorax or peritonitis) was noted in 3/15 (20%) in group 1
172 (abdominal effusion only, transudate) and 2/9 (22%) in group 2 (abdominal effusion only,
173 modified transudate).

174 **Laboratory analysis**

175 The correlation between the two measurements delivered by the Osmomat 050 was very high:
176 ICC=0.995 (CI95% 0.99-1). Table 1 presents the laboratory results for variables of interest and
177 COP values measured at T0, T6, T12, and T24. There were no statistically significant differences
178 in any of the parameters evaluated at T0 between the two groups, and the COP values were
179 below the lower reference range in all dogs (reference range from 17 to 26 mmHg). No
180 statistically significant differences in the COP results were found by the multilevel models nor
181 between the times, neither between the two different doses. A high level of intraindividual
182 variability was identified (figure 1a-b and table 2). No statistically significant differences

183 between values of the variables at each sampling time were observed even after correction by
184 disease.

185 In group 1, from T0 to T24: COP was increased in 9/15 dogs (60%), in which only 5/9 dogs
186 presented increase in ALB (55%); COP was decreased in 6/15 dogs (60%), in which only 3/6
187 dogs presented decrease in ALB (50%). In group 2, from T0 to T24: COP was increased in 2/15
188 dogs (22%), in which 2/2 dogs presented increase in ALB (100%); COP was decreased in 5/9
189 dogs (55%), in which only 2/5 dogs presented decrease in ALB (40%); COP and ALB remained
190 unchanged in 2/9 dogs.

191 A statistically significant decrease in PCV was noted in group 1 at T12 and T24 *versus* T0
192 ($p < 0.001$); a significant increase in total protein was shown in group 2 at T12 and T24 *versus* T0
193 ($p < 0.008$).

194 **Discussion**

195 This study found no **significant** differences in COP measurements between the two rates of
196 HES 130/0.4 infusion of 1ml/Kg/h and 2 ml/Kg/h administered in hypoalbuminemic dogs over
197 24 hours; as well as no difference in COP measurements over time. However there was high
198 level of intraindividual variability with both rates of infusion, and each dog **responded** to the
199 infusion of HES 130/0.4 in an unpredictable way. That variability was influenced by individual
200 factors and could also be attributed to the different amount of crystalloids administered and to
201 the underlying disease status, although **adjustments for the specific disease were made**, and the
202 results suggested that it did not influence the COP trends (figure 1a-b). The intraindividual
203 variability comes out from the results of the multilevel model: no changes can be attributed to the
204 group (neither increase, nor decrease) but it lies on the individual response to the treatment,

205 independently from the belonging group. This great variability has determined the lack of
206 statistical significance (figure 1a-b).

207 Regarding the other results obtained in this study, a decrease in PCV was observed only in
208 group 1 at T12 and T24. This result could indicate a certain degree of **hemodilution**, but the
209 concentration of albumin was unchanged and no hemodilution was seen in group 2 which
210 received twice the volume of HES 130/0.4. This unexpected finding could be due to changes in
211 clinical conditions or a difference in the total dose of fluid administered.

212 In group 1, no change in the values of total protein as measured by refractometry was found at
213 any time point, whereas in group 2 an increase was seen only at T12 and T24, though it has been
214 reported that this measurement could be affected by the colloid solutions.³⁰ Since the refractive
215 index of tetrastarch 130/0.4 is 42 g/L (4.2 g/dl), dilution of blood with colloids could change the
216 refractive index of plasma, but a high dose of infused volume is probably needed to interfere
217 with the refractometer reading.^{28,29}

218 Revision of Starling equation has questioned the clinical utility of measuring plasma COP, as
219 the main factor responsible for fluids exchange could be the subglycocalix COP and the integrity
220 of the glycocalyx.^{5,6,31} In the early 2000s, it was discovered that Starling's equation
221 overestimates the effect of interstitial COP on fluid exchange between the intravascular and the
222 interstitial space.^{5,31} Above the normal capillary pressure of 20 mmHg, an infusion of colloid
223 solution should increase capillary pressure, raise the volume of filtration to the interstice, but
224 preserve plasma COP; whereas, an infusion of crystalloids should decrease COP and raise the
225 filtration volume more than colloid solutions.^{32,33} In our study, although COP did not differ
226 before and after HES administration in our population of hypoalbuminemic dogs, colloid
227 infusion may probably have helped to maintain the COP and the fluid in the intravascular space,

228 decreasing the rate and amount of fluids lost in the interstitial space. This statement remains a
229 hypothesis, since there was no control group that received only crystalloid infusion in the present
230 study. Moreover, the introduction of the concept of glycocalyx raises the question of the need to
231 restore normal COP value in hypoalbuminemic dogs. Indeed, glycocalyx integrity could be more
232 important than increasing plasma COP and products like pooled albumin, plasma and plasma
233 substitutes could contribute to capillary sealing, rather than acting on the plasma COP.^{34,35}
234 Previous studies evaluating the changes in COP after HES administration in dogs obtained
235 different results. Direct comparison with our data would be challenging because of the
236 differences in pathological conditions, type of colloid solutions, and doses and rate of
237 administration.²⁶⁻²⁹ Two studies have evaluated the effects of a HES in hypoalbuminemic dogs.
238 Smiley et al (1994) have administered HES 450/0.7 at a dose ranging from 9 to 26 ml/Kg over 6
239 to 8 hours. They noted a significant increase in mean COP, but no relationship between dose and
240 magnitude of increase.²⁶ Furthermore, it was reported an improvement in edema or effusion after
241 HES administration. It is difficult to relate this result with an increase intravascular COP,
242 because the revised Starling law states that, since no absorption occurs by the capillaries, filtered
243 fluid returns to the intravascular compartment by the lymphatic vessels.^{5,6} Moore et al. (1996)
244 have measured the duration of action of a single dose of HES 450/0.7 (dose ranged from 7.7 to
245 43.9 ml/Kg) administered over approximately 6 hours, and have found a significant increase in
246 mean COP after HES administration in all dogs, but the effect disappeared within 12 hours after
247 administration.²⁷ It was also observed that the increase in COP was not significant in the dogs
248 with acute gastrointestinal protein loss, whereas in our study the underlying disease did not
249 appear to influence the COP. This difference could be related to a different studied population:
250 patients in the previous study were mainly affected by canine parvovirus. For this reason, it is

251 likely that they were puppies or young dogs. Younger animals might differently respond to HES
252 administration.

253 One study compared the effects of an equal dose of synthetic colloid (40 ml/Kg of HES
254 130/0.4) or saline, administered over a period of 30 minutes in healthy dogs and dogs with
255 induced SIRS. An increase in COP was observed in both groups treated with HES 130/0.4, with
256 a major increase in the healthy dogs.²⁹ Interestingly, the rise in COP was greater in the healthy
257 dogs and lasted for 1 to 4 hours, as compared to the 1 to 2 hours noted for the ill dogs. We can
258 hypothesize that, in light of the revised Starling equation, this difference might have been related
259 to an acquired alteration in the glycocalyx in the ill dogs, leading to an increase in capillary flow
260 towards the interstitium.^{5,34}

261 Chohan et al. (2011) showed different results after evaluating the administration of HES
262 600/0.75 or lactated Ringer's (both fluids at a dose of 10 ml/Kg, over 20 minutes) in healthy
263 dogs.²⁸ At 1 hour post-infusion, a significant decrease in COP was observed in both groups, with
264 significantly lower COP in the lactated Ringer's group than in the HES 600/0.75 group.²⁸ The
265 explanation could be that the dogs were evaluated during anesthesia and a study of Dismukes et
266 al. (2010) has shown that COP decreases on average 5 mmHg in healthy dogs undergoing
267 general anesthesia.³⁶

268 The different results obtained in aforementioned studies could be related to the dose (bolus)
269 and type of colloid (old generation HES with high molecular weight, high grade of molar
270 substitution and different C2/C6 ratio) administered. We used a new generation HES (130/0.4)
271 administered as a CRI and that could be another reason for the unchanged COP. There aren't
272 pharmacokinetic studies evaluating this type of administration **available** in dog, but it is **known**
273 that low molecular weight HES are excreted in greater quantities by renal route.¹⁵ That limits the

274 amount of HES remaining at the intravascular level influencing COP. It would be interesting to
275 evaluate the effect on COP of HES 130/0.4 administered in hypoalbuminemic dogs as a bolus
276 followed by a CRI. Theoretically, the administration of HES as a CRI, rather than a bolus, might
277 be more indicated in normovolemic and hypoalbuminemic dogs, because the increase in
278 capillary pressure above the normal value increases the volume of transendothelial flow of fluids
279 and proteins with a loss of albumin in the interstitial space.^{32,37,38} Despite this, in the current
280 study, the use of HES 130/0.4 as a CRI did not influence significantly COP. Based on previous
281 studies, older generation HES products could possibly be more effective.^{26,27}

282 To the author's knowledge, this is the first study to evaluate the effects of HES 130/0.4,
283 administered as a CRI, on plasma COP in hypoalbuminemic dogs, but some limitations could
284 have affected the generalization of these results. One limitation is the lack of a control group
285 treated with an equal dose of crystalloid to evaluate the trend of COP and confirm whether it
286 decreases during this type of infusion. However, this is a study involving client-owned dogs, and
287 it was considered not ethical to treat some of these patients only with crystalloid, at the time of
288 study design. Another limitation is the small sample size, that could have introduced a Type II
289 error. As set up at the beginning, the study was powered, but there were more exclusions than the
290 expected. Since the total concentration of crystalloid solution, administered for 24 hours, was not
291 recorded, the influence of this variable on our results was not assessed, but no change in the
292 amount of albumin was observed and this is likely to suggest minimal hemodilution, if present.

293 Other studies are needed to evaluate the advantage of HES 130/0.4 administration as a CRI in
294 hypoalbuminemic dogs relating to the morbidity and the outcome. Moreover, in dogs, the side
295 effects (e.g. hemostatic and/or renal effects) associated with the administration of colloid
296 solutions, have to be taken into account to balance positive effects *versus* the negative ones.

297 In conclusion, the administration of HES 130/0.4 as a CRI in hypoalbuminemic dogs did not
298 cause significant changes in plasma COP.

299

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302 Veterinary Emergency and Critical Care Society Congress, Lyon, France, June 2015.

303

304 **Footnotes**

305 a. Voluven, Fresenius Kabi Italia srl., Isola della Scala (VR), Italy.

306 b. Microsoft Excel, Redmond, WA, USA.

307 c. ADVIA 120 Hematology, Siemens Healthcare Diagnostics, Tarrytown, NY, USA.

308 d. ILAB 300 plus, Clinical Chemistry System, Instrumentation Laboratories, Milan, Italy.

309 e. Coagulometer StART, Diagnostica Stago, New York, USA.

310 f. Snap 4 Dx, IDEXX Laboratories, Westbrook, Maine, USA.

311 g. Snap Leishmania Test, IDEXX Laboratories, Westbrook, Maine, USA.

312 h. Osmomat 050, Gonotec, GmbH, Berlin, Germany.

313 i. ABL 800 Flex, A. DE MORI S.p.A., Milano, Italy.

314 l. Osmomat 050-User guide. Gonotec, GmbH, Berlin, Germany; 2011, p 32.

315 m. Stata Corp 14.1, Special Edition College Station, Texas, USA

316

317 **References**

318 1. Hughes D. Transvascular fluid dynamics. *Vet Anaesth Analg* 2000;27:63-69.

319 2. Adamik KN, Yozova ID, Regenscheit N. Controversies in the use of hydroxyethyl

- 320 starch solutions in small animal emergency and critical care. *J Vet Emerg Crit Care* 2015; 25
321 (1):20-47.
- 322 3. Fanali G, Di Masi A, Trezza V, et al. Human serum albumin: from bench to bedside. *Mol*
323 *Aspects Med* 2012;33(3):209-290.
- 324 4. Wellman ML, Di Bartola SP, Kohn W. Applied physiology of body fluids in dogs and cats. In:
325 Di Bartola SP, editor. *Fluid, electrolyte and acid-base disorders in small animal practice*. 4th ed.
326 St. Louis: Saunders Elsevier; 2011, pp. 2-25.
- 327 5. Levick JR, Michel CC. Microvascular fluid exchange and the revised Starling principle.
328 *Cardiovasc Res* 2010;87(2):198-210.
- 329 6. Levick JR. Revision of the Starling principle: new views of tissue fluid balance. *J Physiol*
330 2004;557(pt3):704.
- 331 7. Waddell LS. Colloid osmotic pressure and osmolality monitoring. In: Silverstein DC, Hopper
332 K., editors. *Small animal critical care medicine*. 2th ed. St. Louis: Saunders Elsevier; 2015, pp.
333 978-981.
- 334 8. Rieger A. Blood Volume and plasma protein. 3. Changes in blood Volume and plasma
335 proteins after bleeding and immediate substitution with Macrodex, Rheomacrodex and Physiogel
336 in the splenectomized dog. *Acta Chirurgica Scandinavica – Supplementum* 1967;379:22–38.
- 337 9. Vink H, Duling BR. Capillary endothelial surface layer selectively reduces plasma solute
338 distribution volume. *Am J Physiol Heart Circ Physiol* 2000;278(1):H285–289.
- 339 10. Thomas LA, Brown SA. Relationship between colloid osmotic pressure and plasma protein
340 concentration in cattle, horses, dogs, and cats. *Am J Vet Res* 1992;53(12):2241-2244.
- 341 11. Brown SA, Dusza K, Boehmer J. Comparison of measured and calculated values for colloid
342 osmotic pressure in hospitalized animals. *Am J Vet Res* 1994;55(7):910-915.

- 343 12. Hughes D and Boag A. Fluid therapy with macromolecular plasma volume expanders. In: Di
344 Bartola SP, editor. Fluid, electrolyte and acid-base disorders in small animal practice. 4th ed. St.
345 Louis: Saunders Elsevier; 2011, pp. 647-664.
- 346 13. Westphal M, James MF, Kozek-Langenecker S, et al. . Anesthesiology 2009;111(1):187-
347 202.
- 348 14. Haupt MT, Rackow EC. Colloid osmotic pressure and fluid resuscitation with hetastarch,
349 albumin, and saline solutions. Crit Care Med 1982;10(3):159-162.
- 350 15. Jungheinrich C. The starch family: are they all equal? Pharmacokinetics and
351 pharmacodynamics of hydroxyethyl starches. Trans Altern Trans Med 2007(3);9:152-163.
- 352 16. Kozek-Langenecker A, Scharbert G. Effects of hydroxyethyl starches on hemostasis. Trans
353 Altern Trans Med 2007;9(3):173-181.
- 354 17. Glover PA, Rudloff E, Kirby R. Hydroxyethyl starch: a review of pharmacokinetics,
355 pharmacodynamics, current products, and potential clinical risks, benefits, and use. J Vet Emerg
356 Crit Care 2014;24(6):642-661.
- 357 18. Hayes G, Benedicenti L, Mathews K. Retrospective cohort study on the incidence of acute
358 kidney injury and death following hydroxyethyl starch (HES 10% 250/0.5/5:1) administration in
359 dogs (2007-2010). J Vet Emerg Crit Care 2016;26(1):35-40.
- 360 19. Smart L, Jandrey KE, Kass PH, et al. The effect of hetastarch (670/0.75) in vivo on platelet
361 closure time in the dog. J Vet Emerg Crit Care 2009;19(5):444–449.
- 362 20. Chohan AS, Greene SA, Grubb TL, et al. Effects of 6% hetastarch (600/0.75) or lactated
363 Ringer’s solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for
364 orthopedic surgery. Vet Anaesth Analg 2011;38(2):94–105.
- 365 21. Gauthier V, Holowaychuk MK, Kerr CL, Bersenas AME, Darren Wood R. Effect of

- 366 synthetic colloid administration on coagulation in healthy dogs and dogs with systematic
367 inflammation. *J Vet Intern Med* 2015;29(1):276- 285.
- 368 22. McBride D, Hosgood G, Rasis A, Smart L. Platelet closure time in anesthetized Greyhounds
369 with hemorrhagic shock treated with hydroxyethyl starch 130/0.4 or 0.9% sodium chloride
370 infusion. *J Vet Emerg Crit Care* 2016;26(4):509-515.
- 371 23. Helmbold KA, Mellema MS, Hoper K, Epstein SE. The effect of hetastarch 670/0.75
372 administered in vivo as a constant rate infusion on platelet closure time. *J Vet Emerg Crit Care*
373 2014;24(4):381-387.
- 374 24. Reutler A, Flammer SA, Howard J, Adamik KA. Comparison of the effects of a balanced
375 crystalloid-based and saline based tetrastarch solution on canine whole blood coagulation and
376 platelet function. *J Vet Emerg Crit Care* 2017; 27(1): 23-34.
- 377 25. Botto A, Bruno B, Maurella C, et al. Thromboelastometric assessment of hemostasis
378 following hydroxyethyl starch (130/0.4) administration as a constant rate infusion in
379 hypoalbuminemic dogs. *BMC Vet Res.* 2018 Jan 31;14(1):33
- 380 26. Smiley LE, Garvey MS. The use of hetastarch as adjunct therapy in 26 dogs with
381 hypoalbuminemia: a phase two clinical trial. *J Vet Intern Med* 1994;8(3):195-202.
- 382 27. Moore LE, Garvey MS. The effect of hetastarch on serum colloid oncotic pressure in
383 hypoalbuminemic dogs. *J Vet Intern Med* 1996;10(5):300-3.
- 384 28. Chohan AS, Greene SA, Grubb TL, et al. Effects of 6% hetastarch (600/0.75) or lactated
385 Ringer's solution on hemostatic variables and clinical bleeding in healthy dogs anesthetized for
386 orthopedic surgery. *Vet Anaesth Analg* 2011;38(2):94-105.

- 387 29. Gauthier V, Holowaychuk MK, Kerr CL, et al. Effect of synthetic colloid administration on
388 hemodynamic and laboratory variables in healthy dogs and dogs with systemic inflammation. *J*
389 *Vet Emerg Crit Care* 2014;24(3):251-258.
- 390 30. Bumpus SE, Haskins SC, Kass PH. Effect of synthetic colloids on refractometric readings of
391 total solids. *J Vet Emerg Crit Care* 1998;8(1):21-26.
- 392 31. Schött U, Solomon C, Fries D, et al. The endothelial glycocalyx and its disruption, protection
393 and regeneration: a narrative review. *Scand J Trauma Resusc Emerg Med* 2016;24:48.
- 394 32. Adamson RH, Lenz JF, Zhang X, et al. Oncotic pressures opposing filtration across non-
395 fenestrated rat microvessels. *J Physiol* 2004;557(pt 3):889-907.
- 396 33. Clough G. Relationship between microvascular permeability and ultrastructure. *Prog*
397 *Biophys Mol Biol* 1991;55(1):47-69.
- 398 34. Woodcock TE, Woodcock TM. Revised Starling equation and the glycocalyx model of
399 transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. *Br*
400 *J Anaesth* 2012;108(3):384-394.
- 401 35. Jacob M, Rehm M, Loetsch M, et al. The endothelial glycocalyx prefers albumin for evoking
402 shear stress-induced, nitric oxide-mediated coronary dilatation. *J Vasc Res* 2007; 44(6):435-443.
- 403 36. Dismukes DI, Thomovsky EJ, Mann FA et al. Effects of general anesthesia on plasma colloid
404 oncotic pressure in dogs. *J Am Vet Med Assoc* 2010;236(3):309-311.
- 405 37. Berg S, Golster M, Lisander B. Albumin extravasation and tissue washout of hyaluronan
406 after plasma volume expansion with crystalloid or hypooncotic colloid solutions. *Acta*
407 *Anaesthesiol Scand* 2002;46(2):166-172.
- 408 38. Nicholson JP, Wolmarans MR, Park GR. The role of albumin in critical illness. *Br J Anaesth*
409 2000;85(4):599-610.

410 39. Kudryk B, Okada M, Redman CM, et al. Biosynthesis of dog fibrinogen. Characterization of
411 nascent fibrinogen in the rough endoplasmic reticulum. *Eur J Biochem.* 1982;125(3):673-682.

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432 **Table 1-** Laboratory analysis for variables of interest and COP values measured at T0, T6, T12
 433 and T24 (Group 1 N=15; Group 2 N=9).

Variable	T0	Group 1 [1 ml/kg/h]		
		T6	T12	T24
COP (mmHg)	9.2 (7-10.8)	9.3 (7.5-11.1)	9.3 (7.3-11.1)	8.9 (7.4-10.8)
Packed cell volume (%)	40 (37-45)	39 (36-44)	37 (34-42)*	39 (34-42)*
Total protein (g/L)	33 (30-44) [3.3 (3-4.4) g/dl]	35 (30-44) [3.5 (3-4.4) g/dl]	37 (30-40) [3.7 (3-4) g/dl]	42 (30-44) [4.2 (3-4.4)g/dl]
Albumin (g/L)	16 (16-18) [1.6 (1.6-1.8) g/dl]	16 (15-19) [1.6 (1.5-1.9) g/dl]	16 (15-18) [1.6 (1.5-1.8) g/dl]	16 (14-20) [1.6 (1.4-2)g/dl]
Fibrinogen (µmol/L)	13 (6.8-34.5) [443 (232-1174) mg/dl]			
Variable	T0	Group 2 [2 ml/kg/h]		
		T6	T12	T24
COP (mmHg)	9.5 (7.9-11.7)	8.4 (7.7-9.6)	9.7 (7.3-10.1)	8.6 (7.3-10.2)
Packed cell volume (%)	38 (33-42)	34 (31-36)	35 (31-44)	34 (29-39)
Total protein (g/L)	32 (28-37) [3.2 (2.8-3.7) g/dl]	35 (28-39) [3.5 (2.8-3.9) g/dl]	36 (26-37) * [3.6 (2.6-3.7) g/dl]	35 (32-42) * [3.5 (3.2-4.2) g/dl]
Albumin (g/L)	15 (14-15) [1.5 (1.4-1.5) g/dl]	15 (13-15) [1.5 (1.3-1.5) g/dl]	15 (13-16) [1.5 (1.3-1.6) g/dl]	14 (13-17) [1.4 (1.3-1.7)g/dl]
Fibrinogen (µmol/L)	9.3 (6.6-21.8) [318 (223-741) mg/dl]			

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435 Legend of table 1: Data are reported as median (range, 25th-75th percentile). Group 1, CRI at 1
 436 ml/kg/h of HES 130/0.4 administered in hypoalbuminemic dogs, Group 2, CRI at 2 ml/kg/h of
 437 HES 130/0.4 administered in hypoalbuminemic dogs, COP, colloid osmotic pressure. The
 438 institutional reference interval for COP ranges from 17 to 26 mmHg. Fibrinogen level was
 439 measured at T0 because marked increase can influence COP; it was not repeated because it
 440 **changes** very slowly over time.³⁹

441 * statistically significant difference between T0 and T12 and between T0 and T24, p<0.05

442 **Table 2-** Part of the multilevel model showing the variability within the dogs.

Random-effects parameters	Estimate	95% CI	
Individual variance	5.21	2.89	9.38
Residual variance	0.82	0.59	1.13

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444 On a total of about 6 of variance, 5.21 was attributable to the single dog, and just a minor part of
445 variance was due to the differences among the two groups and among the times (0.82).

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462 **Figure 1**

463 **Figure 1a: Trend of COP values measured at different sampling times in each dog of group**

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465 Legend: COP, colloid osmotic pressure; Group 1, CRI at 1 ml/kg/h of HES 130/0.4 administered
466 in hypoalbuminemic dogs; T0, baseline; T1, 6 hour after the start of CRI; T2, 12 hour after the
467 start of CRI; T3, 24 hour the after start of CRI.

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469 **Figure 1b: Trend of COP values measured at different sampling times in each dog of group**

470 **2**

471 Legend: COP, colloid osmotic pressure; Group 2, CRI at 2 ml/kg/h of HES 130/0.4 administered
472 in hypoalbuminemic dogs; T0, baseline; T1, 6 hour after the start of CRI; T2, 12 hour after the
473 start of CRI; T3, 24 hour the after start of CRI.

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