Fluid resuscitation-related coagulation impairment in a porcine hemorrhagic shock model

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36 **Abstract:**

37 **Background:** Fast and effective treatment of hemorrhagic shock is one of the most important

38 preclinical trauma care tasks e.g. in combat casualties in avoiding severe end-organ damage or

39 death. In scenarios without immediate availability of blood products, alternate regimens of fluid

40 resuscitation represent the only possibility of maintaining sufficient circulation and regaining

41 adequate end-organ oxygen supply. However, the fluid choice alone may affect the extent of the

42 bleeding by interfering with coagulation pathways. This study investigates the impact of

43 hydroxyethyl starch (HES), gelatine-polysuccinate (GP) and balanced electrolyte solution (BES)

44 as commonly used agents for fluid resuscitation on coagulation using a porcine hemorrhagic shock

45 model.

46

47 **Methods:** Following approval by the State and Institutional Animal Care Committee, life-

48 threatening hemorrhagic shock was induced via arterial blood withdrawal in 24 anesthetized pigs.

49 Isovolumetric fluid resuscitation with either HES, GP or BES (n = 3 x 8) was performed to

50 compensate for the blood loss. Over four hours, hemodynamics, laboratory parameters and

51 rotational thromboelastometry-derived coagulation were analyzed. As secondary endpoint the

52 porcine values were compared to human blood.

53

54 **Results:** All the agents used for fluid resuscitation significantly affected coagulation. We

55 measured a restriction of laboratory parameters, clot development and clot firmness, particularly

56 in HES- and GP-treated animals. Hemoglobin content dropped in all groups but showed a more

57 pronounced decline in colloid-treated pigs. This effect was not maintained over the four-hour

58 monitoring period.

59

60 **Conclusion:** HES, GP, and BEL sufficiently stabilized the macrocirculation, but significantly

61 affected coagulation. These effects were most pronounced after colloid and particularly HES

62 administration. Despite suitability for rapid hemodynamic stabilization, colloids have to be chosen

63 with caution, because their molecular properties may affect coagulation directly and as a

64   consequence of pronounced hemodilution. Our comparison of porcine and human coagulation

65   showed increased coagulation activity in pig blood.

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Background:

70   Massive blood loss as a hemorrhagic shock causes requires resolute and efficient action to prevent

71   severe injury or death. As a factor in 15% of all fatal injuries, it is one of the most challenging

72   issues in prehospital and early clinical scenarios (1, 2). The instability that results from massive

73   blood loss induces an undersupply of oxygen and can cause irreversible organ failure (3, 4). To

74   combat this problem, different regimens and guidelines focusing on fluid resuscitation have been

75   established (5, 6). For years, colloids and especially hydroxyethyl starch (HES) have been

76   indispensable for nearly all forms of shock resulting from relative or absolute hypovolemia. Their

77   efficacy relies on macromolecule-related increases in oncotic pressure and decreased

78   extravasation. Crystalloids, however, act only temporarily due to a free volume-specific shift into

79   the extravascular space (7). Several clinical studies have questioned the safety of HES in the

80   treatment of critical ill patients (8-10). Side effects such as increased risk of acute kidney injury,

81   which requires renal replacement therapy and can even lead to mortality, have challenged the role

82   of HES in the intensive care unit (11-14). Other detrimental effects of HES include increased

83   perioperative blood loss and coagulopathy. Some studies, however still regard HES administration

84   as reliable option, particularly in isolated, manageable bleeding scenarios and patient without risk

85   constellation for acute kidney injury, which has led to controversial discussions (15, 16). In the

86   past, the European Medicines Agency (EMA) recommended avoiding HES and prohibited its use

87   in patients with severe sepsis or burn injuries (6). In 2018 and 2019, however, the EMA re-

88   permitted administration of HES for the management of acute blood loss-induced hypovolemia

89   while pointing out several contraindications, including sepsis, severe coagulopathy and renal

90   impairment or replacement therapy (6, 17). Nevertheless, these recommendations are undermined

91   by lack of alternative solutions and clinical scenarios that represent a grey zone between defined

92   indications and prohibitions. Gelatine-polysuccinate (GP) represents an alternative but less

93   frequently applied colloid based on bovine collagen. Apart from its shorter intravascular

94   persistence, GP appears to be comparable to modern HES 130/0.4 (18, 19). Interestingly,

95   knowledge of the disadvantages and advantages of GP is limited (20-23), with the exception of a

96   higher risk of anaphylaxis compared to other colloids (24, 25). Bleeding control is a crucial task

97   during hemorrhagic shock and requires sufficient coagulation maintenance. Though indicated in

98   blood loss-related hypervolemia, HES bears the risk of coagulation impairment. (26-31).

99   Comparisons between HES and GP have shown similar detrimental effects, but only few studies

100 have investigated these effects during hemorrhagic shock (32-34).

101 Our aim was to explore the influence of HES and GP on coagulation with a focus on point-of-care

102 rotational thromboelastometry (ROTEM), which enables to fibrin polymerization analysis, clot

103 firmness and formation, platelet function and fibrinolysis through different assays (27).

104 Viscoelastic point-of-care methods are explicitly discussed in the European guidelines for the

105 management of traumatic and perioperative bleeding (6, 35) and are an important instrument in

106 early goal-directed coagulation therapy (36). We hypothesized that comparable amounts of HES

107 and GP would impair the coagulation cascade more so than balanced electrolyte solution (BES) in

108 a pig model simulating a preclinical hemorrhagic shock scenario.

109

110 **Methods**

111 This study was approved by the State and Institutional Animal Care Committee

112 (Landesuntersuchungsamt Rheinland-Pfalz, Koblenz, Germany; Chairperson: Dr Silvia Eisch-

113 Wolf; reference number: 23 177-07/G 15-1-092; 01/2016) in accordance with the ARRIVE

114 guidelines. This study represents an independent sub-project and complementary hypothesis of a

115 research project that investigates the cerebral effects of solutions for fluid resuscitation and did not

116 increase the overall number of animal experiments within this project. We included 24 juvenile

117 male pigs (*sus scrofa domestica*; mean weight 28 ± 2 kg; age: 8-12 weeks) in a prospective

118 randomized animal experiment.

119

120 *Anesthesia and instrumentation*

121 The anesthesia, instrumentation and shock induction were performed as previously described (37,

122 38). The animals stayed in their known environment for as long as possible to minimize stress, and

123 breeder controlled environmental conditions. The animals were sedated via intramuscular

124 injections of ketamine (8 mg/kg) and midazolam (0.2 mg/kg) to minimize their stress during

125 transport. Fentanyl (4 µg/kg), propofol (4 mg/kg) and atracurium (0.5 mg/kg) were injected

126 intravenously via an ear-vein for general anesthesia and endotracheal intubation. Continuous

127 infusion of fentanyl (0.1-0.2 mg/kg/h) and propofol (8-12 mg/kg/h) maintained the anesthesia.

128 During the experiment, the animals were held under general anesthesia by means of continuous

129 propofol (8-12 mg/kg/h) and fentanyl (0.1-0.2 mg/kg/h) and ventilated in the following volume-

130 controlled mode (Evita 2, Draeger, Lübeck, Germany): positive end-expiratory pressure of 5

131 cmH_2O, a tidal volume of 8 ml/kg, fraction of inspired oxygen of 0.4, an inspiration to expiration

132 ratio of 1:2 and a variable respiration rate to achieve end-tidal $CO_2 < 6$ kPa.

133 Four femoral vascular catheters were placed using ultrasound guidance as follows: central venous

134 line (drug administration and thermodilution-related cold saline application, left femoral vein),

135 pulse contour cardiac output system (PiCCO, Pulsion Medical Systems, Germany, right femoral

136 artery), arterial line (blood withdrawal for shock model, left femoral artery) and large-bore venous

137 introducer (fluid resuscitation, right femoral vein). Hemodynamic and spirometric parameters

138 were constantly measured and recorded (LabVIEW, AD-Instruments, Sydney, Australia).

139

140 *Experimental design*

141 Instrumentation was performed, followed by 30 minutes of consolidation and baseline

142 documentation. Hemorrhagic shock was induced via arterial blood withdrawal (35-40 ml/kg) over

143 15 minutes until mean arterial pressure (<45 mmHg) and cardiac index (<40% of the baseline

144 value) decreased (38). Figure 1 displays a short summary of this protocol.

145 Thirty minutes after the shock induction, the animals were randomized and treated according to a

146 group-specific fluid resuscitation regime and adjusted, according to the volume of removed blood,

147 as follows:

148

149   GP (Gelafundin iso 4%, B. Braun, Germany; n = 8)

150   HES 130/0.4 (Volulyte 6%, Fresenius Kabi AG, Germany; n = 8)

151   BES (Sterofundin iso, B. Braun, Germany; n = 8).

152

153 No specific cardio-circulatory parameters influenced this approach. No further fluids or

154 catecholamine were administered other than a permanent BES infusion (5 ml/kg/h). At the

155   baseline, following fluid resuscitation and at the end of the experiment (baseline, 0 h and 4 h),

156   blood was withdrawn for coagulation and laboratory analysis into standard test tubes. After four

157   hours of monitoring, the animals were killed during deep general anesthesia via central venous

158   administration of propofol and potassium chloride.

159

160   *Laboratory assessment*

161   Blood samples were collected at the baseline, following fluid resuscitation (0 h) and after four

162   hours of observation (4 h). The blood was stored in tubes with 0.14 ml citrate solution (S-

163   Monovette®, Sarstedt, Nuembrecht, Germany). Laboratory blood tests, including partial

164   thromboplastin time (PTT), prothrombin time (PT), fibrinogen, platelet count, hemoglobin content

165   and hematocrit, were used to analyze standard coagulation and blood parameters.

166

167   *Rotational thromboelastometry*

168   Rotational thromboelastometry (ROTEM® delta; TEM International GmbH, Munich, Germany)

169   analyses were performed according to operation instructions. Thromboelastometry was able to

170   detect changes in the viscoelastic characteristics of a citrate blood clot. The blood samples were

171   mixed with specific reagents to allow for analysis of coagulation properties through the following

172   five tests: EXTEM, which investigates the tissue factor pathway (extrinsic); INTEM, which

173   determines the contact activation pathway (intrinsic); HEPTEM, which analyses heparin-induced

174   effects in comparison to INTEM; FIBTEM, which examines fibrinogen deficiency or restricted

175   fibrin polymerization in clots through the addition of cytochalasin D to inhibit platelet

176   contribution; and APTEM, which uses aprotinin to analyze fibrinolysis. We performed all tests

177   excluding HEPTEM, as no heparin was provided in the study protocol. To validate the measured

178   values, we compared the baseline parameters with human references (39). Thromboelastometry

179   analyses depict three phases of coagulation: clot formation (clotting time = CT, time until clot

180   starts to form; clot formation time = CFT, time between CT and an amplitude of 20 mm clot

181   firmness; alpha-angle = alpha, angle between 0 and 2 mm of amplitude), clot firmness (A10 and

182   A20, amplitude of clot firmness at 10 and 20 minutes; maximum clot firmness = MCF, the absolute

183   highest amplitude of the curve) and lysis (maximum lysis = ML, maximum percentage loss of clot

184   strength compare to MCF) (40, 41) .

185

186   *Statistics*

187   We displayed the data in terms of mean and standard deviation (SD). The intergroup effects were

188   analyzed using two-way analysis of variance (ANOVA). The Student-Newman-Keuls method was

189   used for pairwise multiple comparison corrections. P values lower than 5% were considered

190   significant. The statistical analysis was performed utilizing software package SigmaPlot 12.5

191   (Systat Software, USA). The porcine reference values were displayed as confidence intervals of a

192   confidence level of 95%.

193

194   **Results:**

195   Comparable amounts of blood were withdrawn (33 ± 5 ml/kg) for shock induction from all three

196   groups. Arterial blood pressure ($58 \pm 11\%$) and cardiac index ($50 \pm 12\%$) significantly decreased

197   in a shock-like fashion versus the baseline (each $p < 0.01$) (Table 1). The amounts of fluid

198   administered were also comparable.

199   In the HES and BES groups, a survival rate of 100% was achieved. In the GP group, one animal

200   died during a tachyarrhythmia episode one hour after the fluid resuscitation. The different infusion

201   regimens led to adequate hemodynamic stabilization in all groups. The cardiac index increase was

202   significantly greater following colloid infusion. After four hours, all values returned to the baseline

203   level, with the exception of heart rate in the BES group (Table 1). Immediately following shock

204   induction, the hemoglobin content was relatively unaltered; however, it significantly decreased

205   throughout fluid resuscitation, with a pronounced decrease in the colloid groups (each $p < 0.01$).

206   Fibrinogen, PT and PTT became considerably impaired in the colloid groups, whereas the BES

207   group was only affected to a minor extent (Table 1 and Figure 2).

208   The rotational thromboelastometry data showed no intergroup differences at the baseline.

209   Parameters concerning the temporal sequence (CT, CFT, alpha) as well as clot firmness (A10,

210   A20, MCF) were significantly impaired following administration of GP and HES, particularly in

211   EXTEM but also in INTEM. HES exerted the most detrimental effects, followed by GP. The BES

212   group varied significantly from the baseline, as-well, but were still higher compared to the colloid

213   groups (Figure 3-4). Similar to EXTEM significant impairment of FIBTEM and APTEM in the

214   colloid groups was observed (Figure 5-6). Regrettably, the FIBTEM clotting time in the GP and

215   HES groups were indeterminable due to technical errors.

216   All rotational thromboelastometry-derived parameters recovered over the four hours in all groups,

217   but the extent of recovery differed between the different regimes. In both EXTEM and APTEM,

218   CT and CFT were most prolonged through HES administration compared to the baseline. and to

219   the other groups. CFT returned to the baseline level only in the BES group (Figures 3&6).

220   Amplitude and MCF were restricted in all groups but nearly reached the baseline level in the BES-

221   treated animals (Figures 3-4). INTEM results were not as pronounced as the EXTEM, FIBTEM

222   and APTEM results. We observed no restrictions in ML and did not detect any signs of

223   hyperfibrinolysis in any of the groups (Figures 3-6).

224   Our comparison of porcine and human coagulation showed increased coagulation activity in pig

225   blood. This effect could be observed in all parameters and involved decreased clotting times and

226   increased amplitudes (Table 2).

227

228   **Discussion:**

229   This study investigated the influence of HES, GP and BES on coagulation during early

230   hemorrhagic shock in a pig model, with a focus on rotational thromboelastometry. Accordingly,

231   we found an impaired coagulatory activity that concerns temporal kinetics as well as clot firmness

232   as a consequence of fluid resuscitation in all groups, but with the most deleterious effects through

233   HES administration followed by GP. We simulated a frequent preclinical or clinical blood loss

234   scenario with subsequent bleeding control (i.e. during surgery). Shock was induced by defined

235   blood loss in combination with hemodynamic deterioration. If untreated, this model leads to

236   cardio-circulatory failure and death in 66% of the animals within two hours (38, 42). In the scenario

237   sufficient and quick action is necessary, and ongoing fluid therapy will not stop immediately after

238   bleeding control. Hemodynamic values were allowed to fall below the acceptable thresholds of

239   permissive hypotension (6) Isovolemic amounts of fluids were applied using a 1:1 replacement

240   regime. The chosen group size was adjusted on account of comparable large animal studies, that

241   also focused on hemorrhagic shock or cardiorespiratory effects (37, 43).The scenario concentrated

242   on rapid stabilization to avoid the undersupply of oxygen to the vital organs and did not stop

243   immediately after the bleeding was controlled.

244   Sufficient hemodynamic recovery through fluid resuscitation was registered for all three regimens.

245   In the colloid groups particularly, the baseline level was surpassed. After four hours, we did not

246   detect any significant intergroup differences, with the exception of heart rate, which remained

247   increased in the GP and BES groups. These results were expected due to the high effectivity of

248   colloids in increasing osmotic pressure leading to increased intravascular volume. A ratio of 1:1.5

249   between colloids and crystalloids would theoretically achieve similar hemodynamics but would

250   not have simulated a realistic clinical scenario (11). As expected, we observed significantly lower

251   hemoglobin content following the HES and GP administration, a sign of hemodilution.

252   Hemodilution partially explains the impaired coagulation in the laboratory parameters and

253   thromboelastometry results. Conventional coagulation parameters, clot formatting and firmness

254   decreased in all groups, with the highest restriction following HES administration, then GP. These

255   effects were more expressed in the extrinsic pathway, fibrinogen content and platelet count.

256   Additionally, we compared our porcine baseline values with published human reference values;

257   the porcine coagulation activity seemed to be higher than that of humans. This can especially be

258   seen in the decreased clotting and clot formatting time and the pronounced clot firmness among

259   the porcine specimens. The related results in FIBTEM still after four hours can be explain by a

260   lack of fibrinogen that is not displayed into the conventional coagulation parameters. These

261   findings correspond with results from other studies in which porcine hypercoagulation was

262   described (44, 45). These findings must be considered when interpreting the results in order for

263   the right conclusions from this model to be drawn.

264   As described above, crystalloids are less effective (25%) due to fluid dispersion into the

265   interstitium (7, 46). Zaar et al. presented the consequences of hemodilution and demonstrated

266   exacerbated liver bleeding following HES administration, whereas crystalloids mitigated the effect

267   (46). Other studies have claimed that colloids affect coagulation by themselves (40, 47). We

268   observed similar results in our study directly after fluid resuscitation. However, intergroup

269   differences were temporally limited, as hemoglobin content and hematocrit remained unchanged

270   after four hours. Hemodilution reasonably explains coagulation deterioration directly following

271   fluid resuscitation, but not its long-term effects. Several mechanisms have been described to

272   explain this coagulopathy through colloids, including the negative influences of factor VIII (28),

273   factor XIII, fibrinolysis (29), the von Willebrand factor (30) and surface receptor GPIIb/IIIa on

274   platelets (31). Administration of HES can particularly trigger these effects, which are pronounced

275   by different types of HES. In this context, negative side effects of the new HES generation

276   (130/0.4) are less relevant than those of older ones (e.g. HES 200/0.5), in which coagulation is

277   impaired to a higher degree (27). Mauch et al. reported on reduced fibrin polymerization, which

278   depends on decreased factor XIII activity for restricted clot firmness (40, 47). This corresponds
279   with our thromboelastometry results, including the decreases in MCF, which can be interpreted as
280   a sign of impaired fibrin cross-linking in the colloid groups (e.g. Figure 3) (29). We also detected
281   further decreases in CT and alpha-angle as an indication of impaired clot development related to
282   factor VIII and the von Willebrand factor. These results are in line with Haas et al., who reported
283   compromised clot firmness following HES administration compared to GP in pigs (33). This effect
284   appears to be dose-dependent (32). Furthermore, the direct influence of HES on thrombocyte
285   function, which impairs rolling and adhesion capabilities, restricts GPIIb/IIIa receptor binding and
286   direct degeneration through increased osmotic pressure (29, 31). In theory, an effect of colloids on
287   fibrinolysis is possible, but we did not detect any signs of hyperfibrinolysis. Lower degrees of
288   hyperfibrinolysis may exceed the detection ability of a rotational thromboelastometry assay. The
289   minor sensitivity to detect beginning hyperfibrinolysis and the time consuming analysis is a
290   relevant limitation in emergency situations (48). As another item of our study, we compared the
291   measured porcine baseline values with standard parameters for human assays. We demonstrated
292   that human assessments are suitable for this kind of research and generate useful values with
293   general increased porcine coagulation (Table 2) (39). This might also help to established regimes
294   for fluid resuscitation that can used likewise in veterinary medicine, where the number of relevant
295   studies is limited (33, 49).

296   In recent years, use of gelatine-based colloids has increased due to the unclear status of HES.
297   However, beneath the risk of anaphylaxis and kidney injury, evidence concerning the risk profile
298   of GP remains scarce (24, 50). Fast and effective therapy for hemorrhagic shock is indispensable.
299   Even if the bleeding is controlled, regeneration of adequate cardio-circulatory functions and
300   microcircular perfusion is essential to secure sufficient organ supply. If control of the bleeding is
301   not possible and blood products are not immediately available, fluid resuscitation under permissive
302   hypotension is inevitable (51). We designed our model according to a clinical scenario in which
303   immediate fluid resuscitation is considered lifesaving (52). This study demonstrated that the effect
304   of different infusion regimes and their potential consequences on coagulation are relevant even
305   after several hours. Especially, this long-term effect was an essential key finding of this study:
306   coagulopathy following colloid administration is not only a temporary phenomenon, but traceable
307   for hours after bleeding control and hemodynamic stabilization, but may become crucial with re-
308   occurrence of blood loss or demand for further surgery (33). In this context, we found a partial

309   recovery four hours after the initial event, which suggests time-dependent progress. On the other

310   hand, experimental factors and the sub-project character limited to address these effects over an

311   even longer time interval to examine full spontaneous recovery. Additionally, we did not

312   retransfuse the removed blood to simulate an early phase without the availability of blood products

313   and assess the mere effects of the different colloids. In this context, specific thrombocyte function

314   analysis or extended coagulation factor determination could deliver additional findings.

315   Regrettably, these were not available for this study. Finally, the comparability of porcine to human

316   physiology allowed for a realistic clinical-like scenario in this study. Notably, however, several

317   physiological differences exist concerning lower hemoglobin content and hypercoagulation in

318   pigs, which must be considered with respect to translational research.

319   **Conclusion:** In a porcine hemorrhagic shock model HES, GP, but also standard BES sufficiently

320   stabilized the macrocirculation, but significantly affected coagulation. These effects are most

321   pronounced after colloid and particularly HES administration. The choice of solution in fluid

322   resuscitation affects coagulation, may exacerbate bleeding situation and therefore increase

323   patient´s risk. Colloids have to be chosen with caution, because their molecular properties may

324   affect coagulation directly and as a consequence of pronounced hemodilution.

325

326   **Abbreviations**

327   GP: gelatine-polysuccinate; HES: hydroxyethyl starch; BES: balanced electrolyte solution;

328   PiCCO: pulse contour cardiac output system; SD: standard deviation; ANOVA: analysis of

329   variance; PTT: activated partial thromboplastin time; PT: prothrombin time; ROTEM: rotational

330   thromboelastometry; CT: clotting time; CFT: clot formation time, alpha; alpha-angle; A10:

331   amplitude 10; A20: amplitude 20; MCF: maximum clot firmness; MCL: maximum clot lysis; HR:

332   heart rate; MAP: mean arterial pressure; CI: cardiac index; Hb: hemoglobin content; Hct:

333   hematocrit; CI: cardiac index

334   **Availability of data and materials**

335   All data relevant to the study are included in this published article. This study belongs to a larger

336   research project that is investigated the effect of different solutions for fluid resuscitation on

337   cerebral perfusion, which has not yet been analyzed or published. Further data sets analyzed during

338   the current study are available from the corresponding author upon reasonable request.

339

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344

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346   pig model, comparison of porcine and human coagulation

347

348   **Authors' contributions**

349   EKH, AZ and AGB designed the study. AZ, CM, RR, JK and AGB conducted the study. CM and

350   MS analyzed the data, which were interpreted by AZ, EKH and ST. AZ, RR and EKH performed

351   the literature review and wrote the manuscript. CM, AGB, JK and ST proofread and revised the

352   manuscript. All authors read and approved the final manuscript. The manuscript was edited by

353   Scribendi editing service.

354

355   **Competing interests**

356   The authors have no competing interests to declare.

357

358   **Consent for publication**

359   Not applicable.

360

361   **Ethics approval and consent to participate**

362   This study was conducted after approval by the State and Institutional Animal Care Committee

363   (Landesuntersuchungsamt Rheinland-Pfalz, Mainzer Straße 112, 56068 Koblenz, Germany;

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371

Presentation

Preliminary data were presented as a poster presentation at the German Anesthesia Congress (DAC) in April 2017 in Nurnberg, Germany, and the annual meeting of the American Society of Anesthesiologists (ASA) in October 2019 in Orlando, USA.

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Figure 1

Figure 1: Experimental flow chart

GP = gelatine-polysuccinate; HES = hydroxyethyl starch; BES = balanced electrolyte solution; BLH = baseline healthy
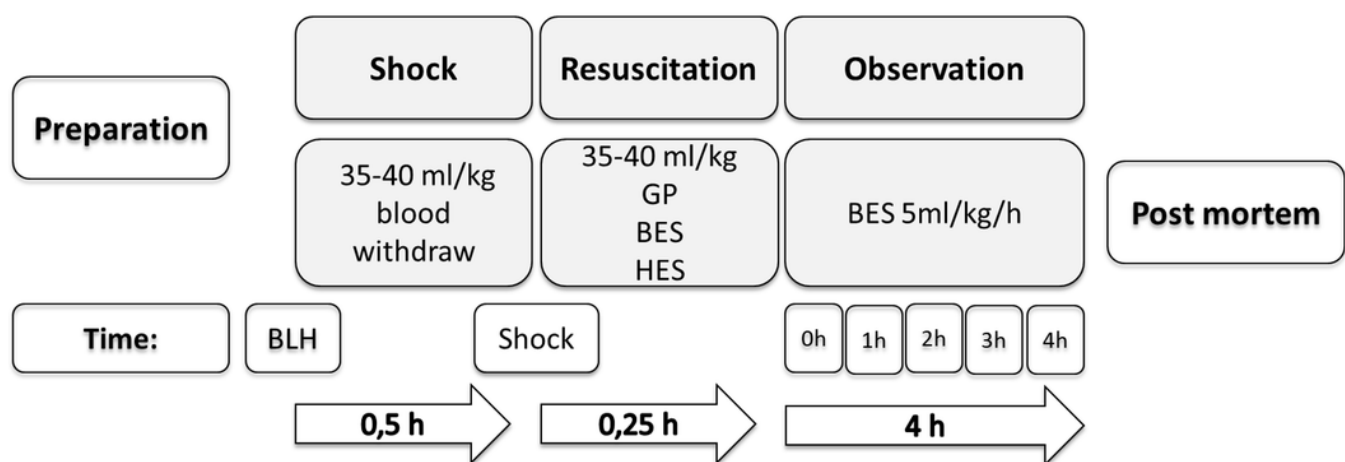
Figure 2

Figure 2: Laboratory coagulation parameters.

A = prothrombin time; B = activated partial thromboplastin time; C = platelet count; D = fibrinogen

Group effects over time were analyzed using two-way ANOVA and the post-hoc Student-Newman-Keuls method.

* = p < 0.05 timepoint comparison to the baseline (1 = HES; 2 = GP; 3 = BES);

= p < 0.05 in the intergroup comparison (1 = HES vs. GP; 2 = GP vs. BES; 3 = HES vs. BES);

GP = gelatine-polysuccinate; HES = hydroxyethyl starch; BES = balanced electrolyte solution
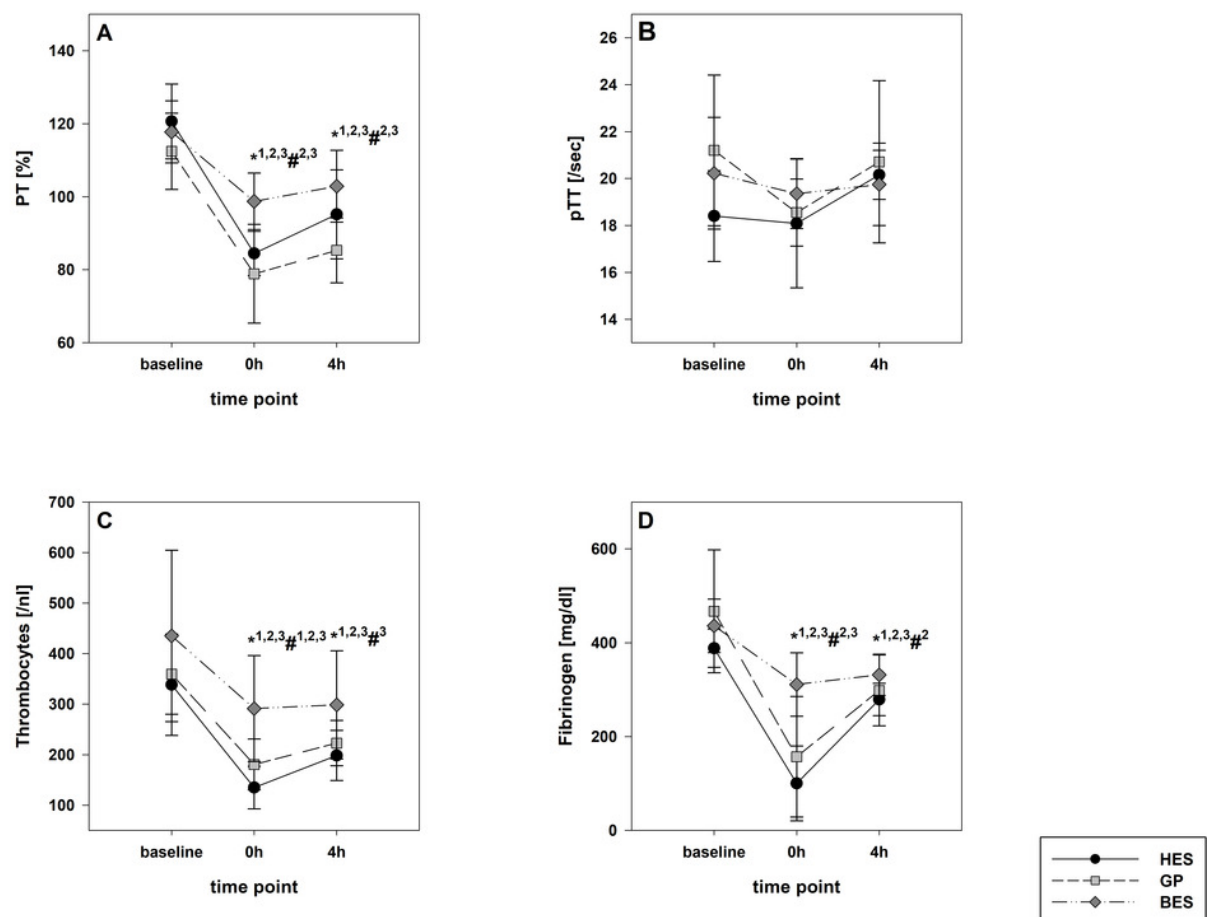
Figure 3

Figure 3: EXTEM (tissue factor pathway)

A = clotting time; B = clot formatting time; C = amplitude 20; D = maximum clot firmness

Group effects over time were analyzed using two-way ANOVA and the post-hoc Student-Newman-Keuls method.

* = p < 0.05 timepoint comparison to the baseline (1 = HES; 2 = GP; 3 = BES);

= p < 0.05 in the intergroup comparison (1 = HES vs. GP; 2 = GP vs. BES; 3 = HES vs. BES);

GP = gelatine-polysuccinate; HES = hydroxyethyl starch; BES = balanced electrolyte solution
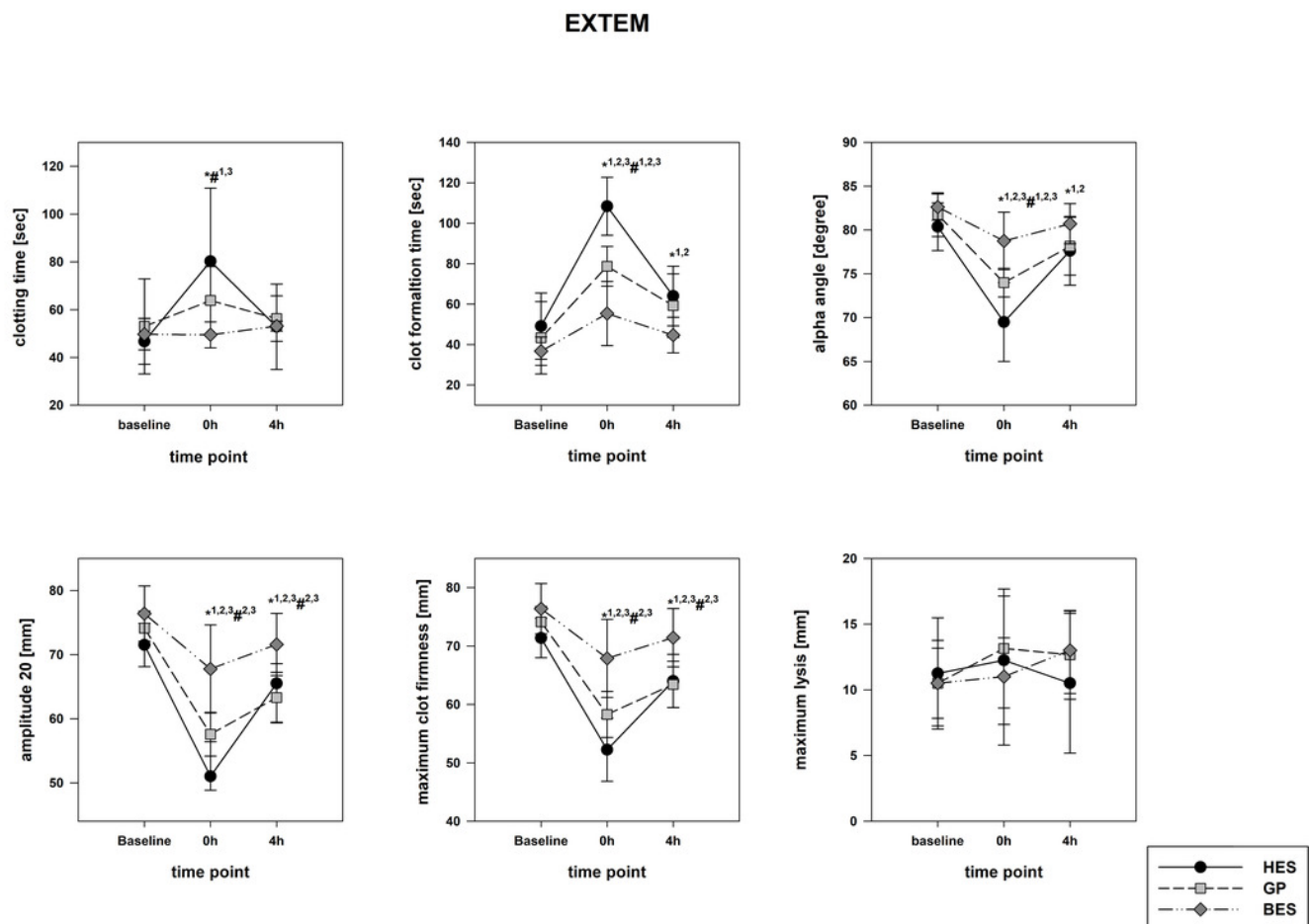
Figure 4

Figure 4: INTEM (contact activation pathway)

A = clotting time; B = clot formatting time; C = amplitude 20; D = maximum clot firmness Group effects over time were analyzed using two-way ANOVA and the post-hoc Student-Newman-Keuls method.

* = p < 0.05 timepoint comparison to the baseline (1 = HES; 2 = GP; 3 = BES);

= p < 0.05 in the intergroup comparison (1 = HES vs. GP; 2 = GP vs. BES; 3 = HES vs. BES);

GP = gelatine-polysuccinate; HES = hydroxyethyl starch; BES = balanced electrolyte solution
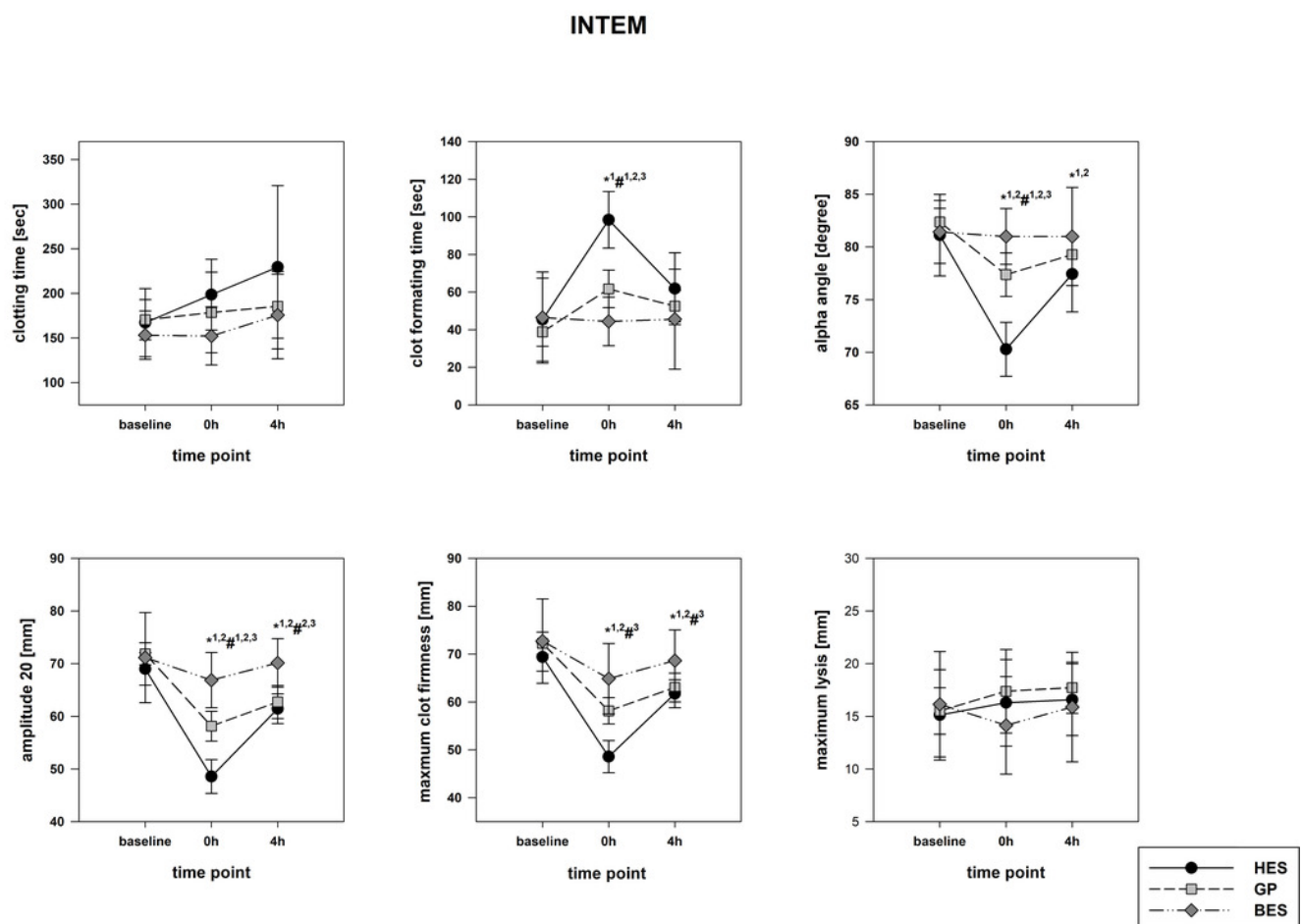
Figure 5

Figure 5: FIBTEM-Test (fibrinogen deficiency detection)

A = clotting time; B = clot formatting time; C = amplitude 20; D = maximum clot firmness

Group effects over time were analyzed using two-way ANOVA and the post-hoc Student-Newman-Keuls method.

* = p < 0.05 timepoint comparison to the baseline (1 = HES; 2 = GP; 3 = BES);

= p < 0.05 in the intergroup comparison (1 = HES vs. GP; 2 = GP vs. BES; 3 = HES vs. BES);

GP = gelatine-polysuccinate; HES = hydroxyethyl starch; BES = balanced electrolyte solution
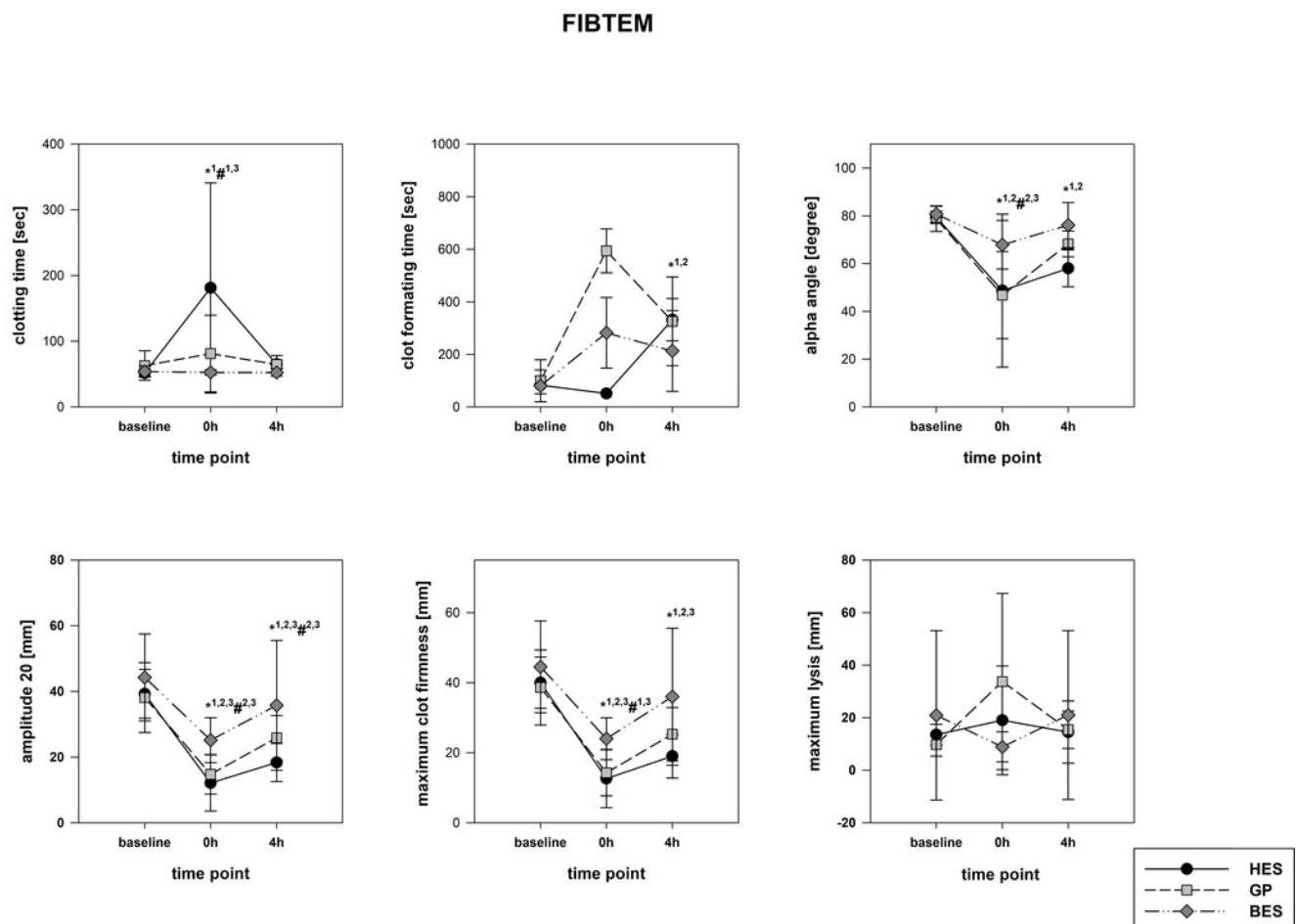
Figure 6

Figure 6: APTEM-Test (hyper-fibrinolysis-detection)

A = clotting time; B = clot formatting time; C = amplitude 20; D = maximum clot firmness

Group effects over time were analyzed using two-way ANOVA and the post-hoc Student-Newman-Keuls method.

* = $p < 0.05$ timepoint comparison to the baseline (1 = HES; 2 = GP; 3 = BES);

= $p < 0.05$ in the intergroup comparison (1 = HES vs. GP; 2 = GP vs. BES; 3 = HES vs. BES);

GP = gelatine-polysuccinate; HES = hydroxyethyl starch; BES = balanced electrolyte solution

APTEM
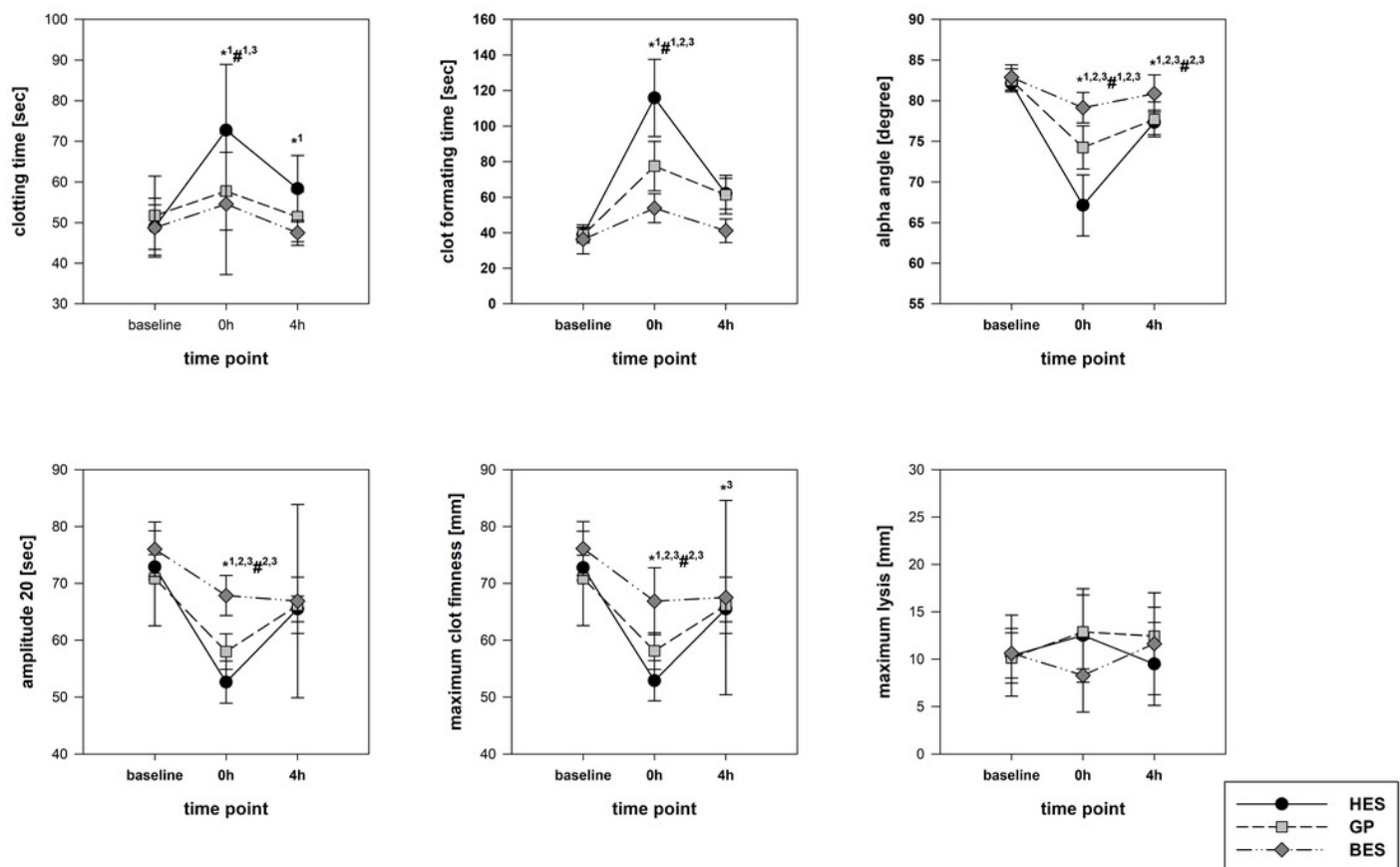
Table 1: Extended hemodynamic and blood parameters

* = indicates p<0.05 timepoint comparison to baseline;

\# = indicates p<0.05 in intergroup comparison (1 = HES vs. GP; 2 = GP vs. BES; 3 = HES vs. BES)

SD: Standard deviation; MAP: mean arterial pressure (mmHg); HR: heart rate (min^{-1}); CI: cardiac index [l/min/m^2]; Hct = Hematocrit (%); Hb = Hemoglobin (g/dl)

Parameter Mean (SD)		BLH	Shock	0h	2h	4h
MAP	HES	66 (8)	31 (10)*	80 (11)*	67 (8)	65 (7)
	GP	70 (9)	25 (5)*	76 (16)	59 (10)*	61 (6)*
	BES	61 (11)	29 (4)*	73 (10)	58 (8)	58 (9)
HR	HES	75 (17)	116 (38)*	123 (42)*	99 (33)	99 (33)
	GP	84 (10)	145 (53)*	137 (26)*	106 (27)	104 (21)
	BES	78 (22)	144 (46)*	118 (31)*	127 (48)*	134 (40)*
CI	HES	4.2 (0.7)	2.1 (0.4)*	7.5 (2.6)*#^{3}	5.7 (1.4)*	4.7 (0.8)
	GP	4.8 (1)	2 (0.3)*	7.6 (2)*#^{2}	5.4 (1.3)	5.6 (1.6)
	BES	4.1 (1.2)	2.2 (0.4)*	5.4 (0.9)*#^{2,3}	4.4 (0.6)	4.7 (0.6)
HCT	HES	26.6 (2.5)	23.2 (1.7)	13.4 (2)*#^{3}	16.1 (3.2)*#^{3}	17 (2.1)*
	GP	28.5 (1.4)	23 (2.1)	11,9 (1,7)*#^{2}	18.2 (0.5)*#^{2}	14.2 (1.6)*
	BES	26.1 (2.3)	23.6 (2.5)	17 (1.8)*#^{2,3}	19.6 (1.4)*#^{2,3}	16.8 (1)*
HB	HES	8.6 (0.8)	7.5 (0.5)	4.8 (0.8)*#^{3}	5.1 (0.7)*#^{3}	5.1 (0.6)*
	GP	9.2 (0.5)	7.5 (0.7)	4.7 (0.9)*#^{2}	4.7 (0.5)*#^{2}	4.6 (0.5)*
	BES	8.5 (0.7)	7.7 (0.8)	5.9 (0.3)*#^{2,3}	5.4 (0.3)*#^{2,3}	5.4 (0.3)*

Table 2: Comparison of human and porcine thromboelastometric references(n = 24) (37)

Table 2: Comparison of human and porcine thromboelastometric references(n = 24) (37)

Data are displayed in the 95% confidence interval of absolute values (AV) and in relation (R) (displayed in percentage) to human values. CT=clotting time (sec); CFT=clot formation time (sec); APLHA = alpha-angle (degree); A10 = amplitude 10% (mm); A20 = amplitude 20% (mm); MCF = Maximum clot firmness (mm); ML= maximum lysis (mm)
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TEST	EXTEM				INTEM				FIBTEM				APTEM			
SPECIES	HUMAN		PIG		HUMAN		PIG		HUMAN		PIG		HUMAN		PIG	
UNIT	AV	R	AV	R	AV	R	AV	R	AV	R	AV	R	AV	R	AV	R
CT	42-74	100	45-55	86	137-246	100	149-207	93	43-69	100	50-62	100	42-74	100	47-53	86
CFT	46-148	100	36-46	42	42-100	100	36-51	61	46-148	100	60-107	86	46-148	100	36-40	39
ALPHA	63-81	100	81-83	114	71-82	100	81-83	106	63-81	100	78-81	110	63-81	100	82-83	115
A10	43-65	100	70-74	133	44-68	100	68-72	125	9-24	100	50-62	339	43-65	100	69-74	132
A20	50-69	100	72-76	124	50-71	100	69-73	117	8-21	100	36-45	279	50-69	100	71-76	124
MCF	49-71	100	72-76	123	52-72	100	69-73	115	9-25	100	37-45	241	49-71	100	71-76	123
ML	0-18	100	9-12	117	0-12	100	14-17	258	13-27	100	9-13	55	0-18	100	9-11	111