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Demonstrating the safety and efficacy of the Hemafuse device for autotransfusion in a swine model of intraperitoneal pelvic hemorrhage

Rebecca N Treffalls (rtreffalls@gmail.com)
R. Adams Cowley Shock Trauma Center, Baltimore, MD https://orcid.org/0000-0002-2436-754X
David P Stonko
Department of Surgery, Johns Hopkins Hospital, Baltimore, MD https://orcid.org/0000-0002-2804-2857
Eric Lang
R. Adams Cowley Shock Trauma Center, Baltimore, MD
Matthew Lubas
Sisu Global Health, Baltimore, MD
Jonathan J Morrison (jonathan.morrison@som.umaryland.edu)
R. Adams Cowley Shock Trauma Center, Baltimore, MD https://orcid.org/0000-0001-7462-8456

Method Article

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Abstract

Noncompressible hemorrhage, such as pelvic hemorrhage, is associated with a high mortality rate and is a potentially preventable cause of death. Blood transfusion is a critical part of resuscitation, however, the availability or storage of blood products in austere and rural settings can impede or delay necessary transfusion. Similarly, large animal laboratories may also have limited resources, such as no banked animal blood. To minimize the need for donor blood products, blood recovery and autotransfusion can be performed using an intra-operative cell salvage machine. While this technique has proven to be beneficial, it is expensive, requires electricity, and can delay the time of transfusion. Recently, a handheld autotransfusion device was created for rural, remote, or low-resource facilities to return shed blood back to a patient or animal undergoing surgery quickly. To determine the resuscitative feasibility of the device, we sought to develop a swine model of open intraperitoneal pelvic hemorrhage, which is a common clinical scenario in low resource settings where cesarean section is one of the most common operations.

The aim of this study is to use a model of pelvic hemorrhage to compare resuscitation strategies encountered in austere environments:

Strategy 1 (control arm): a crystalloid-based resuscitation.

Strategy 2 (therapeutic arm): salvaged, filtered blood recovered by a novel handheld device.

Primary outcome:

· Red blood cell mass (hematocrit) at four hours following resuscitation.

Secondary outcomes:

· Hematologic: complete blood count (WBC, RBC, Hb, Hct, platelet count), free Hb, blood smear, blood culture, and potassium.

· Coagulation: thromboelastography (TEG), INR, PT, aPTT, ACT.

· Hemodynamic measures: mean arterial pressure and heart rate trends.

· Device: time to evacuate blood and time to re-transfusion.

Introduction

Noncompressible hemorrhage, such as traumatic pelvic hemorrhage and post-partum hemorrhage, is a potentially preventable cause of death, yet is associated with high mortality rates (20-30%). [1-5] In patients with pelvic hemorrhage, particularly those in extremis, blood transfusion is a critical part of resuscitation. [6] However, the lack of availability or storage of blood products in austere and rural settings, or in large animal laboratories, can impede or delay necessary transfusion and resuscitation for such patients. [7-8] To minimize the need for donor blood products, an autotransfusion system can be performed using an intra-operative cell salvage (ICS) technique. [9] The ICS machine suctions shed blood, anti-coagulates the blood, and is filtered prior to being transfused into the patient. [9] While this technique has proven to be clinically and financially beneficial [11-12], there is a risk of hemolysis associated with the suctioning and filtering of the blood. [13-14] To mitigate this risk, it has been shown that a pooled source of blood can decrease hemolysis compared to continuous suctioning of ongoing hemorrhage. [14] This is especially feasible in pelvic hemorrhage, ruptured ectopic pregnancy, and post-partum hemorrhage where the blood quickly pools into the pelvic cavity, where following hemorrhage control it may be possible to salvage the shed blood.

An important limitation to the ICS system is the need for electricity, which may be unreliable or unavailable in austere or rural settings. A perfusionists is often required to operate the ICS machine, which can be unfavorable in areas without extensive personnel and can become another costly deterrent. [15] For low-resource facilities, an ICS machine may can be a costly upfront expensive, difficult to obtain in certain locations, cumbersome to transport, and increase time to transfusion as the blood is processed. [16] Another method of direct transfusion is used by the United States military where soldiers provide fresh whole blood directly to injured soldiers, known as a walking blood bank. [7,17] This method of blood transfusion in austere environments increased in popularity as data in recent years showed the benefit of whole blood resuscitation. [18-19] While a walking blood bank is not ideal in unpredictable situations, it demonstrates the potential for fresh whole blood transfusion in austere settings. To develop a simple, mechanical method of autotransfusion, the Hemafuse device from Sisu Global Health was engineered to auto-transfuse shed blood back into the patient using a handheld, electricity-free method. [19] The pooled blood in the pelvic cavity is suctioned through a cannula, filtered through a handheld device, pushed into a pre-anticoagulated bag, and then subsequently re-transfused into the patient.

We sought to develop a swine model of open intraperitoneal pelvic hemorrhage to determine the resuscitative feasibility of the device. A model of endovascular pelvic hemorrhage in swine has previously been established by Abdou et al and was used as a guide for an open method of pelvic hemorrhage. [20] Once a model of pelvic hemorrhage was constructed, a handheld autotransfusion device was used for resuscitation. Two study groups are assessed: a therapeutic group, who received shed blood transfusion following hemorrhage, and a control group, who received intravenous fluid following hemorrhage. The aim of the study is to evaluate the post-transfusion red blood cell mass, as well as hematological

laboratory values, hemodynamic trends, and device characteristics between study cohorts (therapeutic vs control).

Reagents

1. Telazol (4-5 mg/kg)
2. Xylazine (1.8-2.2 mg/kg)
3. Midazolam (0.2-0.4 mg/kg)
4. Isoflurane via endotracheal (1.5-3%) (Sigma-Aldrich, SKU 792632-250MG)
5. Heparin Sodium (10,000 Units/10mL)
6. Dextrose 50% (0.5 g/mL)
7. Norepinephrine (4mg/4mL dosed 0.2-0.3 mcg/kg)
8. Sodium bicarbonate (8.4%)
9. Magnesium sulfate (0.5g/1mL dosed 1-4g)
10. Calcium chloride (10%)
11. Potassium Chloride (20mEq/100mL)

Equipment

Autotransfusion Device:

1. Hemafuse pump (9200 Rev D; Sisu Global Health, Baltimore, MD)
2. Hemafuse filter and accessories kit (8100 Rev A; Sisu Global Health, Baltimore, MD)
3. 1mL syringes for blood samples
4. Standard blood bags with 1,000 Units of Heparin

Vascular Access:

1. 5 Fr micro-puncture access kit (Cook Medical, Bloomington, USA; MPIS-502-NT-U-SST)
2. 10 cm 6 Fr sheath (Terumo, Elkton, NJ; REF/Product Code RM*RS7F10PA)

3. 11 cm 8 Fr sheath (Terumo, Elkton, NJ; REF/Product Code RM*RS7F10PA)

4. 11 cm 8.5 Fr sheath (Cordis, Santa Clara, CA; REF/Product Code 401011M)

Monitoring:

1. Pressure catheter (5 F, Dual, Straight, 3 cm, 120 cm, PU/WD; SPR-751S or SPR-75)

2. Rectal temperature probe (ADInstruments, Large Animal Rectal Probe (RET-1))

3. Flow Probes, 5-6mm (ADInstruments, Series MA-n-PS-ori)

4. Electrocardiogram monitoring electrodes (3M, St. Paul, MN; Red Dot, 2600 Series)

5. Telemetry 5-lead monitor (3M, St. Paul, MN; YMA5SD)

6. $EtCO_2$ capnography monitor, airway adaptor, and sample lines

Imaging:

1. Bedside US system, such as Phillips Lumify App and US Probes (Phillips, NV, USA; https://www.usa.philips.com/healthcare/sites/lumify/lumify-android-app)

Labs:

1. TEG 5000 Thrombelastograph Hemostasis Analyzer system or equivalent TEG capable device (Haemonetics, Boston, MA)

2. ABL800 FLEX blood gas analyzer with 18 STAT parameters (Radiometer, Brea, CA)

3. iSTAT 1 (Abbott Labs; https://www.pointofcare.abbott/us/en/offerings/istat/istat-handheld#specs)

4. iSTAT test cartridges for activate clotting time (ACT) (Abbott Labs; https://www.globalpointofcare.abbott/en/product-details/apoc/istat-actc-us.html)

5. iSTAT test cartridges for INR/PT (Abbott Labs; https://www.globalpointofcare.abbott/en/product-details/apoc/istat-ptinr-us.html)

6. iSTAT test cartridges for chemistry 8+ (Abbott Labs; https://www.globalpointofcare.abbott/en/product-details/apoc/istat-chem8.html)

7. Blood collection tubes with K2 EDTA or appropriate additive (BD Vacutainer, Franklin Lakes, NJ)

a. Blood sample will be sent to the institution core lab for CBC, blood smear, and blood culture analysis.

Anesthesia Supplies:

1. Mechanical ventilator with isoflurane vaporizer (Drager Fabius GS; DFABIUSGS)
2. Endotracheal Tube 28 French 7.0 mm 10/bx Endotrol (SAM Medical: 026351)
3. Ventilator tubing, air, gas tanks, lines, and CO_2 absorber (Dragersorb 800+)

Surgical Equipment:

1. Operating Room Table
2. Bookwalter Retractor Set
3. Standard Lap Sponges (Medline, Northfield, IL)
4. Mayo Instrument Stand
5. General Surgical Instruments
6. Vascular Surgical instruments

Other:

1. 0.9% Normal Saline, 1L bags
2. Infusion Tubing (BD: SKU 10013365)
3. Prefilled 10 cc 0.9% Saline Syringes (BD-9104 BD PosiFlush Saline Syringe)
4. Various sutures (Silk, 0 to 4-0, Proline, 3-0 to 5-0; Vicryl, 0 to 5-0)
5. Foley bag and Foley catheter (SKU: JOR1027 and FC30X12)
6. Digital laboratory scale

Procedure

Animal Model: 50-70kg adult castrated male Yorkshire swine obtained from a USDA approved vendor.

Pre-Procedure Husbandry and Preparation*:* Using our usual animal husbandry protocol [21-26], we animals will be housed in communal pens, under veterinary supervision, with free access to food and water for at least 72 hours in the vivarium to allow acclimatization to their new environment. They will be fasted the night before surgery for at least 8 hours prior to the induction of general anesthesia.

Continuous Monitoring: Mean arterial pressure, central venous pressure, iliac artery blood flow, heart rate, oxygen saturation, $EtCO_2$, and temperature.

Study Groups: Animals will be grouped into two cohorts: a therapeutic group (n=6) and a control group (n=6). Both study groups will undergo instrumentation, hemorrhage, and suction of shed blood using the Hemafuse device. The therapeutic group will be resuscitated with shed blood transfusion and the control group will be resuscitated with IV fluid transfusion.

Phase 1: Animal Preparation and Instrumentation

1. Sedate the animal Midazolam (2 mg/kg) followed by Telazol (5 mg/kg) and Xylazine (2 mg/kg) and transport animal to procedure room.

2. Initiate isoflurane via facemask with a targeted MAC of 1.0 and FiO2 of 40%.

3. Intubate with a 7.0 mm endotracheal tube and initiate general anesthesia at 10cc/kg tidal volume and respiratory rate of 12-14 with a target pCO2 of 30-45 and FiO2 of 40%.

4. Place Bovie pad and EKG leads after shaving and prepping skin.

5. Place the animal in a supine position and restrain the animal onto the operating table.

6. Place 8 Fr sheath into the femoral artery percutaneously with a US-guided modified Seldinger technique. Reserve sheath for hemorrhage.

7. Place 6 Fr sheath into the carotid artery percutaneously and insert a solid-state pressure catheter in the aorta for mean arterial pressure monitoring.

8. Place 6 Fr sheath into the femoral vein percutaneously and insert a solid-state pressure catheter in the inferior vena cava for central venous pressure monitoring.

9. Place 8.5 Fr Cordis Catheter (chosen because this allows for rapid massive re-transfusion) into the external jugular vein percutaneously and reserve for blood transfusion, intravenous fluids, and drug administration.

10. Perform a lower midline laparotomy to access the pelvis.

11. Perform a cystostomy with foley catheter placement into the bladder.

12. Perform splenectomy to prevent splenic autotransfusion.

13. Provide cephalolateral retraction to the abdominal side wall with a Bookwalter retractor, with cephalad retraction of the small and large intestine to create a “pelvic well” suited for at least 1L of hemorrhage (see Figure 2A and B).

14. Expose the iliac artery and place a 5 or 6mm flow probe around the vessel (Figure 2A) and connect to PowerLab. Apply surgilube as needed for appropriate conduction.

15. Administer 1L of 0.9% normalized saline (NS) and 25ccs of D50 prior to baseline data collection (given that the animals are NPO for at least 8 hours prior to anesthesia).

16. Perform a timeout to identify any technical or instrumentation errors while IV fluid and D50 is infused.

Phase 2: Baseline Data (30 minutes)

1. Obtain arterial blood (minimum 95µL sample) for an arterial blood gas (ABG) test.

2. Obtain arterial blood (min. 95µL) for a Chem8+ test.

3. Obtain arterial blood (min. 95µL) for an activated clotting time (ACT) test.

4. Collect blood sample into Vacutainer EDTA tube for laboratory analysis.

Phase 3: Initiate Hemorrhage

1. Place the femoral artery catheter stopcock into the intraperitoneal space of the pelvis.

2. Open stopcock and allow free hemorrhage into the pelvis until 20% of blood volume (which is approximately 13mL/Kg) is shed.

Phase 4: Suction with Hemafuse Device

1. Once blood begins to pool into the pelvic cavity, we will begin suctioning blood into the barrel of the device.

2. Release air out of the Hemafuse barrel by pushing on the plunger with the outlet port facing up and allowing air to flow out of the open end of the stopcock on the device (Figure 3A).

3. After removing the air, rotate the barrel so the outlet port faces downwards and adjust the stopcock to flow into the blood bag.

4. Push blood into the attached blood bag (Figure 3B).

5. Repeat steps 1-3, as needed, to fill a 1000mL heparinized bag with 13 mL/kg of exsanguinated blood.

a. To quantify the specific amount of shed blood, measure both the volume of blood in the bag and the weight of the shed blood bag on a tared scale.

6. Obtain arterial blood sample from the bag, including EDTA tube sample for laboratory analysis.

7. Obtain arterial blood sample directly from the femoral arterial line on the animal, including EDTA tube sample for laboratory analysis.

Phase 5a: Resuscitation and Monitoring – Therapeutic Group (4 hours)

1. Transfuse the shed blood from the heparinized bag using a standard pressure bag inflated to a pressure of 150 mmHg.

2. Immediately at the end of transfusion, obtain arterial blood sample directly from the animal.

3. Run ABG, Chem8+, INR/PT, and ACT tests on the post-transfusion arterial blood sample.

4. Collect blood sample in EDTA tube for laboratory analysis.

5. Continue IV fluids during the remaining resuscitation period, as needed.

6. Repeat animal blood draw and laboratory tests at 30-, 60-, 120-, 180-, and 240-minutes.

Phase 5b: Resuscitation and Monitoring – Control Group (4 hours)

1. Provide an equivalent amount of crystalloid IV fluid (based on shed blood in therapeutic group) using a standard pressure bag inflated to a pressure of 150 mmHg.

2. Immediately at the end of transfusion, obtain arterial blood sample directly from the animal.

3. Run ABG, Chem8+, INR/PT, and ACT tests on the post-transfusion arterial blood sample.

4. Collect blood sample in EDTA tube for laboratory analysis.

1. The primary outcome is red cell mass at the end of follow up. However, the ABGs and coagulation profile will be trended.

5. Continue IV fluids during the remaining resuscitation period, as needed.

6. Repeat animal blood draw and laboratory tests at 30-, 60-, 120-, 180-, and 240-minutes.

Phase 6: End of Study

1. Euthanize the animal and properly dispose following protocol.

2. Clean all equipment and instruments used during the study.

Troubleshooting

Time Taken

Estimated 1 hour for instrumentation; 30 minutes for baseline laboratory tests; 30 minutes for hemorrhage, suction using autotransfusion device, and laboratory tests; and 240 minutes for transfusion, resuscitation, and laboratory tests. **Total time is approximately 6 hours per animal.**

Anticipated Results

To assess the primary outcome:

RBC mass at end of study: Student t-tests of final Hematocrit values at the end of the 4-hour resuscitation period will be performed to evaluate the therapeutic group vs control group.

To assess the secondary outcomes:

Hematologic tests: Multiple t-tests of each time point of laboratory values (WBC, RBC, Hb, Hct, platelet count, free Hb, blood smear, blood culture, and potassium) will be performed to evaluate the therapeutic

group vs control group.

Coagulation tests: Multiple t-tests of each time point of laboratory values (TEG values, INR, PT, aPTT, ACT) will be performed to evaluate the therapeutic group vs control group.

Hemodynamic trends: Multiple t-tests of each time point of hemodynamic variables (MAP, heart rate, iliac artery blood flow, and CVP) will be performed to evaluate the therapeutic group vs control group.

References

1. Morrison JJ. Noncompressible torso hemorrhage. *Crit Care Clin*. 2017;33:37–54.

2. Dalby B, Jones J. The effect of hemorrhage control adjuncts on outcome in severe pelvic fracture: a multi-institutional study. *J Emerg Med*. 2019;57:591–592.

3. Patel N, Harfouche M, Stonko DP, Elansary N, Scalea TM, Morrison JJ. Factors Associated With Increased Mortality in Severe Abdominopelvic Injury. Shock. 2022 Feb 1;57(2):175-180.

4. Goffman D., Nathan L., Chazotte C.: Obstetric hemorrhage: a global review. Semin Perinatol 2016; 40: pp. 96-98.

5, Stonko DP, Azar FK, Betzold RD, et al. Contemporary Management and Outcomes of Injuries to the Inferior Vena Cava: A Prospective Multicenter Trial From PROspective Observational Vascular Injury Treatment. *The American Surgeon*. August 2021.

6. Kogutt BK, Vaught AJ. Postpartum hemorrhage: Blood product management and massive transfusion. Semin Perinatol. 2019 Feb;43(1):44-50.

7. Braverman MA, Smith A, Shahan CP, Axtman B, Epley E, Hitchman S, Waltman E, Winckler C, Nicholson SE, Eastridge BJ, Stewart RM, Jenkins DH. From battlefront to homefront: creation of a civilian walking blood bank. Transfusion. 2020 Jun;60 Suppl 3:S167-S172.

8. Kubio C, Tierney G, Quaye T, Nabilisi JW, Ziemah C, Zagbeeb SM, Shaw S, Murphy WG. Blood transfusion practice in a rural hospital in Northern Ghana, Damongo, West Gonja District. Transfusion. 2012 Oct;52(10):2161-6.

9. Tawes, R. L. (Ed.). (1997). *Autotransfusion: Therapeutic Principles and Trends*. Appleton Communications, Incorporated.

10. Wilson AJ, Cuddigan BJ, Wyatt AP. Early Experience of Intraoperative Autotransfusion. *Journal of the Royal Society of Medicine*. 1988;81(7):389-391.

11. Blevins FT, Shaw B, Valeri CR, Kasser J, Hall J. Reinfusion of shed blood after orthopaedic procedures in children and adolescents. The Journal of Bone and Joint surgery. American Volume. 1993

Mar;75(3):363-371.

12. Frank SM, Sikorski RA, Konig G, Tsilimigras DI, Hartmann J, Popovsky MA, Pawlik TM, Waters JH. Clinical Utility of Autologous Salvaged Blood: a Review. J Gastrointest Surg. 2020 Feb;24(2):464-472.

13. Dyer RH Jr, Alexander JT, Brighton CT. Atraumatic aspiration of whole blood for intraoperative autotransfusion. Am J Surg. 1972 May;123(5):510-4.

14. Sullivan IJ, Faulds JN. Assessment of intra-operative cell salvage haemolysis in the obstetric and orthopaedic clinical setting, in comparison with allogeneic blood. Transfus Med. 2014 Oct;24(5):280-5.

15. Bassett AK, Auten JD, Zieber TJ, Lunceford NL. Early, Prehospital Activation of the Walking Blood Bank Based on Mechanism of Injury Improves Time to Fresh Whole Blood Transfusion. J Spec Oper Med. 2016 summer;16(2):5-8.

16. Zelinka ES, Brevig J, McDonald J, Jin R. The perfusionist's role in a collaborative multidisciplinary approach to blood transfusion reduction in cardiac surgery. J Extra Corpor Technol. 2010 Mar;42(1):45-51.

17. Amanvermez Senarslan D, Yildirim F, Kurdal AT, Damar A, Ozturk T, Tetik O. Efficacy and cost-effectiveness of cell saver usage in the repair of thoracic aortic aneurysms and dissections. Perfusion. 2021 Jun 30:2676591211028178.

18. Black JA, Pierce VS, Kerby JD, Holcomb JB. The Evolution of Blood Transfusion in the Trauma Patient: Whole Blood Has Come Full Circle. Semin Thromb Hemost. 2020 Mar;46(2):215-220.

19. Spinella PC. Warm fresh whole blood transfusion for severe hemorrhage: U.S. military and potential civilian applications. Crit Care Med. 2008 Jul;36(7 Suppl):S340-5. doi: 10.1097/CCM.0b013e31817e2ef9.

20. Sisu Global The Hemafuse [Internet]. [cited 2022 Jul 1]. Available from: https://sisuglobal.health/hemafuse

21. Abdou H, Du J, Harfouche MN, et al. Development of an Endovascular Model of Pelvic Hemorrhage Using Volumetric Computed Tomography Validation. *Journal of Endovascular Therapy*. 2021;28(4):614-622.

22. Hossam Abdou, Joseph Edwards, Neerav Patel, David P. Stonko, Noha Elansary, Eric Lang, Michael J. Richmond, Thomas Ptak, Joseph M. White, Thomas M. Scalea, Jonathan J. Morrison, Characterizing Brain Perfusion in a Swine Model of Raised Intracranial Pressure, Journal of Surgical Research, Volume 278,2022, Pages 64-69, ISSN 0022-4804.

23. Madurska MJ, Abdou H, Elansary NN, et al. Whole Blood Selective Aortic Arch Perfusion for Exsanguination Cardiac Arrest: Assessing Myocardial Tolerance to the Duration of Cardiac Arrest. Shock (Augusta, Ga.). 2022 Jun;57(6):243-250.

24. Stonko DP, Edwards J, Abdou H, Elansary NN, Lang E, Savidge SG, et al. PV loops and REBOA during hemorrhage and resuscitation. Protocol Exchange. Available at: https://www.researchsquare.com. Accessed July 1, 2022.

25. Treffalls RN, Stonko DP, Edwards J, Abdou H, Savidge SG, Morrison JJ. Effects of hemorrhagic shock and resuscitation on mesenteric circulation. Protocol Exchange. Available at: https://www.researchsquare.com. Accessed July 1, 2022.

26. David P. Stonko, Joseph Edwards, Hossam Abdou, Noha N. Elansary, Eric Lang, Samuel G. Savidge, Jonathan J. Morrison, A technical and data analytic approach to pressure-volume loops over numerous cardiac cycles, JVS-Vascular Science, Volume 3, 2022, Pages 73-84, ISSN 2666-3503.

Figures

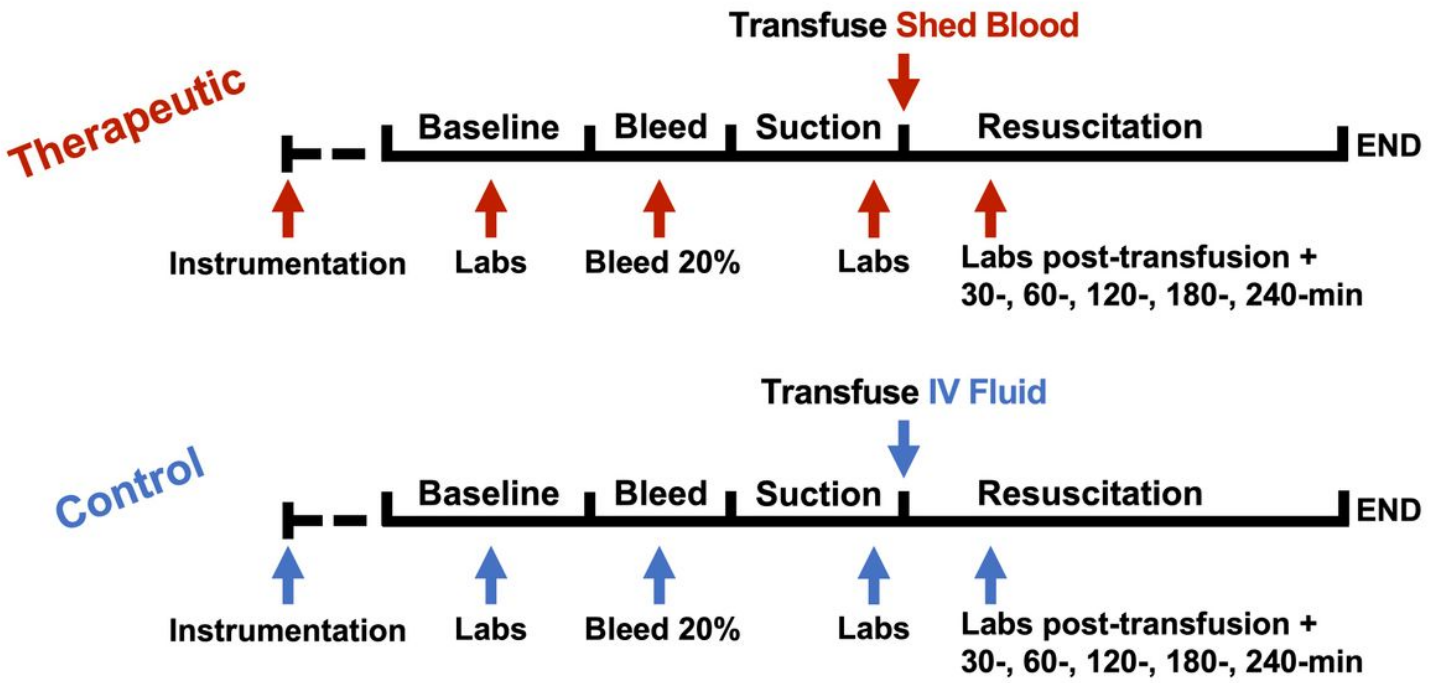

Figure 1

The study timeline for both study cohorts: therapeutic group and control group. Each cohort utilizes a swine model of intraperitoneal pelvic hemorrhage managed with an autotransfusion device to suction blood from the pelvis. The protocol begins with instrumentation, baseline, hemorrhage (20% volume) into pelvis, and suction of blood from the pelvis into a heparinized bag using the Hemafuse device. The study cohorts have different transfusion methods during the four hours of resuscitation as depicted in the figure. Arterial blood samples were taken at baseline from the animal, from the device post-suction, from the animal post-transfusion, and at 30-, 60, 120-, 180-, and 240-minutes during resuscitation.

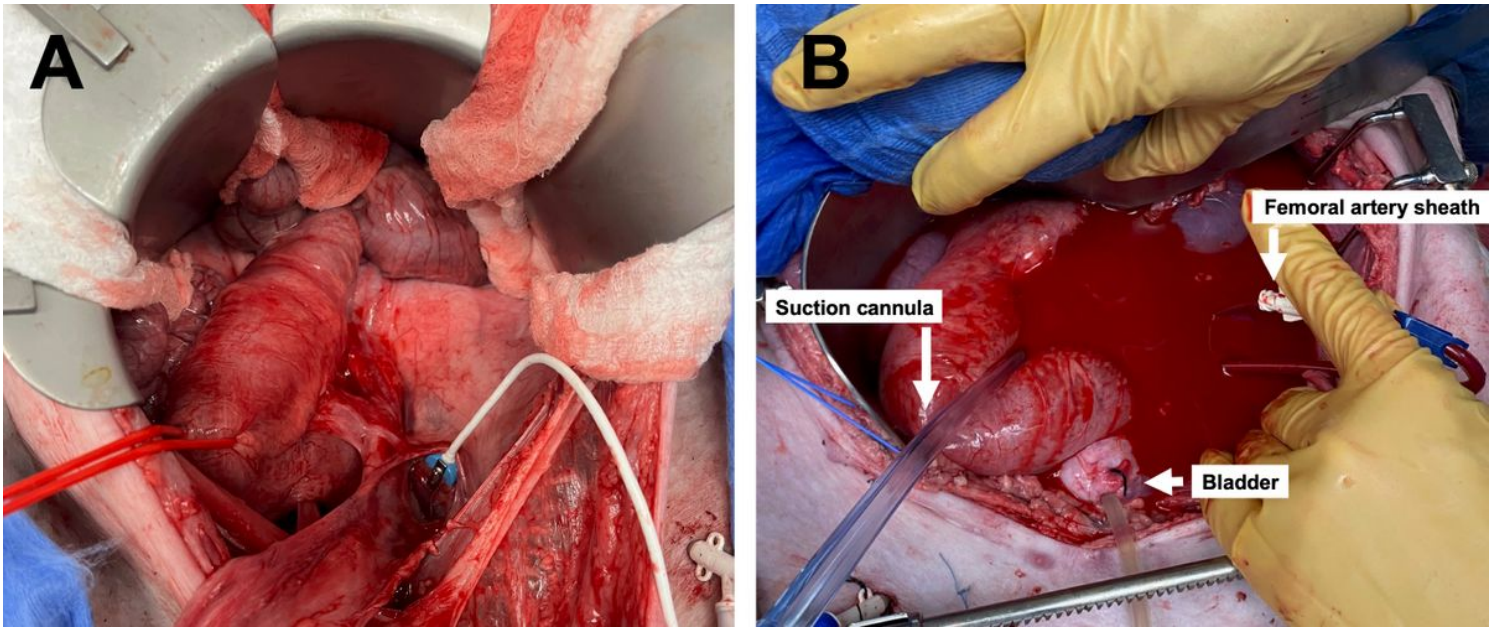

Figure 2

(A) The pelvic cavity exposed and retracted prior to the initiation of hemorrhage and (B) the pelvic cavity after the femoral artery sheath stopcock was opened for hemorrhage prior to suctioning.

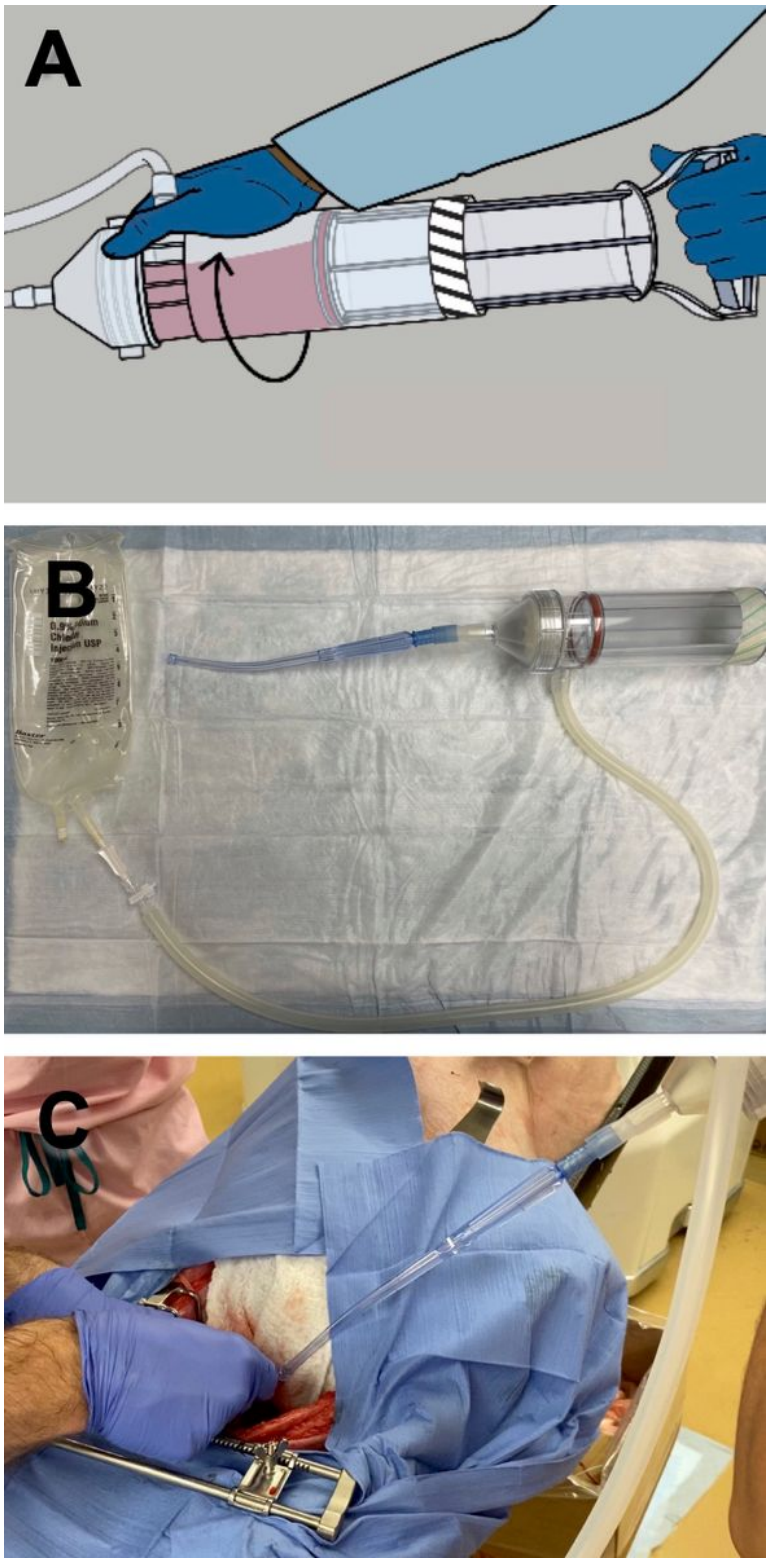

Figure 3

(A) The Sisu Global Hemafuse device illustration, (B) the Sisu Global Hemafuse device in real life connected to a bag for shed blood, and (C) set-up of pelvic cavity with Hemafuse device prior to intraperitoneal hemorrhage.

Variable	Baseline	Post-Suction Blood in Bag	Immediately Post-transfusion	t=30 min	t=60 min	t=120 min	t=180 min	t=240 min
Temp (C)								
Complete Blood Count								
WBC (10^3/μL)								
Lymphocytes (10^3/μL)								
RBC (10^6/μL)								
Hemoglobin (g/dL)								
Free Hb (g/dL)								
Hematocrit (%)								
Platelet count (10^3/μL)								
Potassium (mmol/L)								
Coagulation Studies								
INR								
PT (sec)								
aPTT (sec)								
ACT (sec)								
TEG Results								
TEG-ACT (sec)								
R time (sec)								
K time (sec)								
a-angle								
Maximum Amplitude								
LY-30								

Figure 4

Sample table for data collection of each time point during the study: animal pre-hemorrhage, shed blood in in blood bag, animal immediately post-transfusion, and at 30-, 60-, 120-, 180-, and 240-minute intervals during resuscitation.

Variable	Control Group						Therapeutic Group					
Animal	1	2	3	4	5	6	1	2	3	4	5	6
Shed Blood (mL)												
Shed Blood (g)												
Time to shed blood evacuation (min)												
Time to shed blood retransfusion (min)												

Figure 5

Sample table for data collection of the shed blood volume (mL) and weight (g), time to shed blood evacuation (minutes), and time to shed blood retransfusion (minutes) for each animal in the control group and therapeutic group.