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# A prospective randomized open-label trial on the comparative effects of 6% hydroxyethyl starch 130/0.4 versus polyionic isotonic crystalloids on coagulation parameters in dogs with spontaneous hemoperitoneum

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## Abstract

**Objective:** To evaluate the effects of 6% hydroxyethyl starch 130/0.4 (HES) and a polyionic isotonic crystalloid (CRYS) on standard coagulation tests and rotational thromboelastometry (ROTEM) in dogs with spontaneous hemoperitoneum (SHP).

**Design:** Prospective randomized open-label clinical study.

**Setting:** University teaching hospital.

**Animals:** Forty-two client-owned dogs presented with SHP.

**Interventions:** Dogs diagnosed with SHP and hypovolemic shock were randomly allocated to receive HES (10 mL/kg, n = 22) or CRYS (30 mL/kg, n = 20) intravenously over 20 minutes for hemodynamic stabilization.

**Measurements and main results:** Parameters measured before ($T_0$) and after ($T_1$) treatment were HCT, platelet counts, prothrombin time, activated partial thromboplastin time, fibrinogen concentrations, and extrinsic activated (EXTEM), intrinsic activated (INTEM), and extrinsic activated with platelet inhibition ROTEM assays. Data were analyzed as absolute values and as the percentage change from $T_0$ to $T_1$. No significant differences between groups were detected in any variable at $T_0$, and for HCT, platelet counts, prothrombin time, activated thromboplastin time, and fibrinogen concentrations at $T_1$. Clot formation time in EXTEM was significantly prolonged ($P = 0.037$), and maximum clot firmness was significantly decreased ($P = 0.038$) in the HES group compared to the CRYS group at $T_1$. The percentage change in EXTEM clotting time ($P = 0.012$) and INTEM clot formation time ($P = 0.031$) was greater after HES than CRYS. Lysis indices remained at 100% for all ROTEM assays in both groups.

**Abbreviations:** APPLEfast, acute patient physiologic and laboratory evaluation score; aPTT, activated partial thromboplastin time; CFT, clot formation time; CRYS, crystalloid; CT, clotting time; EXTEM, extrinsically activated thromboelastometric assay; FIBTEM, extrinsically activated thromboelastometric assay with additional cytochalasin D; HES, hydroxyethyl starch; INTEM, intrinsically activated thromboelastometric assay; IQR, interquartile range; LI30, percentage of clot firmness after 30 minutes; LI45, percentage of clot firmness after 45 minutes; MCF, maximum clot firmness; PT, prothrombin time; RI, reference interval; ROTEM, rotational thromboelastometry; SHP, spontaneous hemoperitoneum; TEG, thromboelastography

**Conclusion:** Compared to a 3-fold volume of CRYS, administration of HES was associated with impairment in ROTEM parameters in dogs with SHP, but no evidence of hyperfibrinolysis was detected.

KEYWORDS
dog, hemoperitoneum, hemorrhage, hemostasis, synthetic colloids, thromboelastometry

## 1 | INTRODUCTION

Hemoperitoneum is a frequent finding in dogs presented with hypovolemic shock in emergency practice.[1–4] Causes of hemoperitoneum in dogs include blunt trauma and nontraumatic (spontaneous) hemorrhage associated with intraabdominal neoplasia, coagulation disorders, gastric dilatation–volvulus, and splenic or hepatic lobe torsion.[5] The most common cause of spontaneous hemoperitoneum (SHP) in dogs is splenic hemangiosarcoma.[5–8] Dogs presented with SHP frequently have hemostatic abnormalities exacerbated by consumption of platelets and coagulation factors that are associated with impaired standard and viscoelastic coagulation assays and increased fibrinolysis.[8–12]

Acute hemoperitoneum may be life-threatening and requires immediate volume resuscitation. Electrolyte-balanced, buffered isotonic crystalloids (CRYSs) are the most frequently used and currently recommended solutions for fluid resuscitation in dogs with hypovolemic shock.[13–15] However, these solutions undergo rapid extravasation, and large volumes are required to expand the intravascular space and restore tissue perfusion, which may lead to fluid overload, vascular damage, tissue edema, and dilutional coagulopathy.[16] Given that hydroxyethyl starch (HES) has 3 to 4 times greater volume-expanding effects in both healthy dogs and dogs with hemorrhagic shock,[17,18] volume resuscitation with HES may be used for low-volume resuscitation or if CRYSs are insufficient for hemodynamic stabilization.[16,19–21] Nevertheless, HES administration impairs primary and secondary hemostasis,[22–24] which is associated with increased transfusion requirements in people.[25] Previous in vitro and in vivo studies showed dose-dependent, albeit short-lived, impairments in platelet function and viscoelastic coagulation testing in healthy dogs administered 6% tetrastarch (HES 130/0.4 or 130/0.42).[26–28] However, no differences in platelet function were found between 6% HES 130/0.4 and a 3- to 4-fold volume of 0.9% NaCl in an in vitro study in healthy dogs[27] and an in vivo study in dogs with controlled hemorrhagic shock.[29] These findings may suggest that 6% tetrastarch does not cause platelet dysfunction beyond the effects of hemodilution alone. However, more specific methods to assess platelet function (eg, flow cytometry and platelet aggregometry) are necessary to confirm this. In contrast, the aforementioned experimental study in dogs with controlled hemorrhagic shock demonstrated enhanced hypocoagulability by means of viscoelastic coagulation testing after HES administration compared with a 4-fold volume of 0.9% NaCl.[30] However, clinical studies comparing the effects of tetrastarch and isotonic CRYSs on coagulation assays in dogs with SHP are lacking.

To assess hemostasis in dogs, both standard plasmatic coagulation tests, such as prothrombin time (PT), activated partial thromboplastin time (aPTT), and fibrinogen concentration, and viscoelastic assays, including thromboelastography (TEG) and rotational thromboelastometry (ROTEM), have been used.[14–20] In contrast to standard coagulation tests, ROTEM monitors clot formation and firmness over time. As whole blood is used for analysis, the cellular contribution to hemostasis is more appropriately evaluated.[31] By using a combination of standard and viscoelastic assays, coagulation disorders due to deficiencies in extrinsic and intrinsic coagulation factors, hypofibrinogenemia, impaired fibrin polymerization, and thrombocytopenia can be discriminated.[32] Furthermore, viscoelastic assays have been used to detect both hypo- and hyperfibrinolytic states.[33]

The purpose of this prospective randomized open-label study was to compare the effects of treatment with 6% tetrastarch with the 3-fold greater volume of a polyionic isotonic CRYS on standard coagulation tests and ROTEM in dogs with SHP. Furthermore, the effects on HCT, platelet counts, heart rate, shock index, and plasma lactate concentrations were compared. Our first hypothesis was that dogs with SHP would have coagulopathies prior to treatment and that treatment with either fluid will exacerbate the existing coagulopathy. The second hypothesis was that HES would cause greater impairment in clot formation and fibrinolysis compared to the 3-fold volume of a CRYS.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals

Dogs with SHP and hypovolemic shock presented to the emergency service of the Small Animal Teaching Hospital of the Vetsuisse Faculty of Bern between January 2015 and June 2016 were included in the study. Hemoperitoneum was confirmed by removal of nonclotting blood with a PCV ≥ 20% via paracentesis during abdominal focused assessment with sonography for trauma (AFAST) at admission.[19] Dogs were considered to be in shock if at least 2 of the following criteria were fulfilled: heart rate > 140/min, shock index > 0.9 (shock index = heart rate divided by the systolic blood pressure),[3] and blood lactate concentration > 2.5 mmol/L. An acute patient physiologic and laboratory evaluation ($APPLE_{fast}$) score, calculated from blood

glucose, albumin, and lactate concentrations, platelet counts, and mentation score, was compiled for each dog at admission.[34] Exclusion criteria included age < 10 months, body weight < 8 kg, known trauma, a previous history of coagulopathy or disorders associated with coagulopathies (hyperadrenocorticism, protein-losing enteropathy or nephropathy, and hepatic or renal insufficiency), and administration of steroidal or nonsteroidal anti-inflammatory drugs, artificial colloids, or blood products within 2 weeks prior to admission.[21,24,35]

The study protocol was approved by the institutional ethics committee of the Veterinary University of Bern (BE19/14, No. 24972), and informed owner consent was acquired prior to study enrollment for every dog.

## 2.2 | Fluid resuscitation protocol

Dogs were randomly allocated to receive fluid resuscitation with either a polyionic isotonic CRYS solution[*] (CRYS group) or 6% HES 130/0.4[†] (HES group). Randomization was performed by choosing 1 of 10 sealed envelopes containing equal allocations to the CRYS and HES group. After randomizing the first 10 cases, 10 further envelopes were used to randomize the next group of dogs in the same manner. Dogs in the CRYS group received a bolus of 30 mL/kg, and dogs in the HES group received a bolus of 10 mL/kg within 20 minutes using 1 or 2 volumetric infusion pumps.[‡] In large-breed dogs, 2 large-bore venous catheters were placed to ensure bolus administration within 20 minutes. Heart rate, shock index, and blood sampling were assessed immediately prior to ($T_0$) and 5 minutes after ($T_1$) the end of the fluid bolus. Thereafter, any further fluid administration or other treatments were administered at the discretion of clinicians.

## 2.3 | Monitoring

Mentation, mucous membrane color, capillary refill time, heart rate, respiratory rate, body temperature, and oscillometric blood pressure[§] were recorded during resuscitation based on the institution's fluid resuscitation monitoring protocol.

## 2.4 | Blood sampling and analysis

Blood was sampled by atraumatic jugular venipuncture using a 21-Ga needle connected to a 10-mL syringe and a total volume of 8.0 mL of whole blood sampled. Venipuncture was performed on the right jugular vein for the first sample ($T_0$) and the left jugular vein for the second sample ($T_1$) to minimize sample contamination with tissue factor.[36,37] After venipuncture, each sample was immediately distributed into one 1.3-mL lithium heparin tube[¶] (for plasma lactate, plasma glucose, and albumin measurements), two 2.7-mL vacutainer tubes containing 3.2% trisodium citrate[#] (for ROTEM and standard coagulation assays), and one 1.3-mL EDTA tube[‖] (for HCT and platelet counts). Plasma lactate measurements were performed on whole blood within 5 minutes of sampling using a point-of-care blood gas analyzer.[**] The HCT and platelet counts,[††] glucose and albumin,[‡‡] and standard coagulation assays[§§] were performed by the Clinical Diagnostic Laboratory, Department of Clinical Veterinary Medicine, Vetsuisse Faculty, University of Bern within 2 hours of blood sampling. When platelet count was below 120,000/L, platelet count was estimated by blood smear evaluation (average platelet number in 10 high-power fields on a blood film microscopically and multiplying by 15,000). If samples were obtained after laboratory opening hours, 1 of the 2 citrate tube and lithium heparin tubes were centrifuged and the plasma separated; EDTA whole blood tubes and plasma separated from lithium heparin tubes were stored at 4°C, and plasma separated from citrate tubes was stored at −20°C for analyses the next day. Standard coagulation assays included PT, aPTT, and fibrinogen concentrations using the Clauss method.

All ROTEM[¶¶] assays were performed after venipuncture and samples were allowed to rest for 30 minutes at room temperature immediately after. Single portion reagents[##] were used for all assays. Each sample was extrinsically (EXTEM, tissue factor activation; FIBTEM; tissue factor activation plus cytochalasin D) and intrinsically (INTEM; contact activation with ellagic acid) activated, according to manufacturer's instructions. Data collected included clotting time (CT; the time from the start of the measurement until the onset of clotting), clot formation time (CFT; the time between the onset of clotting and a clot firmness of 20 mm amplitude), and maximum clot firmness (MCF; the maximum amplitude of the curve measured in millimeters MCF) for EXTEM and INTEM assays and MCF for FIBTEM assays. The CT represents the initiation to initial fibrin formation and is influenced by factor levels, CFT is an indicator of clot kinetics and is more strongly influenced by fibrinogen and platelets (usually not detectable in FIBTEM tracings), and MCF depends on both platelet and fibrinogen activation and the function of coagulation factor XIII.[31] Furthermore, lysis index at 30 ($LI_{30}$; percentage of clot firmness after 30 min relative to the MCF) and 45 ($LI_{45}$) minutes and maximum lysis for EXTEM and INTEM assays were determined. Samples were considered hypocoagulable if PT, aPTT, CT, or CFT was prolonged or fibrinogen or MCF was decreased compared to canine institutional reference intervals (RIs).[38] These were established based on 37 clinically healthy dogs including different sexes and ages. Dogs were considered healthy based on unremarkable clinical examination, complete blood cell count, biochemistry, and plasmatic coagulation analyses. Hyperfibrinolysis was defined as $LI_{30}$ or $LI_{45}$ below 100% or maximum lysis >10.[38]

## 2.5 | Statistical analyses

Statistical analyses were performed using commercial statistical software.[‖‖] Data were analyzed for normality using D'Agostino–Pearson tests and by examining normal plots. As some parameters were not normally distributed, nonparametric statistical analyses were used throughout. Data at $T_1$ were compared to baseline data at $T_0$, and the percentage change from $T_0$ to $T_1$ was calculated as $[(T_1 - T_0)/T_0] \times 100$. Mann–Whitney tests were used to compare paired data between

**TABLE 1** Standard coagulation parameters in 42 dogs with spontaneous hemoperitoneum before ($T_0$) and after ($T_1$) treatment with either 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group)

| Variable | Time point | HES group | CRYS group | *P*-value[a] |
|---|---|---|---|---|
| PT (s) (RI: 6.3–8.5) | $T_0$ | 10.1 (8.9–11.2)[c] | 9.2 (8.2–10.5)[c] | .215 |
| | $T_1$ | 10.5 (9.6–11.9)[b c] | 10.2 (8.7–13.4)[b c] | .261 |
| | % change from $T_0$ to $T_1$ | +8.1 (+5.9 to +11.2) | +7.6 (+4.9 to +12.8) | .899 |
| aPTT (s) (RI:9.6–16.1) | $T_0$ | 13.2 (12.0–16.1) | 13.0 (11.5–14.9) | .804 |
| | $T_1$ | 14.6 (13.4–17.6)[b] | 14.7 (13.1–16.5)[b] | .882 |
| | % change from $T_0$ to $T_1$ | +10.3 (+6.3 to +20.9) | +9.9 (+6.5 to +17.8) | .603 |
| Fibrinogen (g/L) (RI:1.50–3.00) | $T_0$ | 1.71 (0.99–2.24)[c] | 1.77 (0.96–2.50)[c] | .776 |
| | $T_1$ | 1.17 (0.78–1.50)[b c] | 1.26 (0.83–1.71)[b c] | .539 |
| | % change from $T_0$ to $T_1$ | −26.5 (−37.4 to −11.0) | −22.9 (−35.3 to −12.4) | .704 |

*Note.* Values are presented as median (interquartile range).
[a]*P*-value for between group comparison of hemostatic variables by Mann–Whitney test.
[b]Values differing significantly from $T_0$ ($P < 0.05$).
[c]Values outside the reference interval.
Abbreviations: aPTT, activated partial thromboplastin time; PT, prothrombin time; RI, reference interval.

groups, and Wilcoxon tests were used to compare paired data within groups between time points. Fisher's exact tests were used for analyses of categorical data. Spearman's rank correlation was used to compare percentage changes of hematocrit and fibrinogen concentration with ROTEM parameters. All tests were 2-tailed, and statistical significance was set at $P < 0.05$ throughout. Results are reported as median and interquartile range (IQR) unless otherwise indicated.

## 3 | RESULTS

### 3.1 | Cohort characteristics

Sixty-two dogs with hemoperitoneum and shock were presented during the study period. Of those, 20 dogs were excluded due to lack of informed owner consent (n = 7), traumatic hemoperitoneum (n = 11), and immediate euthanasia (n = 2). Of the 42 dogs included in the study, 20 were in the HES group and 22 were in the CRYS group.

Dogs represented 22 different breeds, the most common of which were mixed breed (n = 7), German Shepherd Dog (n = 6), Labrador Retriever (n = 3), Golden Retriever (n = 3), Flat-Coated Retriever (n = 3), and Rhodesian Ridgeback (n = 3). There were 17 females (CRYS group: 1 sexually intact and 8 neutered; HES group: 1 sexually intact and 7 neutered) and 25 male dogs (CRYS group: 3 sexually intact and 9 neutered; HES group: 6 sexually intact and 6 neutered). Dogs had a median age of 10.7 years (IQR, 2.0) in the CRYS group and 10.0 years (IQR, 2.6) in the HES group. The median body weight was 33.0 kg (IQR, 14.6) in the CRYS group and 34.8 kg (IQR, 17.6) in the HES group. No significant difference was found between the 2 groups for sex ($P = 0.83$), age ($P = 0.60$), or body weight ($P = 0.71$).

All dogs underwent abdominal ultrasound performed by a board-certified radiologist. Seventeen dogs (HES group, n = 7; CRYS group, n = 10) were sent to surgery. Of these, 15 survived to discharge, 1 dog was euthanized at surgery (CRYS group), and 1 dog died during the postoperative period (HES group). Histopathology was available in all 17 cases, and the following diagnoses were obtained: hemangiosarcoma (13; 76%), benign hematoma (3; 18%), and splenic fibrohistiocytic nodules (1; 6%). No significant difference was found in the number of dogs diagnosed with malignant neoplasia between the 2 groups ($P = 1.000$). The remaining 25 dogs were euthanized without surgery due to suspicion of diffuse metastatic neoplasia on abdominal (eg, presence of multiple cavitated splenic or hepatic lesions) or cardiac (right atrial lesion) ultrasound. Seven of these dogs had full body necropsy and were diagnosed with hemangiosarcoma.

### 3.2 | Standard coagulation assays

No significant differences were found between the groups for PT, aPTT, and fibrinogen concentrations at $T_0$ or $T_1$ or for the percentage change from $T_0$ to $T_1$ (Table 1). Within the CRYS group, PT ($P < 0.001$) and aPTT ($P = 0.008$) were significantly prolonged and fibrinogen concentrations ($P < 0.001$) decreased at $T_1$ compared to $T_0$ (Figure 1). Likewise, within the HES group, PT ($P < 0.001$) and aPTT ($P < 0.001$) were significantly prolonged and fibrinogen concentrations ($P < 0.001$) decreased at $T_1$ compared to $T_0$ (Figure 1). At $T_0$, 12 of 22 dogs in the CRYS group and 16 of 20 dogs in the HES group were classified as hypocoagulable ($P = 0.108$). At $T_1$, 20 of 22 dogs in the CRYS group and 19 of 20 dogs in the HES group were classified as hypocoagulable ($P = 1.000$).

### 3.3 | Rotational thromboelastometry

No significant differences were found in any ROTEM variable between the groups at $T_0$ (Table 2). At $T_1$, $CFT_{EXTEM}$ was significantly prolonged

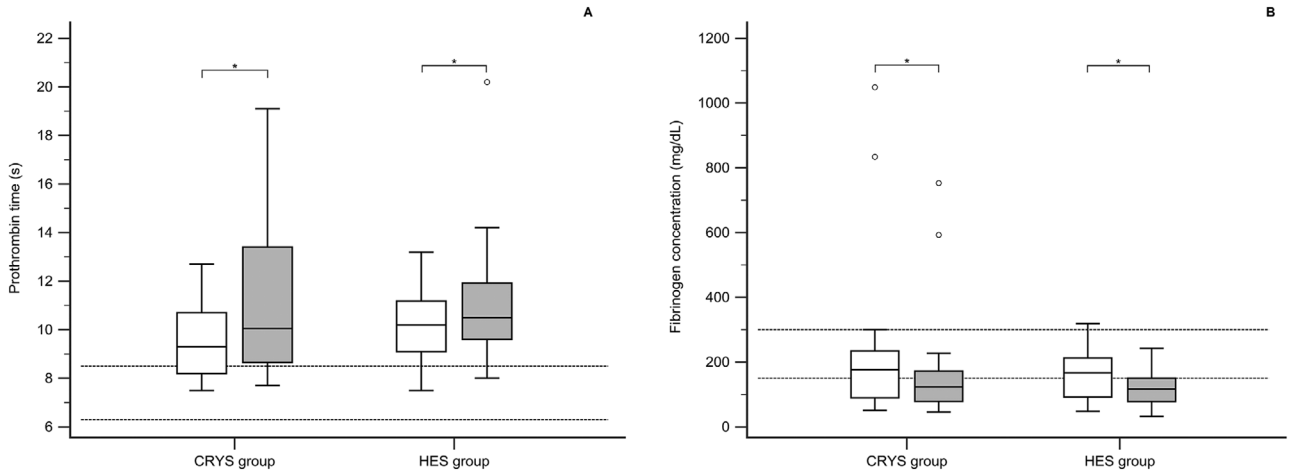


**FIGURE 1** Boxplots of (A) prothrombin time (PT) and (B) fibrinogen prior to ($T_0$, white boxplots) and 5 minutes after ($T_1$, gray boxplots) infusion with 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group). The upper and lower borders of the boxes represent interquartile range, whereas the limits of the whiskers denote the minimum and maximum values. The horizontal line within the box represents the median value. The dashed horizontal lines indicate the upper and lower limits of the reference interval. ° denotes an outlier value

**TABLE 2** Rotational thromboelastometry parameters in 42 dogs with spontaneous hemoperitoneum before ($T_0$) and after ($T_1$) treatment with either 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group)

| Variable | Time point | HES group | CRYS group | *P*-value[a] |
|---|---|---|---|---|
| $CT_{EXTEM}$ (s) (RI: 20–85) | $T_0$ | 47 (40–92) | 52 (37–118) | 0.937 |
| | $T_1$ | 111 (43–145)[b c] | 64 (39–114) | 0.291 |
| | % change from $T_0$ to $T_1$ | +32 (+15 to +60) | −4 (−32 to +31) | **0.012** |
| $CFT_{EXTEM}$ (s) (RI: 55–374) | $T_0$ | 291 (158–363) | 240 (134–337) | 0.423 |
| | $T_1$ | 385 (286–625)[b c] | 238 (179–376)[b] | **0.037** |
| | % change from $T_0$ to $T_1$ | +44 (−8 to +95) | +12 (−6 to +25) | 0.129 |
| $MCF_{EXTEM}$ (mm) (RI: 29–75) | $T_0$ | 48 (41–59) | 49 (38–61) | 0.800 |
| | $T_1$ | 42 (33–45)[b] | 48 (38–56)[b] | **0.038** |
| | % change from $T_0$ to $T_1$ | −13 (−25 to −3) | −5 (−9 to −1) | 0.072 |
| $CT_{INTEM}$ (s) (RI: 111–275) | $T_0$ | 166 (149–194) | 171 (139–200) | 0.917 |
| | $T_1$ | 200 (156–231)[b] | 169 (146–188) | 0.251 |
| | % change from $T_0$ to $T_1$ | +11 (−2 to +18) | +8 (−10 to +15) | 0.344 |
| $CFT_{INTEM}$ (s) (RI: 49–163) | $T_0$ | 193 (121–270)[c] | 143 (91–248) | 0.534 |
| | $T_1$ | 243 (107–436)[b c] | 170 (101–260)[b c] | 0.160 |
| | % change from $T_0$ to $T_1$ | +34 (+11 to +73) | +12 (−2 to +30) | **0.031** |
| $MCF_{INTEM}$ (mm) (RI: 47–68) | $T_0$ | 50 (46–56) | 54 (45–63) | 0.297 |
| | $T_1$ | 43 (36–61) | 51 (45–62)* | 0.303 |
| | % change from $T_0$ to $T_1$ | −10 (−20 to −4) | −4 (−8 to −2) | 0.593 |
| $MCF_{FIBTEM}$ (mm) (RI: 3–17) | $T_0$ | +5 (+4 to +8) | +6 (+4 to +9) | 0.613 |
| | $T_1$ | +5 (+3 to +6) | +5 (+3 to +8) | 0.303 |
| | % change from $T_0$ to $T_1$ | −18 (−50 to 6) | −10 (−36 to 4) | 0.927 |

*Note*. Values are presented as median (interquartile range).
[a]*P*-value for between group comparison of ROTEM variables by Mann–Whitney test.
[b]Values differing significantly from $T_0$ ($P < 0.05$).
[c]Values outside the reference interval.
Abbreviations: CFT, clot formation time; CT, clotting time; MCF, maximum clot firmness; RI, reference interval.

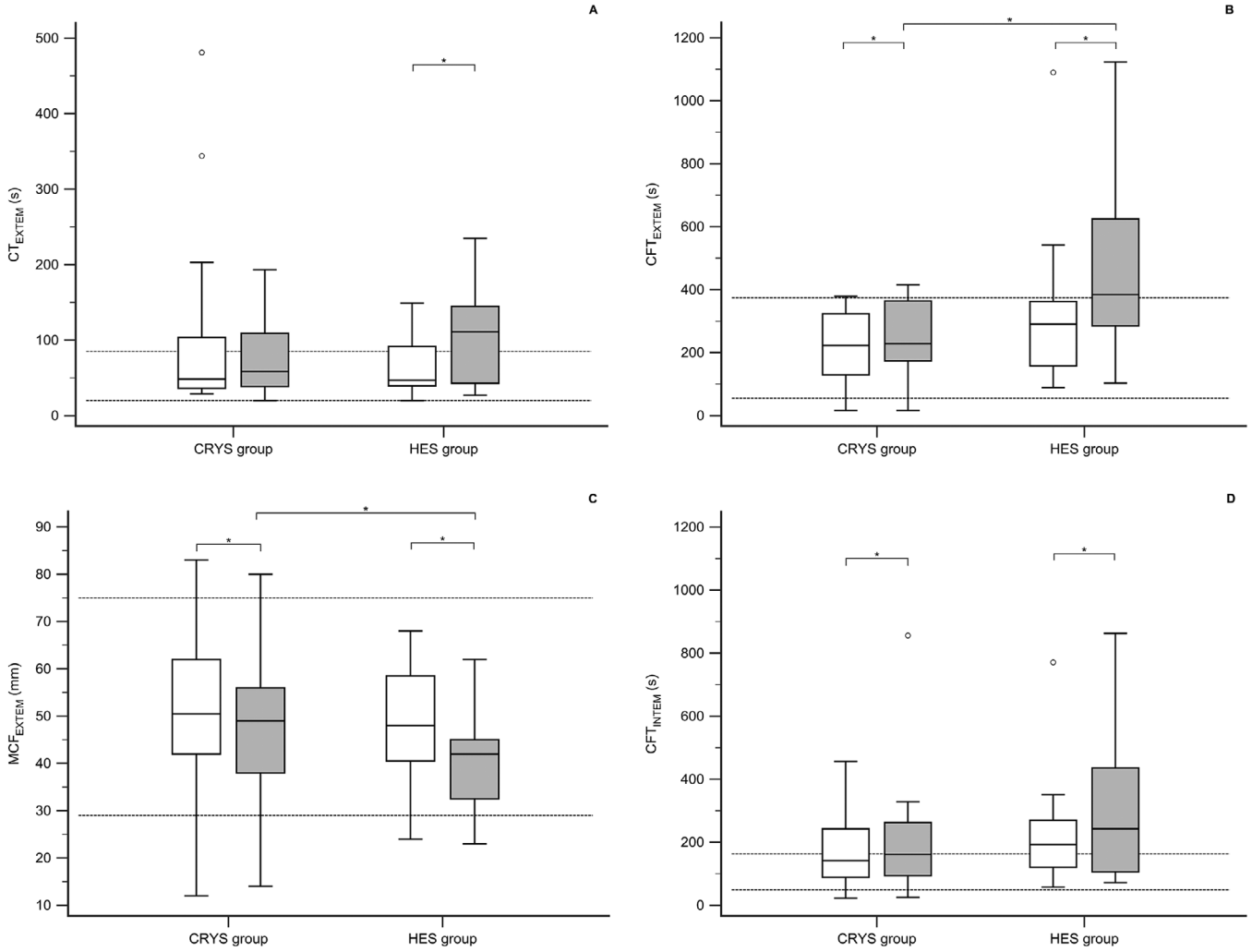


**FIGURE 2** Boxplots of (a) clotting time (CT) in EXTEM, (b) clot formation time (CFT) in EXTEM, (c) maximum clot firmness (MCF) in EXTEM, and (d) clot formation time in INTEM prior to ($T_0$, white boxplots) and 5 minutes after ($T_1$, grey boxplots) infusion with 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group). The upper and lower borders of the boxes represent interquartile range while the limits of the whiskers denote the minimum and maximum values. The horizontal line within the box represents the median value. The dashed horizontal lines indicate the upper and lower limits of the reference interval. ° denotes an outlier value

and $MCF_{EXTEM}$ significantly decreased in the HES group compared to the CRYS group (Table 2). Moreover, a higher percentage change in $CT_{EXTEM}$ and $CFT_{INTEM}$ from $T_0$ to $T_1$ was found in the HES group (Table 2).

Significant differences between $T_0$ and $T_1$ were found in the HES group for $CT_{EXTEM}$ ($P = 0.001$), $CT_{INTEM}$ ($P = 0.006$), $CFT_{EXTEM}$ ($P = 0.012$), $CFT_{INTEM}$ ($P = 0.002$), and $MCF_{EXTEM}$ ($P = 0.004$) (Figure 2). Significant differences between $T_0$ and $T_1$ were found in the CRYS group for $CFT_{EXTEM}$ ($P = 0.045$), $CFT_{INTEM}$ ($P = 0.020$), $MCF_{EXTEM}$ ($P = 0.044$), and $MCF_{INTEM}$ ($P = 0.003$) (Figure 2). Based on ROTEM variables, at $T_0$, 12 of 22 dogs in the CRYS group and 12 of 20 dogs in the HES group were classified as hypocoagulable ($P = 0.764$). At $T_1$, 13 of 22 dogs in the CRYS group and 14 of 20 dogs in the HES group were classified as hypocoagulable ($P = 0.531$). The median $LI_{30}$ and $LI_{45}$ in all 3 assays was 100%, and the maximum lysis index was <10 at both time points in both groups (data not shown).

No significant correlation between percentage changes of HCT or fibrinogen concentration, respectively, and ROTEM parameters was found.

## 3.4 | Hematocrit and platelet counts

No significant differences in HCT and platelet counts were found between the groups at $T_0$ or $T_1$ or for the percentage change from $T_0$ to $T_1$ (Table 3). Significant differences between $T_0$ and $T_1$ within both groups were found for HCT ($P < 0.001$) and platelet counts ($P < 0.001$) (Figure 3). At $T_0$, 17 of 22 dogs in the CRYS group and 14 of 20 dogs in the HES group had HCTs below the RI ($P = 0.730$). At $T_1$, 21 of 22 dogs in the CRYS group and 19 of 20 dogs in the HES group had HCTs below the RI ($P = 1.000$). At $T_0$, 16 of 22 in the CRYS group and 14 of 20 dogs in the HES group had platelet counts below the RI ($P = 1.000$). At $T_1$, 17

**TABLE 3** Hematocrit and platelet counts in 42 dogs with spontaneous hemoperitoneum before ($T_0$) and after ($T_1$) treatment with either 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group)

| Variable | Time point | HES group | CRYS group | *P*-value[a] |
|---|---|---|---|---|
| Hematocrit (%) (RI: 39–57) | $T_0$ | 30.5 (24.0–39.5)[c] | 25.5 (22.0–37.0)[c] | 0.399 |
| | $T_1$ | 22.5 (17.0–27.0)[b c] | 18.5 (16.0–29.0)[b c] | 0.521 |
| | % change from $T_0$ to $T_1$ | −24 (−33 to −19) | −23 (−29 to −18) | 0.529 |
| Platelets ($\times 10^9$/L) RI: 150–400) | $T_0$ | 81 (54–156)[c] | 110 (58–155)[c] | 0.641 |
| | $T_1$ | 64 (36–121)[b c] | 77 (42–124)[b c] | 0.465 |
| | % change from $T_0$ to $T_1$ | −22 (−32 to −15) | −22 (−33 to −12) | 0.886 |

*Note*. Values are presented as median (interquartile range).
[a]*P*-value for between group comparison of hematocrit and platelet counts by Mann–Whitney test.
[b]Values differing significantly from $T_0$ ($P < 0.05$).
[c]Values outside the reference interval.

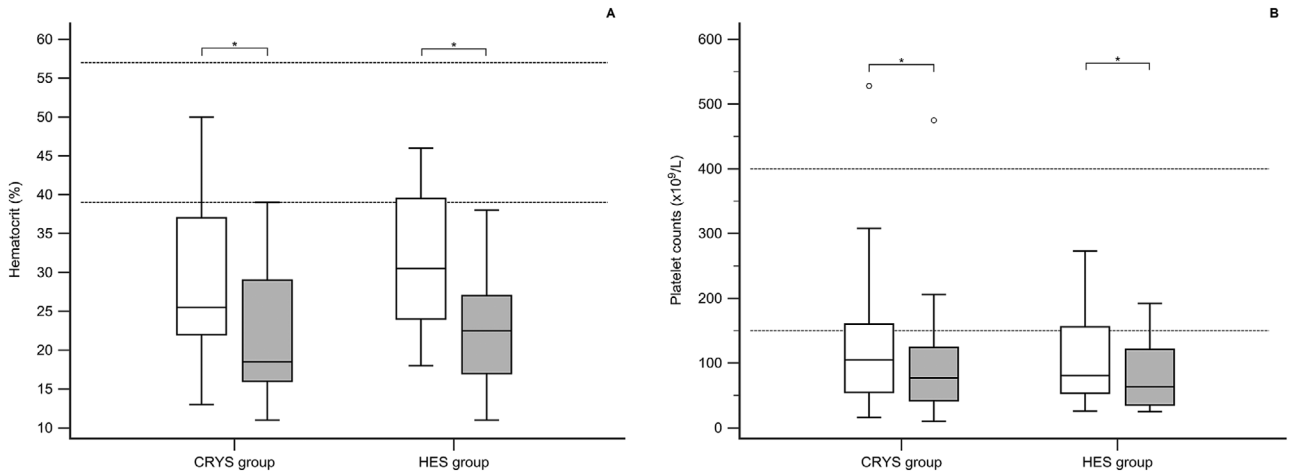


**FIGURE 3** Boxplots of (A) HCT and (B) platelet counts (PLTs) prior to ($T_0$, white boxplots) and 5 minutes after ($T_1$, gray boxplots) infusion with 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group). The upper and lower borders of the boxes represent interquartile range, whereas the limits of the whiskers denote the minimum and maximum values. The horizontal line within the box represents the median value. The dashed horizontal lines indicate the upper and lower limits of the reference interval. ° denotes an outlier value

of 22 dogs in the CRYS group and 18 of 20 dogs in the HES group had platelet counts below the RI ($P = .414$).

### 3.5 | Hemodynamic parameters, $APPLE_{fast}$ scores, and lactate concentrations

No significant differences in heart rate, shock index, lactate concentrations, or $APPLE_{fast}$ scores were found between the groups at $T_0$ or $T_1$ or for the percentage change from $T_0$ to $T_1$ (Table 4). No significant differences were found between $T_0$ and $T_1$ within either group for heart rates or shock index, and median shock index remained above the RI in the HES group at $T_1$ (Figure 4). However, lactate concentrations were significantly decreased after fluid therapy in both the HES ($P = 0.004$) and CRYS groups ($P = 0.001$) (Figure 4).

## 4 | DISCUSSION

This is the first clinical study comparing the effects of 6% tetrastarch with the 3-fold volume of a polyionic isotonic buffered CRYS on standard and viscoelastic coagulation assays in dogs with SHP. Dogs in the present study were hypocoagulable at admission as previously described,[8–11] and fluid administration exacerbated coagulation impairment based on both standard and viscoelastic assays in both groups. However, only ROTEM variables were more severely affected by administration of HES compared to administration of polyionic CRYSs. Significant differences compared to CRYS were observed in $CT_{EXTEM}$, $CFT_{EXTEM/INTEM}$, and $MCF_{EXTEM}$, which suggests delayed clot formation, a weaker clot, and a less stable fibrin network. The fact that PT and aPTT did not differ significantly between the groups may support their limited value in discovering global coagulopathies, as these tests do not incorporate effects of cellular elements.[31]

**TABLE 4** Cardiovascular parameters, lactate concentrations, and $APPLE_{fast}$ scores in 42 dogs with spontaneous hemoperitoneum before ($T_0$) and after ($T_1$) treatment with either 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group)

| Variable | Time point | HES group | CRYS group | *P*-value[a] |
|---|---|---|---|---|
| $APPLE_{fast}$ score | $T_0$ | 29.5 (26.5–35.5) | 28.0 (25.0–29.0) | 0.115 |
| Heart rate (per minute) | $T_0$ | 148 (127–160) | 132 (120–149) | 0.075 |
| | $T_1$ | 120 (106–155) | 120 (110–140) | 0.747 |
| | % change from $T_0$ to $T_1$ | −6 (−25 to +10) | −3 (−33 to +4) | 0.580 |
| Shock index (RI: <0.9) | $T_0$ | 1.1 (1.0–1.4)[b] | 1.1 (0.9–1.2)[b] | 0.225 |
| | $T_1$ | 1.1 (0.8–1.2)[b] | 0.8 (0.8–1.2) | 0.477 |
| | % change from $T_0$ to $T_1$ | + 8 (−27 to +25) | −11 (−33 to −10) | 0.373 |
| Lactate (mmol/L) (RI: <2.5) | $T_0$ | 4.0 (3.3–6.0)[b] | 3.6 (2.8–4.9)[b] | 0.286 |
| | $T_1$ | 3.5 (2.4–4.6)[b] | 2.5 (1.7–3.1)[b] | 0.065 |
| | % change from $T_0$ to $T_1$ | −18 (−28 to −10) | −32 (−50 to −26) | 0.074 |

*Note*. Values are presented as median (interquartile range).

[a]*P*-value for between groups comparison of heart rate, shock index, lactate concentrations, and APPLEfast by Mann–Whitney test.

[b]Values differing significantly from $T_0$ ($P < 0.05$).

[c]Values outside the reference interval.

Abbreviation: $APPLE_{fast}$, acute patient physiologic and laboratory evaluation score.

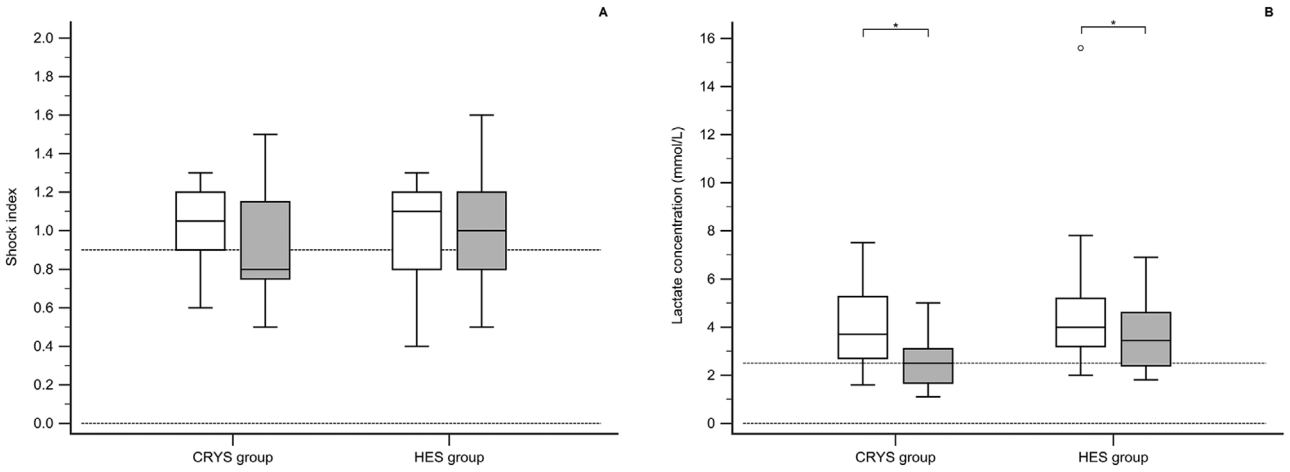


**FIGURE 4** Boxplots of (A) shock index (SI) and (B) blood lactate concentration (lactate) prior to ($T_0$, white boxplots) and 5 minutes after ($T_1$, gray boxplots) infusion with 30 mL/kg polyionic isotonic crystalloid (CRYS group) or 10 mL/kg 6% hydroxyethyl starch 130/0.4 (HES group). The upper and lower borders of the boxes represent interquartile range, whereas the limits of the whiskers denote the minimum and maximum values. The horizontal line within the box represents the median value. The dashed horizontal lines indicate the upper and lower limits of the reference interval. ° denotes an outlier value

Our findings corroborate results observed in previous in vitro and in vivo studies using different tetrastarch preparations in healthy dogs, in which a dose-dependent hypocoagulability in ROTEM after HES, and beyond dilution with a CRYS, was found.[26–28] Likewise, in a study in anesthetized dogs with controlled hemorrhagic shock, $CT_{EXTEM}$ was found to be significantly more prolonged after HES compared with the 4-fold volume of a balanced isotonic CRYS.[30] However, our results are in contrast to the findings of an in vitro study in healthy dogs, in which no significant difference in ROTEM parameters was found when clinical doses of HES and saline (eg, mimicking 10 mL/kg of HES and 30 mL/kg saline) were compared, except a greater decrease in $MCF_{EXTEM}$ after saline.[27] These findings suggest that in vitro studies may not adequately reflect the effects of HES on coagulation in dogs or that ROTEM parameters are affected more severely in dogs with SHP and preexisting hypocoagulability than in healthy dogs.

More ROTEM parameters were beyond the institutional reference intervals after HES compared to CRYS, and more dogs in this group classified as hypocoagulable (based on the definition of hypocoagulability used in this study). Few studies were able to predict the risk of bleeding by viscoelastometric coagulation assays in dogs.[39,40] In a prospective study in which clinical signs of bleeding were noted at time of coagulation analysis, tissue factor-activated TEG assay

(corresponding to the EXTEM assay) correctly identified dogs with clinical signs of bleeding and did so better than a standard coagulation test.[40] In another retrospective study, $CFT_{EXTEM}$, $MCF_{EXTEM}$, and $CT_{INTEM}$ were significantly different between bleeding and nonbleeding dogs.[39] Dogs in the present study were already bleeding per definition, as this was an inclusion criterion. However, the extent to which the ROTEM changes in the HES group may be associated with a higher clinical risk of bleeding compared to the CRYS group remains unclear, as no bleeding risk scoring system was used.

In contrast to our results, previous studies[26–28] found a significant decrease in fibrinogen function based on $MCF_{FIBTEM}$ in the HES groups. Despite the known HES-associated impairment of fibrin polymerization and subsequent reduction in clot firmness,[41,42] this was not detected in the present study (the percentage change of $MCF_{FIBTEM}$ in the HES group was not significantly greater compared with the CRYS group). Moreover, MCF in all assays remained within references ranges in both groups. This apparent discrepancy to previous studies[18,29,43] may be due to the lower dose of HES used in the present study, indicating that larger fluid volumes may be necessary to detect changes in MCF.

Incorporation of HES molecules into polymerizing fibrin strands with subsequent alteration in clot strength and accelerated clot lysis is believed to underly HES-associated increased fibrinolysis.[44,45] Although hyperfibrinolysis detected by viscoelastometric assays or euglobulin lysis time assays has not been documented in people receiving synthetic colloids,[42,46] a transient increase in TEG clot lysis rates after 30 and 60 minutes was observed in dogs after tetrastarch infusion (40 mL/kg).[47] In the present study, no evidence of hyperfibrinolysis was detected based on $LI_{30}$ and $LI_{45}$ and maximum lysis after HES administration. This discrepancy may be due to the very large dose of tetrastarch used in the previous study assessing TEG clot lysis rates (4 times greater than that used in the present study). As such a large dose does not reflect clinical practice, the apparent hyperfibrinolysis previously detected may not be of clinical relevance. Moreover, no evidence of hyperfibrinolysis prior to fluid administration was found in the present study, whereas hyperfibrinolysis was detected in another previous study in dogs with SHP but only after a tissue plasminogen activator-modified TEG was performed.[11] It remains unclear if tissue plasminogen activator-modified ROTEM would have uncovered hyperfibrinolysis in SHP dogs in the present study.

Two previous studies in dogs have suggested that low RBC mass may be associated with artificial hypercoagulability in TEG or ROTEM tracings (most likely due to larger plasma volume and, subsequently, more clotting factors per volume).[37,48] In contrast, after moderate- and high-degree dilution of canine whole blood samples with fresh frozen plasma (down to a hematocrit of 14%), no significant evidence of hypercoagulability was found in TEG. It remains unclear if anemia-induced artificial hypercoagulability could have attenuated the coagulopathic effects of either HES or CRYS in the present study.

Platelet dysfunction after HES administration has been described in both people and dogs.[42] Possible mechanisms include extracellular coating of platelets with colloidal macromolecules, inhibiting conformational changes, and interactions between glycoproteins IIb–IIIa and Ib and their ligands with subsequent decreased platelet aggregability and adhesion.[42] Previous in vitro and in vivo studies on evaluation of canine platelet function after tetrastarch by using a Platelet Function Analyzer-100 (a device used particularly to detect platelet dysfunction and von Willebrand disease)[49] have shown inconsistent results. Two studies (using HES 130/0.42, a potato-based starch) found a dose-dependent impairment of platelet function beyond the dilutional effect.[28,50] Although 3 other studies (using HES 130/0.4, a maize-based starch) also found some degree of platelet dysfunction after HES, this was not significantly different when compared with the 3- or 4-fold volume of an isotonic CRYS.[27,29,30] In contrast to the Platelet Function Analyzer-100, which is measuring the time in seconds from onset of aspiration of citrated whole blood to complete closure of the aperture by a platelet plug,[49] viscoelastic tests such as the ROTEM assess alterations in the viscosity of coagulating blood, either with or without additional activators.[31] The viscosity of the clotting blood is substantially affected by the number and function of platelets, fibrinogen concentration, and the platelet–fibrinogen interaction (glycoprotein IIb/IIIa receptor action).[31,48] The primary ROTEM variable affected by the contribution of platelets to clot formation is $MCF_{EXTEM/INTEM}$, although severe thrombocytopenia or hypofibrinogenemia will also affect CFT.[31] In the present study, dogs in both groups were thrombocytopenic at both time points, with a significant greater magnitude of thrombocytopenia at $T_1$ and subsequent decrease in $MCF_{EXTEM}$. The significantly lower $MCF_{EXTEM}$ in the HES group at $T_1$ compared with CRYS may be suggestive of possible platelet dysfunction. However, further specific analyses would be needed to rule out platelet dysfunction.

No differences between the groups were detected in heart rate, shock index, and blood lactate concentrations, suggesting similar hemodynamic effects of 10 mL/kg HES and 30 mL/kg CRYS. Moreover, median shock index in the HES group remained above the reference range at $T_1$, suggesting an inadequate volume effect compared to CRYS. Previous experimental studies in dogs have demonstrated that the volume-expanding effects of tetrastarch is 3–4 times that of isotonic CRYSs,[18–20,51] and 3-fold volumes of CRYSs have similar effects on organ perfusion, although some studies found significantly prolonged PT and decreased HCTs and platelet count, which was not detected in the present study.[51]

The present study has several limitations. First, coagulation assays were evaluated at only a single time point after fluid therapy and were likely performed prior to complete hemodynamic stabilization. Indeed, recent research in healthy dogs found transient effect of IV HES on platelets and coagulation parameters that were abolished after 2–4 hours.[28,47] However, in dogs with experimentally induced systemic inflammation, the same volume of HES (40 mL/kg) led to decreased clot formation speed and clot strength over a time period of 4–24 hours. In the study herein, coagulation analyses at later time points may have uncovered time-dependent changes in dogs with SHP. Second, the extent of intraoperative bleeding was not assessed. Evaluation of the correlation between intraoperative bleeding and changes in coagulation with the 2 different study fluids would have helped to interpret the possible clinical relevance of the detected changes.

Third, the current study design precludes strong conclusions about the presence or absence of hyperfibrinolysis in patients with SHP as tissue plasminogen activator-modified ROTEM assays to detect increased fibrinolytic susceptibility of forming clots were not performed.[52] In addition, the presence of hyperfibrinolysis was not further investigated using other markers of hyperfibrinolysis, such as D-dimers or fibrinogen degradation products.[53] Last, platelet function was not separately analyzed in the present study, and a possible influence of low platelet counts or HCTs on ROTEM parameters cannot be ruled out.[54]

In conclusion, fluid resuscitation with HES or with a 3-fold greater volume of CRYS lead to significant changes in both standard coagulation tests and ROTEM assays, with more pronounced effects on ROTEM assays after HES. Clot strength was only mildly affected, and no evidence of hyperfibrinolysis was found in the present study, but further research is warranted. Whether these HES-induced ROTEM changes are associated with an increased risk of bleeding is unclear and needs further investigation. Clinicians should be aware of the potential exacerbation of a preexisting coagulopathy in dogs with SHP when considering HES administration for volume resuscitation.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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## ENDNOTES

* Plasma-Lyte A, Viaflo, Baxter AG, Volketswil, Switzerland.
† Volulyte, Fresenius Kabi AG, Oberdorf, Switzerland.
‡ Infusomat Space, B Braun Melsungen AG, Melsungen, Germany.
§ Cardell 9401, Midmark, 124 Dayton, OH.
¶ Lithium heparin, Sarstedt AG, Sevelen, Switzerland.
# Trisodium-citrate 3.2% (1:10), Sarstedt AG, Sevelen, Switzerland.
‖ K-EDTA, Sarstedt AG, Sevelen, Switzerland.
** RAPIDPoint 500, Siemens Healthcare AG, Zurich, Switzerland.
†† Advia 2120, Siemens Healthcare Diagnostics AG, Zurich, Switzerland.
‡‡ Cobas c501, Roche Diagnostics, Rotkreuz, Switzerland.
§§ Start MAX, Stago CH SA, Zurich, Switzerland.
¶¶ ROTEM, TEM Innovations GmbH, Munich, Germany.
## ex-tem S/in-tem S/fib-tem S, TEM Innovations GmbH, Munich, Germany.
‖‖ MedCalc Statistical Software version 17.1, Medcalc Software bvba, Ostend, Belgium; https://www.medcalc.org; 2017.

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