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# Resuscitation of hemorrhagic shock using normal saline does not damage the glycocalyx in the immediate resuscitation phase

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## Abstract:

**OBJECTIVES:** The objectives were to study the effect of aggressive resuscitation using normal saline on hemodynamics, serum atrial natriuretic peptide (ANP), syndecan-1 (marker of endothelial glycocalyx shedding), and extravascular lung water index (ELWI) following hemorrhagic shock.

**METHODS:** Eleven male piglets (*Sus scrofa*) underwent blood drawing to create 20% drop in mean arterial pressure (MAP). Two-phase resuscitation was performed: Phase 1 using normal saline of an equal volume of blood drawn to create shock and Phase 2 using 40 ml/kg BW of normal saline to simulate hypervolemia and hemodilution. Heart rate, MAP, cardiac index (CI), systemic vascular resistance index, oxygen delivery (DO<sub>2</sub>), global end-diastolic volume index, ELWI, hemoglobin (Hb), lactate, ANP, and syndecan-1 at each phase and up to 60 min following Phase 2 resuscitation were recorded.

**RESULTS:** Phase 2 resuscitation significantly decreased Hb concentration ( $P = 0.006$ ), however, DO<sub>2</sub> was maintained ( $P = 1.000$ ). CI increased from shock to Phase 1 ( $P = 0.029$ ) and further increase in Phase 2 resuscitation ( $P = 0.001$ ). Overall, there was a transient increase of ANP following Phase 1 resuscitation, from  $85.20 \pm 40.86$  ng/L at baseline to  $106.42 \pm 33.71$  ng/L ( $P = 0.260$ ). Serum syndecan-1 and ELWI change at all phases were not significant.

**CONCLUSIONS:** We demonstrate compensatory protective mechanism despite overzealous fluid resuscitation. Compensatory increased CI despite decreased Hb maintained DO<sub>2</sub>. In the absence of inflammation, serum ANP did not increase significantly, no glycocalyx shedding occurred, subsequently no change in ELWI. We show that factors other than volume overload are more dominant in causing glycocalyx shedding.

## Keywords:

Atrial natriuretic factor, extravascular lung water, hypervolemic hemodilution, oxygen delivery, syndecan-1

## Introduction

Most pediatric resuscitation guidelines recommend liberal fluid resuscitation.

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However, excess fluid resuscitation may lead to complications including hemodilution and subsequent impairment of oxygen delivery (DO<sub>2</sub>) and hypothermia.<sup>[1,2]</sup> Clinical

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### Box-ED Section

#### What is already known on the study topic?

- Aggressive fluid resuscitation has negative effects secondary to hemodilution and tissue edema
- Previous *in vivo* study demonstrates that ANP release increases glycocalyx shedding
- Clinical evidence demonstrates ANP release following fluid resuscitation, due to acute atrial wall stretching
- Endothelial glycocalyx degradation causes severe plasma leakage

#### What is the conflict on the issue? Has it importance for readers?

- Fluid resuscitation remains the most important treatment in shock, however, its negative effect is hardly explored
- The causal-effect, and possible chain reaction from aggressive fluid loading, ANP release, glycocalyx shedding and following increase in extravascular water has never been explored in sequence.

#### How is this study structured?

- This was an experimental study on animal models
- Hemorrhagic shock was induced on 11 male piglets (*Sus scrofa*) followed by normovolemic and hypervolemic resuscitation using crystalloid, simulating aggressive fluid resuscitation
- ANP, syndecan-1 (marker of glycocalyx shedding), and hemodynamic monitoring were recorded at each phase

#### What does this study tell us?

- Following aggressive fluid resuscitation, ANP release was transient, and was not followed by glycocalyx shedding
- Hemodynamic parameters showed compensatory effect during shock, normovolemia, and hypervolemia
- In clinical observational studies, factor such as inflammation plays a more determining factor in causing glycocalyx shedding
- In the case of isolated hemorrhagic/hypovolemic shock, aggressive fluid resuscitation is generally safe

studies have demonstrated negative effects of aggressive fluid resuscitation in terms of longer hospital length of stay, cardiopulmonary complications, tissue edema, and increased mortality.<sup>[3,4]</sup>

The effect of aggressive volume resuscitation as illustrated by superimposition of the Frank–Starling and Marik–Phillips curves demonstrates that in a nonfluid responsive subject, fluid loading increases extravascular lung water index (ELWI).<sup>[5]</sup> Fluid resuscitation increases

the pulmonary circulation hydrostatic pressure and stimulates atrial natriuretic peptide (ANP) release due to acute stretching of atrial walls.<sup>[6-8]</sup> A previous study demonstrated that ANP induces endothelial glycocalyx shedding in guinea pigs.<sup>[3]</sup> The endothelial glycocalyx holds a vital role in regulating vascular permeability.<sup>[9]</sup> A previous clinical study measured syndecan-1 as glycocalyx shedding marker showed a strong association with severe plasma leakage.<sup>[10]</sup>

Despite such findings, currently, there is no experimental study that clearly describes the effect of aggressive fluid resuscitation on ANP and syndecan-1 in relation to hemodynamic markers and ELWI. We hypothesize that aggressive fluid loading leads to the shedding of the glycocalyx via increased levels of ANP and subsequently elevates ELWI in hemorrhagic shock model.

## Methods

### Ethics

Ethical approval was obtained from the Animal Ethics Committee of the Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, with ethical approval number 055/KEH/SKE/III/2017, at July 3, 2017. Experiment took place at the Animal Management Unit Laboratory, January–June 2018. Eleven healthy male domestic piglets (*Sus scrofa*), aged 6-10 weeks, were acclimatized for 15 days before the experiment.

### Study design

Each animal subject was anesthetized with ketamine and xylazine, supported by volume control mechanical ventilation, adjusted to blood gas analysis readings. For maintenance, 3 mL/kg/h of 0.9% normal saline was infused. The environmental temperature was maintained with a thermal blanket. Following 1 h of stabilization, hemodynamic parameters and blood sample were drawn for baseline values.

The pressure-targeted shock was then induced via venous blood drawing, to achieve a 20% reduction in mean arterial pressure (MAP). Thirty minutes later, two-step resuscitation was performed. In Phase 1 resuscitation, we administered a bolus of normal saline with a volume equal to the volume of blood loss needed to induce shock. Thirty minutes later, the Phase 2 resuscitation was performed with a bolus of 40 mL/kg of saline to simulate aggressive fluid resuscitation.<sup>[11]</sup> Hemodynamic parameters were measured three times, at 3-min intervals for each stage, and up to 60 min following Phase 2 resuscitation. The numerical means of each data set were used for the statistical analysis.

### Hemodynamic measurements

Heart rate (HR), MAP, cardiac index (CI), systemic

vascular resistance index (SVRI), global end-diastolic volume index (GEDVI), and ELWI were measured using a PiCCO Plus v4.12 System (Pulsion Medical Systems AG, Munich, Germany). Body surface area formula was calculated from  $734 \times (\text{body weight in kg})^{0.65611}$ . Cardiac output was calibrated for each measurement using the thermodilution method, with a 10 mL bolus of cold normal saline. Hemodynamic measurements were done in triplets at each phase (within 5 min), and mean values for each parameter were used. Serum lactate, ANP, and syndecan-1 were measured in duplicates using commercially available enzyme-linked immunosorbent assay for *S. scrofa*, performed according to the manufacturer's recommendations (Cloud-Clone Corp., USA).

### Statistical analysis

The sample size was calculated using Federer's formula.<sup>[12]</sup> Mean and standard error is used to present normally distributed data, otherwise median and ranges were used. A repeated measures ANOVA was used to compare effects between subjects and between phases for normally distributed data, otherwise Friedman test was used. *Post hoc* analysis with a Bonferroni adjustment was used to assess difference at two phases. The strength of linear correlation was measured using Pearson correlation. The statistical analysis was performed using SPSS IBM version 24.0 (IBM Corp, Armonk, USA).

## Results

The baseline data and characteristics of all subjects are shown in Table 1. Shock was achieved via blood drawing within  $8 \pm 4.7$  min. Fluid resuscitation was administered in boluses, and Phase 2 bolus was achieved within 9 (2–24 min).

Upon shock, GEDVI generally decreased with a mean difference of  $-164.66$  (95% confidence interval [CI]:  $-376.41$ – $47.094$ ) ml ( $P = 0.208$ ). At this point, the lowest stroke volume index (SVI), CI, and  $DO_2$  were recorded, while serum lactate was at its highest [Table 2]. In response to resuscitation, MAP was restored, however, there was no difference between the MAP measured at

**Table 1: Subject characteristics and baseline data**

Variable	n	Mean ±SD
Weight (kg)	11	14.7±1.3
BSA (m <sup>2</sup> )	11	0.53±0.02
MAP (mmHg)	11	102.6±14.4
Blood volume drawn (mL)	11	101±56
Time to shock <sup>^</sup> (min)	11	8±4.7
Phase 1 resuscitation time (min)	11	2±1
Phase 2 resuscitation time (min)	11	9 (7–24)

<sup>^</sup>Shock is defined as 20% decrease of MAP from baseline. Values are given as mean±SD or median (range). BSA=Body surface area, MAP=Mean arterial pressure, SD=Standard deviation

**Table 2: Changes in hemodynamic profiles**

	Mean±SD						Post hoc test (Bonferroni), P					
	Baseline (A)	Shock (B)	Phase 1 (C)	Phase 2 (D)	30 min (E) <sup>*</sup>	60 min (F) <sup>*</sup>	B-A	C-B	D-C	E-D	F-E	
MAP (mmHg)	102.6±14.4	82.3±8.7	91.5±13.0	94.6±9.1	86.6±6.7	91.6±9.8	0.001*	0.264	1.000	1.000	0.323	
GEDVI (ml)	678.4±271.3	513.8±167.9	628.0±191.5	641.4±177.2	604.0±173.7	643.9±201.0	1.000	0.055	1.000	1.000	1.000	
CI (l/min/m <sup>2</sup> )	4.0±1.2	3.2±0.8	3.8±0.7	4.7±0.9	3.9±0.8	3.5±0.6	0.395	0.029*	0.001*	0.091	0.143	
SVI (mL/m <sup>2</sup> )	44.3±14.3	33.5±10.0	42.4±11.1	53.6±12.3	42.8±12.9	43.2±14.9	0.191	0.160	0.004*	0.012*	1.000	
SVRI (dyn*sec/cm <sup>5</sup> /m <sup>2</sup> )	10831.0±3700.8	9991.3±2895.3	9034.5±2634.8	8416.1±1945.3	9705±3106.3	8941.1±5561.5	1.000	1.000	1.000	1.000	1.000	
DO <sub>2</sub> (mL/min)	22.64±8.65	14.59±3.54	20.29±4.1	22.81±5.26	20.21±4.67	N/A	0.033*	0.001*	0.120	0.825	N/A	
Lactate (mmol/L)	1.07±0.70	1.57±1.92	0.94±0.46	1.08±0.36	0.79±0.32	N/A	1.000	1.000	1.000	1.000	N/A	

<sup>\*</sup>30 min following Phase 2 fluid resuscitation, <sup>\*</sup>60 min following Phase 2 fluid resuscitation. MAP=Mean arterial pressure, GEDVI=Global end-diastolic volume index, CI=Cardiac index, SVI=Stroke volume index, SVRI=Systemic vascular resistance index, SD=Standard deviation, DO<sub>2</sub>=Oxygen delivery, ELWI=Extravascular lung water index

Phase 1 and Phase 2 resuscitation (mean difference of 2.82 [95% CI: -9.26-14.90] mmHg,  $P = 1.000$ ).

Following Phase 1 resuscitation, there was an increase in GEDVI with a mean difference of 114.21 (95% CI: -1.68-230.11) ml ( $P = 0.055$ ). Further aggressive fluid resuscitation at Phase 2 did not produce a significant increase in GEDVI, with a mean change of 13.40 (95% CI: -77.83-104.63) ml ( $P = 1.000$ ) [Table 2]. The increase in CVP following Phase 1 resuscitation was not significant, with a change of 0.52 (95% CI: -0.48-1.51) mmHg ( $P = 1.000$ ). However, CVP increase following Phase 2 resuscitation was significant, with a change of 2.36 (95% CI: 1.08-3.65) mmHg ( $P = 0.001$ ) [Figure 1].

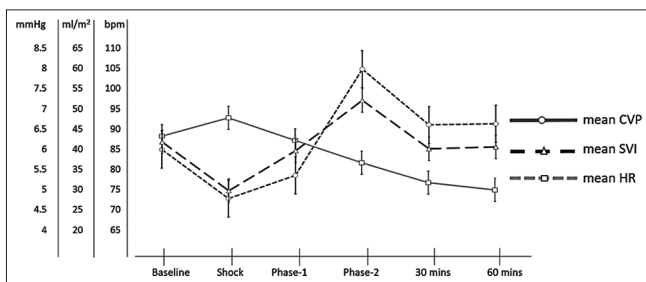
The kinetics of SVI and CI were expectedly similar. Both SVI and CI decreased upon shock, and restored upon Phase 1 resuscitation, peaking at Phase 2 resuscitation. In contrast, SVRI decreased upon resuscitation, and was at its lowest during Phase 2 resuscitation [Table 2]. The measured change in SVRI at Phase 2 resuscitation

from baseline value was -2577.12 (95% CI: -5857.52-724.29) ( $P = 0.195$ ). We found no significant correlation between changes in CI and SVRI in Phase 1 ( $r = 0.004$ ,  $P = 0.990$ ) and in Phase 2 ( $r = -0.587$ ,  $P = 0.057$ ). However, moderate-strong correlation was observed between changes in CI and SVRI 30 min post resuscitation ( $r = -0.679$ ,  $P = 0.031$ )

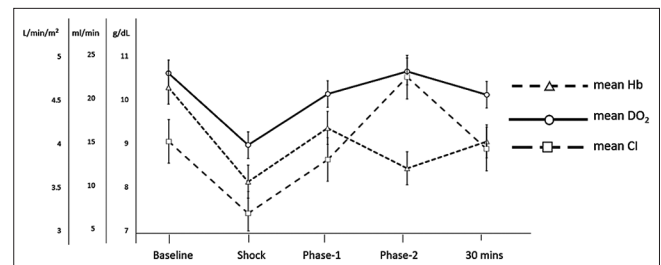
Phase 2 resuscitation significantly decreased hemoglobin concentration with a mean change of -1.79 (95% CI: -3.09--0.49) g/dL ( $P = 0.006$ ) from baseline [Figure 2]. However,  $DO_2$  following Phase 2 resuscitation was not significantly different from baseline with a mean change of -82.51 (95% CI: -920.59-755.57) ( $P = 1.000$ ).

There was an increasing trend of median ELWI following the two-phase resuscitation, peaking at 60 min post Phase 2 resuscitation. There was no significant difference between median baseline ELWI and following Phase 1 resuscitation ( $P = 0.878$ ), nor following Phase 2 resuscitation ( $P = 0.398$ ). The median ELWI 60 min post Phase 2 resuscitation was also not significantly different from baseline ( $P = 0.131$ ) [Figure 3].

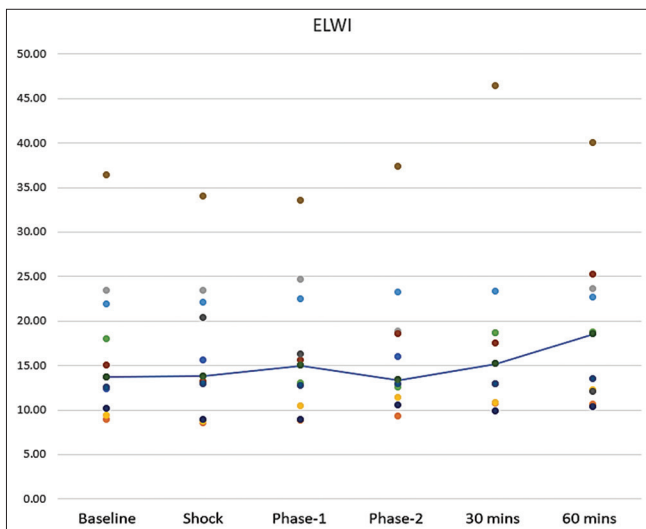
Following Phase 1 resuscitation, the mean serum ANP increased from  $85.20 \pm 40.86$  ng/L at baseline to  $106.42 \pm 33.71$  ng/L ( $P = 0.260$ ). It then decreased to



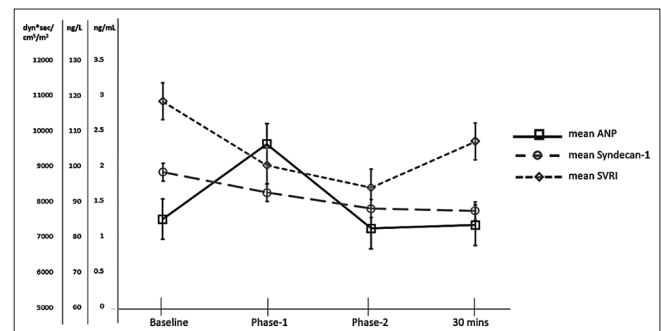
**Figure 1:** Changes in central venous pressure, stroke volume index, and heart rate. Serial measurements of CVP, SVI, and HR. Shock decreased CVP and SVI and increased HR. In response to fluid resuscitation, CVP and SVI increase, while HR decreases. All data are normally distributed. CVP = Central venous pressure, SVI = Stroke volume index, HR = Heart rate



**Figure 2:** Changes in cardiac index and oxygen delivery. Serial measurements of hemoglobin concentration, CI, and  $DO_2$ . Although hemoglobin concentration decreases, a proportionately greater increase of cardiac index results in an increased oxygen delivery. All data are normally distributed. CI = Cardiac index,  $DO_2$  = Oxygen delivery



**Figure 3:** Extravascular lung water index. Serial measurement of ELWI at all phases, showing increase of ELWI following resuscitation, measured up to 60 min. Line represents median ELWI. ELWI = Extravascular lung water index



**Figure 4:** Changes in ANP, syndecan-1, and SVRI. Serial measurement of mean serum ANP, syndecan-1, and SVRI at all phases. All data were normally distributed. ANP = Atrial natriuretic peptide, SVRI = Systemic vascular resistance index

82.60 ± 41.21 ng/L and 83.55 ± 46.09 ng/L following Phase 2 resuscitation and 30 min after, respectively [Figure 4]. There was no correlation between serum ANP and SVRI changes ( $r = 0.106$ ,  $P = 0.281$ ). In contrast, serum syndecan-1 did not change significantly. The mean syndecan-1 at baseline was 1.81 ± 0.3 ng/mL. Following Phase 1, Phase 2, and 30 min after resuscitation, the mean syndecan-1 was 1.54 ± 0.36 ng/mL, 1.32 ± 0.40 ng/mL, and 1.30 ± 0.40 ng/mL, respectively.

## Discussion

We found that despite overzealous fluid loading simulated by Phase 2 resuscitation, all hemorrhagic shock models were fluid responders. Hemodilution did not compromise DO<sub>2</sub> as there was a compensatory increase in CI. Fluid loading immediately causes transient ANP increase, however, not followed by syndecan-1 release. We found that ELWI increased only minimally in the absence of glycocalyx shedding. Clinically, all of the animals survived and none were dependent on mechanical ventilator.

We found no significant difference in MAP following Phase 1 and Phase 2 resuscitation. A meta-analysis by Glassford *et al.* demonstrated only a 4.8–9.5 mmHg increase in MAP in groups in response to fluid resuscitation.<sup>[13]</sup> A previous study found that only 36% of septic patients responded with at least a 10% increase in MAP post fluid resuscitation.<sup>[14]</sup> The relatively stable MAP in this experiment reflects the balance between CI and SVRI. Clinically, this shows that the use of MAP as the sole target for resuscitation is not justified as it poorly reflects hemodynamics.

We observed a decrease in hemoglobin levels due to hemodilution. However, this did not significantly affect DO<sub>2</sub> as there was an increase in CI. From a clinical point of view, central venous oxygen saturation (ScvO<sub>2</sub>) has an inverse relationship with oxygen extraction ratio.<sup>[15]</sup> In clinical setting, ScvO<sub>2</sub> <70% indicates inadequate DO<sub>2</sub> to meet metabolic demand. Currently, there are mixed findings on ScvO<sub>2</sub> >70% targeted resuscitation on mortality.<sup>[16]</sup> This shows that resuscitation cannot be only targeted to DO<sub>2</sub> (and ScvO<sub>2</sub>).

We observed no significant increase in ELWI up to 60 min post hypervolemic resuscitation. This is contrary to a previous study in acute lung injury in swine in which ELWI increased from 6.3 (5.4–7.1) ml/kg to 9.4 (7.9–10.8) ml/kg within 180 min following fluid resuscitation.<sup>[17]</sup> Compared to this study, a median ELWI change of 0.93 ml/kg observed in our experiment has little clinical significance. Although the ELWI value may differ between species, clinically none of our animal subjects required mechanical ventilator during and after the

experiment. Perhaps, this minimal increase in ELWI is limited by no shedding of endothelial glycocalyx that acts as a semi-permeable membrane.

We observed an increase in ANP post Phase 1 resuscitation in hemorrhagic shock model, which then normalized to baseline level [Figure 4]. This finding concurs with previous studies in swine and rat models that demonstrate a temporary increase in ANP following volume loading.<sup>[18,19]</sup> ANP increases in response to atrial wall stretching following volume loading. In their study, Ozer *et al.* demonstrate that intact pericardium becomes the limiting factor to ANP increase.<sup>[20]</sup> We also demonstrate that despite increased CVP from Phase 1 to Phase 2 resuscitation, no significant GEDVI increase was observed. This may explain the returning ANP level in our animal model. However, this finding challenges the finding of Chappel *et al.* that shows no increase in ANP following Phase 1 volume infusion preceded by blood drawing, in contrast to volume loading.<sup>[10]</sup> We think that this may be due to difference in trial methods, i.e., bolus fluid administration in our study compared to slower fluid administration in their study, as well as different types of fluids administered (HES 6% colloid solution) compared to NaCl 0.9% crystalloid saline in this study. Furthermore, Chappel *et al.* did not measure serial ANP, hence whether the increase of ANP was temporary or permanent could not be concluded.

Furthermore, in this study, we found no increase in serum syndecan-1 following fluid administration, thus implying no shedding of endothelial glycocalyx. This is contrary to a previous finding by Bruegger *et al.* on isolated guinea pig's heart which led to ANP increase, subsequently shedding of endothelial glycocalyx.<sup>[3]</sup> We hypothesize that syndecan-1 did not increase as ANP increment was only transient, which may be limited by intact pericardium in animal models, unlike *in vitro* experiment settings. Previous findings also showed that shedding of endothelial glycocalyx is affected by protease activity, free radicals, heparin, and pro-inflammatory cytokines.<sup>[20,21]</sup> Hence, we hypothesized that inflammation plays a more significant role in glycocalyx shedding, which explains syndecan-1 increase and glycocalyx shedding in critically ill patients, and not in our hemorrhagic shock model.

## Limitations

There were some limitations to this study. Firstly, we only observed up to 60 min post Phase 2 resuscitation, hence we cannot draw any conclusion regarding the ELWI trend beyond 60 min. Secondly, the findings of this experiment may only be applicable in isolated hemorrhagic or hypovolemic cases, and not in cases complicated by inflammation or other stressors. We also did not include control group in this study and hence

could not neglect the effect of anesthesia and procedures into the parameters measured. - Clinically, the principal management of hemorrhagic cases includes restoring intravascular volume, maintaining oxygen-carrying capacity while preventing coagulopathy, and most importantly limiting ongoing blood loss. In this study, our animal models underwent controlled bleeding, and resuscitation began after the bleeding stopped. Hence, the improvement in hemodynamic indexes, mortality, and morbidity outcomes should be carefully interpreted.

We recommend future studies using different models (septic shock and cardiogenic shock, presence of anemia, and continuous bleeding) to identify different compensatory changes, as well as to perform clinical observation in patients receiving fluid resuscitation to better distinguish which patients would benefit from liberal fluid resuscitation and to minimize its harmful effect.

## Conclusions

This study is the first to demonstrate that all animal models of hemorrhagic shock were fluid responders. We observed that in compensation to excessive fluid resuscitation,  $DO_2$  is maintained by increased CI despite hemodilution. Following fluid resuscitation, the increase in ANP was only transient, while no glycocalyx shedding occurs even after excessive volume administration. Consequently, we found no significant increase in ELWI in the absence of glycocalyx shedding. This provides new insight on the physiological compensatory mechanisms in response to fluid overload in hemorrhagic cases, which should be taken into consideration for fluid resuscitation and its monitoring.

### Author contribution statement

AHP: conception and study design, data acquisition, analysis and interpretation, drafted and critically revised the work. AF: conception, data analysis, critically revised the work. GS: study design, data acquisition, drafting the work. SB: conception, data analysis and interpretation, drafted the work. SWJ: study design, data analysis, drafted the work. MS: study design, data analysis, drafted the work. ML: study design, data analysis, drafted the work. All of the authors have approved the submitted version and have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

### Conflicts of interest

None Declared.

### Ethical approval

This study obtained ethical approval from the Animal Ethical Committee of Faculty of Veterinary Medicine, Bogor Agricultural University, Bogor, with ethical approval number 055/KEH/SKE/III/2017, at July 3, 2017.

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